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Hepcidin Regulation in the Anemia of Inflammation

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Abstract

Purpose of review—Anemia is prevalent in patients with infections and other inflammatory conditions. Induction of the iron regulatory hormone hepcidin has been implicated in the pathogenesis of anemia of inflammation (AI). This review outlines recent discoveries in understanding how hepcidin and its receptor ferroportin are regulated, how they contribute to AI, and how this knowledge may help guide new diagnostic and therapeutic strategies for this disease.

Recent findings—IL6 is a primary driver for hepcidin induction in many models of AI, but the SMAD1/5/8 pathway also contributes, likely via Activin B and SMAD-STAT3 interactions at the hepcidin promoter. Hepcidin has an important functional role in many, but not all, forms of AI, although hepcidin-independent mechanisms also contribute. In certain populations, hepcidin assays may help target therapy with iron or erythropoiesis stimulating agents to patients who may benefit most. New therapies targeting the hepcidin-ferroportin axis have shown efficacy in pre-clinical and early clinical studies.

Summary—Recent studies confirm an important role for the hepcidin-ferroportin axis in the development of AI, but also highlight the diverse and complex pathogenesis of this disorder depending on the underlying disease. Hepcidin-based diagnostic and therapeutic strategies offer promise to improve anemia treatment, but more work is needed in this area.

Keywords

anemia; inflammation; hepcidin; ferroportin; iron

INTRODUCTION

Anemia is a common complication in patients with infections, autoimmune disorders, malignancy, chronic kidney disease, and other inflammatory disorders. This disorder has been termed anemia of inflammation (AI) or anemia of chronic disease, and is the second most common form of anemia worldwide [1*-2]. A similar condition is seen in the elderly, although often in the absence of a specific underlying disease [3]. Typically, AI is

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J.L.B. has ownership interest in a startup company, Ferrumax Pharmaceuticals Inc., which has licensed technology from the Massachusetts General Hospital based on work cited here and described in prior publications.

normocytic, normochromic, and mild to moderately severe. A hallmark of AI is decreased availability of circulating iron for erythropoiesis despite adequate body iron stores.

pathogenesis of AI

The etiology of AI is multifactorial, and the pathophysiologic mechanisms are still being defined [1*-2]. Erythrocyte survival is shortened, in part due to macrophage activation by inflammatory cytokines, although hemolysis may also contribute. Inflammatory cytokines impair erythropoiesis by inhibiting the production and function of erythropoietin, and by directly inhibiting erythroid progenitor cell proliferation and differentiation. Importantly, inflammation also induces the iron regulatory hormone hepcidin and suppresses the iron exporter ferroportin to restrict the supply of iron for erythropoiesis.

HEPCIDIN AND FERROPORTIN REGULATE SYSTEMIC IRON BALANCE

As recently reviewed [4-5*], iron is provided by dietary absorption in the duodenum, recycling of aged erythrocytes by macrophages, and release from hepatocyte stores. Ferroportin is the sole known mammalian exporter responsible for iron entry into the bloodstream from these sources. Ferroportin is regulated by hepcidin, a 25 amino acid peptide hormone secreted by the liver (Figure 1). Upon binding to hepcidin, ferroportin is ubiquitinated on key lysine residues, endocytosed, and degraded in lysosomes, thereby inhibiting iron entry into the bloodstream. The crystal structure of a putative bacterial homologue of ferroportin was recently solved, which may yield important new insights into how ferroportin transports iron, and how hepcidin interacts with ferroportin to regulate its activity [6**]. Ferroportin also undergoes additional transcriptional and post-transcriptional regulation in a cell-type specific manner [5*].

HEPCIDIN REGULATION

As a key regulator of systemic iron balance, hepcidin transcription in the liver is controlled by a complex interplay of signals, most notably inflammation, iron status, and erythropoietic drive [4,7**]. Circulating and tissue iron upregulate hepcidin to limit further iron entry, while erythropoietic drive suppresses hepcidin to increase iron availability for erythrocyte production. Hepcidin induction by inflammation is presumed to have evolved to sequester iron from pathogenic microorganisms.

Hepcidin regulation by iron and the BMP/SMAD pathway

At the molecular level, the bone morphogenetic protein 6 (BMP6)-SMAD1/5/8 pathway is a central transcriptional regulator of hepcidin in response to iron [8] (Figure 1). BMP6 binds to the co-receptor hemojuvelin and BMP type I and type II receptors to induce phosphorylation of SMAD1/5/8 proteins, which complex with SMAD4 and bind BMP-responsive elements (BREs) on the hepcidin promoter to induce transcription. Interestingly, the crystal structure of hemojuvelin in complex with BMP ligands was recently solved [9**], revealing that the hemojuvelin binding site and the type I receptor binding site overlap. This raises the intriguing question of the true nature of the signaling complex, and how hemojuvelin enhances BMP-SMAD1/5/8 signaling, which is supported by abundant

functional evidence [8]. One model proposes that hemojuvelin binds to BMPs on the cell surface to target ligands for internalization, where type I receptors replace the co-receptor in the acidic endosomal environment to potentiate signaling [9]. This model needs further confirmation by functional studies.

How iron levels are sensed to regulate BMP-SMAD1/5/8 signaling and hepcidin are areas of active investigation [8]. Increases in liver iron induce BMP6 expression, predominantly in liver nonparenchymal cells, while circulating iron induces SMAD1/5/8 phosphorylation downstream or independent of BMP6. Transferrin receptors 1 and 2 likely function as sensors of circulating iron, and interact with the hemochromatosis protein HFE to regulate BMP-SMAD1/5/8 signaling and hepcidin. The transmembrane serine protease 6 (TMPRSS6) was demonstrated to cleave hemojuvelin, and functions as a negative regulator of this pathway in response to iron deficiency. Neogenin is a hemojuvelin interacting protein that may also participate in hepcidin regulation. Although recent studies propose some models for how these pathways and proteins may interact [10-11], the precise molecular mechanisms remain incompletely understood (Figure 1).

Hepcidin regulation by inflammation

Inflammatory cytokines, in particular IL6, regulate hepcidin transcription via the JAK-STAT3 pathway [7] (Figure 1). Recent studies confirm an important role for IL6 in hepcidin induction by many different infections including *streptococcus pneumonia* and influenza A, as well as most extracellular pathogen-associated molecular patterns, since IL6 knockout mice demonstrated impaired or absent hepcidin induction to these stimuli [12]. However, IL22, which can induce hepcidin expression both *in vitro* and *in vivo*, has only a minor role in hepcidin induction by LPS as demonstrated by studies in IL22 knockout mice [13]. A role for IL22 in other infectious or inflammatory conditions *in vivo* remains to be determined. IL1 can also regulate hepcidin, either by inducing IL6 or by IL6-independent mechanisms [14-15]. A recent study confirmed that IL1 β stimulates hepcidin and induces hypoferrremia in mice, and proposed an alternate mechanism through the induction of SMAD1/5/8 signaling [16]. This pathway was implicated as a mechanism for hepcidin induction by commensal intestinal bacteria with potential relevance to inflammatory bowel disease, although the *in vivo* relevance remains to be established. [16].

Crosstalk between inflammation and the SMAD1/5/8 pathway in hepcidin regulation

The inflammatory pathway functionally interacts with the SMAD1/5/8 pathway to regulate hepcidin transcription. On the hepcidin promoter, the proximal BRE is adjacent to the single STAT3 binding element, and cooperativity between SMAD and STAT3 transcription factors at this site was recently explored by mathematical modeling and experimental validation [17*]. This study confirmed that the proximal BRE and a certain basal level of BMP signaling activity are required for hepcidin promoter responsiveness to IL6. Moreover, inflammation reduces hepcidin promoter sensitivity to maximally respond to iron/BMP signals, which may contribute to the pathogenesis of AI. Notably, inflammation also induces SMAD1/5/8 signaling independent of BMP6, likely by inducing hepatic expression of another TGF- β /BMP superfamily ligand, Activin B [18]. Although classically described to utilize distinct type I receptors and SMAD2/3 signaling, Activin B can utilize BMP type I

receptors to stimulate noncanonical SMAD1/5/8 signaling and hepcidin selectively in hepatocytes [19] (Figure 1). A functional role for this pathway *in vivo* was suggested by the ability of the activin inhibitor follistatin-315 to inhibit hepcidin induction in mouse models of inflammation [19]. IL1 β may be one mechanism by which inflammation upregulates Activin B expression in the liver [16].

Hepcidin regulation by endoplasmic reticulum (ER) stress, nutrient signals, hormones, and growth factors

Inflammation is associated with ER stress, which also upregulates hepcidin transcription via CREB3L3 (also known as CREBH) both *in vitro* and in mice [20]. This transcription factor was recently linked to hepcidin regulation by gluconeogenic signals along with the transcriptional co-activator PPARGC1A [21]. Many other nutrient, hormonal, and growth factor stimuli have also been implicated in hepcidin regulation, including hepatocyte growth factor, epidermal growth factor, estrogen, testosterone, progesterone, platelet derived growth factor-BB, and the Ras/RAF and mTOR signaling pathways [8, 22-25*]. The physiologic relevance of the pathways *in vivo* and how they intersect with other hepcidin regulatory pathways are currently being explored.

Hepcidin regulation by anemia

Erythroferrone was recently discovered as an erythroid suppressor of hepcidin expression, and may also have a role in AI [26*-27]. A member of the C1q/TNF-related protein family, erythroferrone was produced in erythroblasts in response to erythropoietin via the JAK2-STAT5 pathway [26*], and levels were increased in β -thalassemia [28] and AI [27] mouse models. Exogenous erythroferrone reduced hepatic hepcidin mRNA via an uncertain mechanism, which appears to be distinct from the BMP-SMAD pathway [26*]. Importantly, erythroferrone knockout mice failed to suppress hepcidin and exhibited delayed recovery from acute blood loss anemia [26*], while they showed some improvement in iron overload and ineffective erythropoiesis in a β -thalassemia model [28]. Notably, erythroferrone knockout mice also had higher hepcidin, more pronounced iron restriction, and more severe anemia in a heat-killed *Brucella abortus* (BA) mouse model of AI, suggesting that erythroferrone may assist in the recovery from AI [27]. Based on the erythroferrone knockout mouse phenotype, there are likely additional unidentified erythroid regulators of hepcidin.

HEPCIDIN AND THE PATHOGENESIS OF AI

Recent studies explored the contribution of hepcidin versus other factors to the pathogenesis of AI, and how this varies in different underlying diseases.

Disease Specific Effects

In BA and turpentine mouse models, hepcidin knockout mice exhibited less severe anemia and a lack of iron restriction compared with wildtype mice, confirming an important functional role for hepcidin in AI, at least in these models [29*-31]. However, hepcidin knockout mice still exhibited some AI features including reduced erythroid progenitor cells, erythropoiesis, and red blood cell number. IL6 knockout mice were also partially protected

against AI in the BA model, and exhibited faster recovery of erythropoiesis, suggesting a distinct role for IL6 in AI pathophysiology by suppressing erythropoiesis [29*]. Recently, IL6 was demonstrated to reduce mitochondrial membrane potential in an erythroleukemic cell line to impair hemoglobin production and erythroid maturation, suggesting at least one mechanism for IL6 to inhibit erythropoiesis [32]. Although these animal models have some limitations, they are valuable tools to dissect the pathophysiology of AI and test new therapeutic strategies.

In a mouse model of anemia of aging [3], older mice exhibited reduced hemoglobin, reduced erythrocyte numbers, increased myeloid lineage cells, and increased IL6 and IFN γ . Although hepcidin was not higher, it may have been inappropriately high relative to the degree of anemia. Aged IL6 and hepcidin knockout mice each exhibited less severe anemia, suggesting that both contribute to the anemia of aging, but other factors also contribute, potentially IFN γ and other modifiers of erythroid and myeloid progenitor commitment.

The etiology of anemia of cancer was also recently examined. In a prospective observational cohort of patients with solid tumors before receiving treatment [33], hemoglobin was inversely associated with inflammatory markers, hepcidin, ferritin, erythropoietin, reactive oxygen species, cancer stage and performance status, while it was positively correlated with serum iron, transferrin, and nutritional markers including albumin and leptin. By multivariate analysis, IL6, leptin, and cancer stage were independent predictors of hemoglobin. Differences were seen with different cancer types, with colorectal cancer exhibiting low hepcidin, serum iron, and ferritin, suggesting a component of iron deficiency anemia (IDA). In 4 mouse models of anemia of cancer [34], most models exhibited inflammation and iron-restricted erythropoiesis, but only one model had elevated hepcidin. An ovarian cancer model exhibited features of IDA without inflammation. Notably, anemia was not ameliorated by hepcidin knockout, suggesting that anemia is predominantly hepcidin-independent in these cancer models. Together, these data highlight the multifactorial nature of anemia of cancer related to inflammation as well as nutritional and metabolic components, and underscore important differences among cancer types.

Pathogen-specific effects

Pathogenic microorganisms require iron for growth and survival and have evolved sophisticated mechanisms for acquiring host iron. Humans and other hosts have likewise evolved to restrict iron from invading pathogens. Recent evidence for this evolutionary struggle comes from analysis of transferrin, which chaperones iron in the bloodstream, and transferrin-binding protein A (TbpA), a bacterial protein which hijacks transferrin to procure iron [35**]. The authors identified several hotspots on transferrin and TbpA that have undergone rapid co-evolution, with transferrin variants selected to preclude TbpA binding, and TbpA mutations counterselected to recapture the modified transferrin. Hepcidin induction by inflammation is presumed to have evolved as another mechanism of nutritional immunity to sequester iron from invading pathogens and possibly also modify the immune response. However, direct experimental evidence for this host defense function of hepcidin and hypoferrinemia is only starting to emerge.

One developing theme is that hepcidin-ferroportin modulation may be different, and the effects may be beneficial or harmful depending on the pathogen and its niche. For example, while many infections upregulate hepcidin and induce hypoferremia, hepatitis B and hepatitis C viruses do not elicit a systemic inflammatory response or induce hepcidin or hypoferremia [36].

A protective role for hepcidin and hypoferremia is most clearly established for siderophilic bacteria, including *Vibrio vulnificus* and *Yersinia enterocolitica*, which are generally rare, but can be lethal in patients with hepcidin deficiency and iron overload characteristic of hereditary hemochromatosis [20]. A pathogenic role for hepcidin deficiency in *Vibrio vulnificus* infections was recently established using hepcidin knockout mice [37*], which exhibited increased bacteremia and decreased survival. This phenotype was partially rescued by dietary iron depletion, and even more effectively rescued by exogenous hepcidin agonists that induced a profound hypoferremia. *Ex vivo* studies in mouse sera demonstrated that the hypoferremic effects of hepcidin agonists, rather than direct antimicrobial effects were responsible for inhibiting bacterial replication.

Conversely, intracellular organisms that reside in macrophages may have enhanced pathogenicity due to hepcidin-induced iron sequestration. Two recent studies in humans [38*] and mice [39*] demonstrated that acute infection caused by *Salmonella* Typhi or *Salmonella* Typhimurium was associated with significantly increased hepcidin and hypoferremia. In the mouse model, hepcidin induction was IL6 dependent, since hepcidin and serum iron were unchanged IL6 knockout mice, and notably, bacterial burden was reduced. These authors identified a novel mechanism by which IL6 regulates hepcidin transcription via estrogen-related receptor (ERR) γ . Notably, an inverse agonist of ERR γ reduced hepcidin, normalized hypoferremia, reduced bacterial burden, and improved survival, suggesting that hepcidin-induced macrophage iron sequestration could have deleterious host effects in this model. Previous studies in this model also revealed local mechanisms in macrophages that limit iron availability, including induction of ferroportin transcription by interferon γ , nitric oxide and nuclear factor erythroid 2-related factor 2 (NRF2) [40]. This may help explain the reduced spleen iron levels seen in more chronic *Salmonella* typhimurium infection [41]. More work is needed to better understand the diverse roles of hepcidin and iron in infection and immunity, and the reader is referred to recent reviews for more in depth discussions [7;40].

Hepcidin-independent ferroportin regulation and hypoferremia in AI

In addition to hepcidin-mediated ferroportin degradation, recent studies demonstrated a functional role for hepcidin-independent ferroportin transcriptional downregulation in the acute hypoferremia induced by Toll-like receptor (TLR) 2/6 ligands [42*] and TLR4 ligands in mice [43]. Inflammatory cytokines also exert a number of other hepcidin-independent effects on iron homeostasis at multiple levels, including increasing macrophage iron uptake, increasing erythrophagocytosis, and promoting efficient iron storage [1*;40]. However, the strong effect of lowering hepcidin on reversing hypoferremia in chronic AI models [29*-30] supports the dominant role of hepcidin in its pathophysiology.

HEPCIDIN-BASED diagnostic strategies and implications for GUIDING THERAPY

AI is diagnosed by the findings of anemia with reduced circulating iron levels despite adequate or elevated body iron stores. Serum ferritin is typically used to assess body iron stores; however, it has limited specificity because it is regulated not only by iron, but also by inflammation and numerous other factors. A particular difficulty is distinguishing isolated AI from IDA and mixed AI with IDA that might benefit from iron therapy [1*-2;44]. In the developing world, where IDA remains a significant global health problem that contributes to poor pregnancy outcome, impaired physical and cognitive development, and reduced work productivity, it is particularly important to identify subpopulations that may benefit from iron without increasing susceptibility to malaria and other infections as was demonstrated in some supplementation initiatives [45]. Erythropoiesis stimulating agents (ESAs) are another therapeutic option with clear benefits for improving quality of life and reducing transfusions in chronic kidney disease patients. However, ESAs also have significant potential adverse effects including an increased risk of stroke and other cardiovascular events, hypertension, clotting, malignancy progression, and mortality [44]. Recent studies investigated the utility of hepcidin assays for identifying patients who may most benefit from iron or ESA treatment.

In a study of African children [46**], serum hepcidin identified IDA and distinguished this from AI with an AUC^{ROC} 0.84-0.88. Serum hepcidin also predicted responsiveness to oral iron therapy with an AUC^{ROC} 0.90. In these respects, hepcidin was statistically similar or outperformed other established measures. The use of hepcidin would have allowed most children with IDA to be identified and treated, while reducing the percent of patients with malaria and AI being treated from 73 and 100% to 20 and 14% respectively. Another study in African children demonstrated that hepcidin levels were lower and the prevalence of iron deficiency increased at the end of the malaria season, which may help guide better timing for iron supplementation strategies in this patient population [47].

In a study of 38 patients with chronic rheumatic diseases, the strongest association with hemoglobin response to iron therapy was the 1 week increase of reticulocyte hemoglobin content, transferrin saturation, serum iron, and reticulocyte count, with baseline hepcidin also predictive to a lesser degree [48]. In rats with AI due to group A streptococcal peptidoglycan polysaccharide (PG-APS), higher pretreatment hepcidin was associated with a poor hematologic response to ESAs [49]. More work is needed to understand where and how hepcidin or other assays may be useful in different patient populations with AI.

NOVEL THERAPEUTIC STRATEGIES FOR AI

Experimental agents targeting the hepcidin-ferroportin axis (Figure 2) have recently been tested in pre-clinical and early clinical trials as new treatments for AI. One strategy targets hepcidin production by inhibiting IL6-STAT3 signaling. The IL6 receptor antibody Tocilizumab reduced hepcidin and improved anemia and iron parameters in patients with rheumatoid arthritis [50-51]. The IL6 antibody Siltuximab lowered hepcidin and improved anemia in patients with multicentric Castleman's Disease [52]. Hydrogen sulfide suppressed

hepcidin and improved hypoferremia in LPS-treated mice by suppressing IL6 production and promoting sirtuin 1-mediated deacetylation of STAT3 to inhibit its activity [53].

A second strategy targets hepcidin production by inhibiting SMAD1/5/8 signaling. A small molecule BMP type I receptor inhibitor (LDN-193189) [49;54-55] and a soluble hemojuvelin fusion protein [55-56] showed efficacy to lower hepcidin and mobilize iron in animal models of AI. Soluble hemojuvelin entered Phase 2 clinical trials to treat anemia in human patients with kidney disease (NCT02228655). Heparins and engineered heparins with low anticoagulant activity, which were shown to inhibit SMAD1/5/8 signaling by sequestering BMP ligands, lowered hepcidin and improved hypoferremia and anemia in rodent AI models [57-58]. The Activin B inhibitor follistatin-315 lowered hepcidin induction by LPS in mice [19]. BMP6 and hemojuvelin neutralizing antibodies reduced hepcidin and mobilized iron in normal rodents and monkeys [59-60].

Additional strategies target hepcidin directly using hepcidin antibodies, anticalins, spiegelmers, and antisense oligonucleotides, or target ferroportin. The hepcidin spiegelmer lexaptetid (NOX-H94) ameliorated IL6-induced anemia in a primate model [61] and reversed LPS-induced hypoferremia in human patients [62*]. Phase 2 clinical trials are underway. A neutralizing hepcidin antibody ameliorated anemia in a rodent AI model and improved iron availability in monkeys [63]. A hepcidin anticalin (PRS-080) and antibody targeting the hepcidin-binding site on ferroportin (LY2928057) have undergone Phase I clinical trials (NCT02340572; NCT01330953).

CONCLUSIONS

Recent studies have enhanced our understanding of the complex pathophysiology of AI and the contribution of the hepcidin-ferroportin axis, which may differ depending on the underlying disease process. This knowledge is leading to the development of new targeted therapeutic strategies, and new diagnostic strategies to tailor therapy to those who may benefit most. There are many key areas for future research. We need to better understand the pathophysiology of AI in humans with different underlying diseases, how iron balance affects susceptibility to many common pathogens and the immune response, and how best to treat patients with AI by recognizing the benefits and risks of iron, ESAs, transfusions, and newer hepcidin lowering therapies in different AI populations.

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REFERENCES AND RECOMMENDED READING

- 1*. Weiss, Guenter. Anemia of Chronic Disorders: New Diagnostic Tools and New Treatment Strategies. *Semin Hematol.* 2015; 52:313–320. This review discusses the epidemiology, pathophysiology, diagnosis, and treatment of anemia of inflammation. [PubMed: 26404443]

2. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am.* 2014; 28:671–81. [PubMed: 25064707]
3. McCranor BJ, Langdon JM, Prince OD, et al. Investigation of the role of interleukin-6 and hepcidin antimicrobial peptide in the development of anemia with age. *Haematologica.* 2013; 98:1633–1640. [PubMed: 23996485]
4. Ganz T. Systemic iron homeostasis. *Physiol Rev.* 2013; 93:1721–1741. [PubMed: 24137020]
- 5*. Drakesmith H, Nemeth E, Ganz T. Ironing out Ferroportin. *Cell Metab.* 2015; 22:777–787. This is a comprehensive review of the iron exporter ferroportin. Topics discussed include the role of ferroportin in systemic and local iron homeostasis, ferroportin regulation by hepcidin and other factors, ferroportin's mechanism of iron transport, genetic disorders of ferroportin, and ferroportin and infection. [PubMed: 26437604]
- 6**. Taniguchi R, Kato HE, Font J, et al. Outward- and inward-facing structures of a putative bacterial transition-metal transporter with homology to ferroportin. *Nat Commun.* 2015; 6:8545. This study reports the crystal structure of a putative bacterial homologue of ferroportin in its inward and outward facing states. Results identify a substrate metal binding site, suggest a possible mechanism of conformational transition, and localize the predicted hepcidin-binding site to the central cavity, providing important new insights into ferroportin function. [PubMed: 26461048]
- 7**. Ganz T, Nemeth E. Iron homeostasis in host defence and inflammation. *Nat Rev Immunol.* 2015; 15:500–510. This review provides an in depth exploration of the role of systemic and local iron regulation by hepcidin and other mechanisms in the context of infection. [PubMed: 26160612]
8. Core AB, Canali S, Babitt JL. Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis. *Front Pharmacol.* 2014; 5:104. [PubMed: 24860505]
- 9**. Healey EG, Bishop B, Elegheert J, et al. Repulsive guidance molecule is a structural bridge between neogenin and bone morphogenetic protein. *Nat Struct Mol Biol.* 2015; 22:458–465. This study reports the crystal structure of hemojuvelin in complex with BMP2 via the N-terminal domain of hemojuvelin that is distinct from the C-terminal binding site for neogenin. Hemojuvelin binds BMP2 in a pH sensitive fashion at a site that overlaps with BMP type I receptors, raising the possibility that endocytosis and acidification may have a role hemojuvelin-mediated BMP signaling and hepcidin regulation. [PubMed: 25938661]
10. Wu XG, Wang Y, Wu Q, et al. HFE interacts with the BMP type I receptor ALK3 to regulate hepcidin expression. *Blood.* 2014; 124:1335–1543. [PubMed: 24904118]
11. Latour C, Besson-Fournier C, Meynard D, et al. Differing impact of the deletion of hemochromatosis-associated molecules HFE and transferrin receptor-2 on the iron phenotype of mice lacking bone morphogenetic protein 6 or hemojuvelin. *Hepatology.* 2016; 63:126–137. [PubMed: 26406355]
- 12*. Rodriguez R, Jung CL, Gabayan V, et al. Hepcidin induction by pathogens and pathogen-derived molecules is strongly dependent on interleukin-6. *Infect Immun.* 2014; 82:745–752. This study employed IL6 knockout mice to solidify a central role for IL6 in hepcidin induction by common bacterial and viral infections, and most pathogen associated molecular patterns. [PubMed: 24478088]
13. Wallace DF, Subramaniam VN. Analysis of IL-22 contribution to hepcidin induction and hypoferrremia during the response to LPS in vivo. *Int Immunol.* 2015; 27:281–287. [PubMed: 25568302]
14. Lee P, Peng H, Gelbart T, et al. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proc Natl Acad Sci U S A.* 2005; 102:1906–1910. [PubMed: 15684062]
15. Kramer F, Torzewski J, Kamenz J, et al. Interleukin-1beta stimulates acute phase response and C-reactive protein synthesis by inducing an NFkappaB- and C/EBPbeta-dependent autocrine interleukin-6 loop. *Mol Immunol.* 2008; 45:2678–2689. [PubMed: 18262272]
16. Shanmugam NK, Chen K, Cherayil BJ. Commensal Bacteria-induced Interleukin 1 β (IL-1 β) Secreted by Macrophages Up-regulates Hepcidin Expression in Hepatocytes by Activating the Bone Morphogenetic Protein Signaling Pathway. *J Biol Chem.* 2015; 290:30637–30647. [PubMed: 26515063]
- 17*. Casanovas G, Banerji A, d'Alessio F, et al. A multi-scale model of hepcidin promoter regulation reveals factors controlling systemic iron homeostasis. *PLoS Comput Biol.* 2014; 10:e1003421. This study utilized mathematical modeling and *in vitro* experimental validation to investigate

how IL6-STAT3 and BMP6-SMAD1/5/8 signaling interact to control hepcidin transcription. Results suggest that the hepcidin response to iron and inflammatory stimuli is shaped by cooperativity and competition between SMAD and STAT transcription factors at the hepcidin promoter. [PubMed: 24391488]

18. Besson-Fournier C, Latour C, Kautz L, et al. Induction of activin B by inflammatory stimuli up-regulates expression of the iron-regulatory peptide hepcidin through Smad1/5/8 signaling. *Blood*. 2012; 120:431–439. [PubMed: 22611157]
19. Canali S, Core AB, Zumbrennen-Bullough KB, et al. Activin B induces noncanonical SMAD1/5/8 signaling via BMP type I receptors in hepatocytes: evidence for a role in hepcidin induction by inflammation in male mice. *Endocrinology*. Jan 6.2016 :en20151747.
20. Pietrangelo A. Genetics, Genetic Testing, and Management of Hemochromatosis: 15 Years Since Hepcidin. *Gastroenterology*. 2015; 149:1240–1251. e4. [PubMed: 26164493]
21. Vecchi C, Montosi G, Garuti C, et al. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. *Gastroenterology*. 2014; 146:1060–1069. [PubMed: 24361124]
22. Latour C, Kautz L, Besson-Fournier C, et al. Testosterone perturbs systemic iron balance through activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. *Hepatology*. 2014; 59:683–694. [PubMed: 23907767]
23. Li X, Rhee DK, Malhotra R, et al. Progesterone receptor membrane component-1 regulates hepcidin biosynthesis. *J Clin Invest*. 2016; 126:389–401. [PubMed: 26657863]
24. Sonnweber T, Nachbaur D, Schroll A, et al. Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. *Gut*. 2014; 63:1951–1959. [PubMed: 24598129]
- 25*. Mleczo-Sanecka K, Roche F, da Silva AR, et al. Unbiased RNAi screen for hepcidin regulators links hepcidin suppression to proliferative Ras/RAF and nutrient-dependent mTOR signaling. *Blood*. 2014; 123:1574–1585. This study used an RNAi screen and hepcidin promoter luciferase reporter assay in hepatoma cells to identify novel hepcidin regulators. Fifteen novel regulators were validated, and results implicate the Ras/RAF MAPK and mTOR pathways in hepcidin suppression, and new components of the transcriptional machinery that may affect steady-state, IL6-, or BMP-induced hepcidin levels. [PubMed: 24385536]
- 26**. Kautz L, Jung G, Valore EV, et al. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet*. 2014; 46:678–684. Using bone marrow gene expression profiling after phlebotomy, this study identified erythroferrone as a novel erythroid suppressor of hepcidin expression. Importantly, a functional role for erythroferrone in recovery from acute blood loss anemia, hepcidin suppression in thalassemia, and recovery from anemia of inflammation is supported by this and companion studies [27, 28] in erythroferrone knockout mice. [PubMed: 24880340]
27. Kautz L, Jung G, Nemeth E, Ganz T. Erythroferrone contributes to recovery from anemia of inflammation. *Blood*. 2014; 124:2569–2574. [PubMed: 25193872]
28. Kautz L, Jung G, Du X, et al. Erythroferrone contributes to hepcidin suppression and iron overload in a mouse model of β -thalassemia. *Blood*. 2015; 126:2031–2037. [PubMed: 26276665]
- 29**. Gardenghi S, Renaud TM, Meloni A, et al. Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed *Brucella abortus*. *Blood*. 2014; 123:1137–1145. In conjunction with 2 other studies [30-31], this study used IL6 and hepcidin knockout mice to delineate their relative contribution to AI. Both IL6 and hepcidin knockout mice displayed milder anemia and faster recovery compared with wildtype mice but with different characteristic features, confirming their important, but distinct, functional roles. [PubMed: 24357729]
30. Kim A, Fung E, Parikh SG, et al. A mouse model of anemia of inflammation: complex pathogenesis with partial dependence on hepcidin. *Blood*. 2014; 123:1129–1136. [PubMed: 24357728]
31. Langdon JM, Yates SC, Femnou LK, et al. Hepcidin-dependent and hepcidin-independent regulation of erythropoiesis in a mouse model of anemia of chronic inflammation. *Am J Hematol*. 2014; 89:470–479. [PubMed: 24415655]
32. McCranor BJ, Kim MJ, Cruz NM, et al. Interleukin-6 directly impairs the erythroid development of human TF-1 erythroleukemic cells. *Blood Cells Mol Dis*. 2014; 52:126–133. [PubMed: 24119518]

33. Macciò A, Madeddu C, Gramignano G, et al. The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large, prospective, observational study. *Haematologica*. 2015; 100:124–132. [PubMed: 25239265]
34. Kim A, Rivera S, Shprung D, et al. Mouse models of anemia of cancer. *PLoS One*. 2014; 9:e93283. [PubMed: 24681760]
- 35**. Barber MF, Elde NC. Nutritional immunity. Escape from bacterial iron piracy through rapid evolution of transferrin. *Science*. 2014; 346:1362–1366. This study compared transferrin orthologue sequences among humans and other primates, as well as sequence variants of bacterial transferrin binding protein A (TbpA). Results reveal hot spots of rapidly evolving sites at the transferrin-TbpA binding interface, suggesting evolutionary pressure for bacteria to capture host iron and for primates to evade iron piracy. [PubMed: 25504720]
36. Armitage AE, Stacey AR, Giannoulatou E, et al. Distinct patterns of hepcidin and iron regulation during HIV-1, HBV, and HCV infections. *Proc Natl Acad Sci U S A*. 2014; 111:12187–12192. [PubMed: 25092293]
- 37*. Arezes J, Jung G, Gabayan V, et al. Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium *Vibrio vulnificus*. *Cell Host Microbe*. 2015; 17:47–57. Hepcidin knockout mice, dietary iron loading and depletion, and administration of hepcidin agonists were employed to elucidate the mechanism of increased susceptibility to the siderophilic bacteria *Vibrio vulnificus* that is reported in hereditary hemochromatosis. Results suggest that hepcidin-mediated hypoferremia is an important host defense mechanism against this extracellular pathogen. [PubMed: 25590758]
- 38*. Darton TC, Blohmke CJ, Giannoulatou E, et al. Rapidly Escalating Hepcidin and Associated Serum Iron Starvation Are Features of the Acute Response to Typhoid Infection in Humans. *PLoS Negl Trop Dis*. 2015; 9:e0004029. This study explored the effects of acute *Salmonella* Typhi infection in human volunteers. Results demonstrate that acute typhoid infection is associated with elevated hepcidin and hypoferremia in humans. [PubMed: 26394303]
- 39*. Kim DK, Jeong JH, Lee JM, et al. Inverse agonist of estrogen-related receptor γ controls *Salmonella* typhimurium infection by modulating host iron homeostasis. *Nat Med*. 2014; 20:419–424. This study examined *Salmonella* Typhimurium infection in mice to discover a novel mechanism for hepcidin induction via the estrogen-related receptor γ (ERR γ). The antimicrobial effect of an inverse agonist of ERR γ in mice support a harmful role of hepcidin-mediated macrophage iron sequestration in infections with this intracellular pathogen. [PubMed: 24658075]
40. Nairz M, Haschka D, Demetz E, Weiss G. Iron at the interface of immunity and infection. *Front Pharmacol*. 2014; 5:152. [PubMed: 25076907]
41. Brown DE, Nick HJ, McCoy MW, et al. Increased ferroportin-1 expression and rapid splenic iron loss occur with anemia caused by *Salmonella* enterica Serovar Typhimurium infection in mice. *Infect Immun*. 2015; 83:2290–2299. [PubMed: 25824831]
- 42*. Guida C, Altamura S, Klein FA, et al. A novel inflammatory pathway mediating rapid hepcidin-independent hypoferremia. *Blood*. 2015; 125:2265–2275. This study provides *in vivo* evidence in mice for a hepcidin-independent mechanism of acute hypoferremia induced by TLR2/6 ligands via inhibiting ferroportin mRNA. [PubMed: 25662334]
43. Deschemin JC, Vaulont S. Role of hepcidin in the setting of hypoferremia during acute inflammation. *PLoS One*. 2013; 8:e61050. [PubMed: 23637785]
44. Zumbrennen-Bullough K, Babitt JL. The iron cycle in chronic kidney disease (CKD): from genetics and experimental models to CKD patients. *Nephrol Dial Transplant*. 2014; 29:263–273. [PubMed: 24235084]
45. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet*. 2006; 367:133–143. [PubMed: 16413877]
- 46**. Pasricha SR, Atkinson SH, Armitage AE, et al. Expression of the iron hormone hepcidin distinguishes different types of anemia in African children. *Sci Transl Med*. 2014; 6:235re3. This study demonstrates that a serum hepcidin assay can differentiate iron deficiency anemia from

anemia of inflammation, and identify iron-responsive subpopulations of African children to guide more targeted iron supplementation strategies.

47. Atkinson SH, Armitage AE, Khandwala S, et al. Combinatorial effects of malaria season, iron deficiency, and inflammation determine plasma hepcidin concentration in African children. *Blood*. 2014; 123:3221–3229. [PubMed: 24596418]
48. van Santen S, de Mast Q, Oosting JD, et al. Hematologic parameters predicting a response to oral iron therapy in chronic inflammation. *Haematologica*. 2014; 99:e171–173. [PubMed: 24895340]
49. Theurl M, Nairz M, Schroll A, et al. Hepcidin as a predictive factor and therapeutic target in erythropoiesis-stimulating agent treatment for anemia of chronic disease in rats. *Haematologica*. 2014; 99:1516–1524. [PubMed: 24895335]
50. Song SN, Iwahashi M, Tomosugi N, et al. Comparative evaluation of the effects of treatment with tocilizumab and TNF- α inhibitors on serum hepcidin, anemia response and disease activity in rheumatoid arthritis patients. *Arthritis Res Ther*. 2013; 15:R141. [PubMed: 24286116]
51. Isaacs JD, Harari O, Kobold U, et al. Effect of tocilizumab on haematological markers implicates interleukin-6 signalling in the anaemia of rheumatoid arthritis. *Arthritis Res Ther*. 2013; 15:R204. [PubMed: 24295403]
52. Casper C, Chaturvedi S, Munshi N, et al. Analysis of Inflammatory and Anemia-Related Biomarkers in a Randomized, Double-Blind, Placebo-Controlled Study of Siltuximab (Anti-IL6 Monoclonal Antibody) in Patients With Multicentric Castleman Disease. *Clin Cancer Res*. 2015; 21:4294–4304. [PubMed: 26124203]
53. Xin H, Wang M, Tang W, et al. Hydrogen Sulfide Attenuates Inflammatory Hepcidin by Reducing IL-6 Secretion and Promoting SIRT1-Mediated STAT3 Deacetylation. *Antioxid Redox Signal*. Sep 3.2015 [Epub ahead of print].
54. Mayeur C, Kolodziej SA, Wang A, et al. Oral administration of a bone morphogenetic protein type I receptor inhibitor prevents the development of anemia of inflammation. *Haematologica*. 2015; 100:e68–71. [PubMed: 25326432]
55. Theurl I, Schroll A, Sonnweber T, et al. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. *Blood*. 2011; 118:4977–4984. [PubMed: 21730356]
56. Sun CC, Vaja V, Chen S, et al. A hepcidin lowering agent mobilizes iron for incorporation into red blood cells in an adenine-induced kidney disease model of anemia in rats. *Nephrol Dial Transplant*. 2013; 28:1733–1743. [PubMed: 23345622]
57. Poli M, Asperti M, Naggi A, et al. Glycol-split nonanticoagulant heparins are inhibitors of hepcidin expression in vitro and in vivo. *Blood*. 2014; 123:1564–1573. [PubMed: 24398330]
58. Poli M, Asperti M, Ruzzenenti P, et al. Oversulfated heparins with low anticoagulant activity are strong and fast inhibitors of hepcidin expression in vitro and in vivo. *Biochem Pharmacol*. 2014; 92:467–475. [PubMed: 25241290]
59. Andriopoulos B Jr, Corradini E, Xia Y, et al. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat Genet*. 2009; 41:482–487. [PubMed: 19252486]
60. Böser P, Seemann D, Liguori MJ, et al. Anti-repulsive Guidance Molecule C (RGMc) Antibodies Increases Serum Iron in Rats and Cynomolgus Monkeys by Hepcidin Downregulation. *AAPS J*. 2015; 17:930–938. [PubMed: 25896304]
61. Schwoebel F, van Eijk LT, Zboralski D, et al. The effects of the anti-hepcidin Spiegelmer NOX-H94 on inflammation-induced anemia in cynomolgus monkeys. *Blood*. 2013; 121:2311–2315. [PubMed: 23349391]
- 62*. van Eijk LT, John AS, Schwoebel F, et al. Effect of the antihepcidin Spiegelmer lexaptapeid on inflammation-induced decrease in serum iron in humans. *Blood*. 2014; 124:2643–2646. This double-blind randomized placebo controlled trial in humans demonstrated that the antihepcidin spiegelmer lexaptapeid ameliorated the hypoferrremia induced by LPS injection. This is the first published human study demonstrating a potential therapeutic effect for a direct hepcidin antagonist in an inflammatory model. [PubMed: 25163699]
63. Cooke KS, Hinkle B, Salimi-Moosavi H, et al. A fully human anti-hepcidin antibody modulates iron metabolism in both mice and nonhuman primates. *Blood*. 2013; 122:3054–3061. [PubMed: 23945155]

KEY POINTS (3-5)

1. 1) Anemia of inflammation (AI) has a complex pathophysiology depending on the underlying disease process, but major contributing factors include reduced iron availability, impaired erythrocyte production, and shortened erythrocyte survival.
2. 2) The hypoferremia of AI is largely mediated by hepcidin, which acts to degrade ferroportin and inhibit iron entry into plasma, although some hepcidin-independent mechanisms may also have a role.
3. 3) IL6 is a key inducer of hepcidin in most models of AI by promoting phosphorylation of STAT3, which acts together with SMAD1/5/8 to activate the hepcidin promoter.
4. 4) Low hepcidin levels may help distinguish patients with iron deficiency anemia versus AI, and patients who may benefit most from iron or ESAs in certain populations, but more work is needed to understand the clinical utility of hepcidin assays.
5. 5) Numerous inhibitors of hepcidin production or action have shown promise to treat AI in pre-clinical studies, and are now entering human clinical trials.

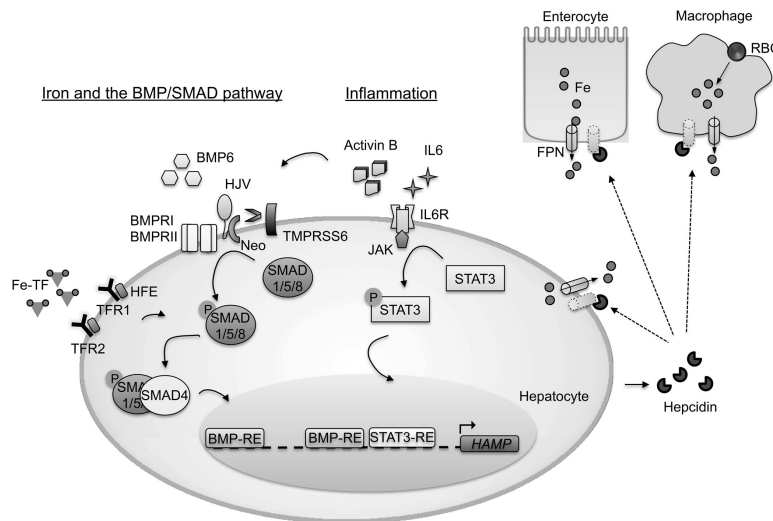


Figure 1. Current model of hepcidin regulation by iron and inflammation

Iron stimulates hepcidin (*HAMP*) transcription through holo-transferrin (Fe-TF) and BMP6. Liver iron increases BMP6 expression in nonparenchymal cells through an unknown mechanism. Fe-TF is sensed by binding to transferrin receptor 1 (TFR1) and transferrin receptor 2 (TFR2). The hemochromatosis protein HFE is displaced from TFR1 by Fe-Tf binding. HFE and TFR2 functionally intersect with the BMP-SMAD1/5/8 pathway to modulate hepcidin transcription through mechanisms that are still being worked out, but may involve interactions with the BMP co-receptor hemojuvelin (HJV) and/or the BMP type I receptor ALK3. BMP6 binding to HJV, type II receptors (BMPRII) and type I receptors (BMPRI) induces phosphorylation of SMAD1/5/8 proteins, which complex with SMAD4 and translocate to the nucleus to bind 2 BMP responsive elements (BMP-RE) on the *HAMP* promoter, thereby inducing transcription. TMPRSS6 cleaves HJV to reduce BMP-SMAD1/5/8 signaling in response to iron deficiency. Neogenin (Neo) is an HJV interacting protein that may also be involved in hepcidin regulation. Inflammatory stimuli induce expression of IL6 and Activin B, which activate the JAK/STAT3 and BMPR/SMAD pathways respectively to induce hepcidin transcription. Hepcidin promotes the degradation of ferroportin (FPN) in enterocytes, macrophages, and hepatocytes to limit iron entry into the bloodstream.

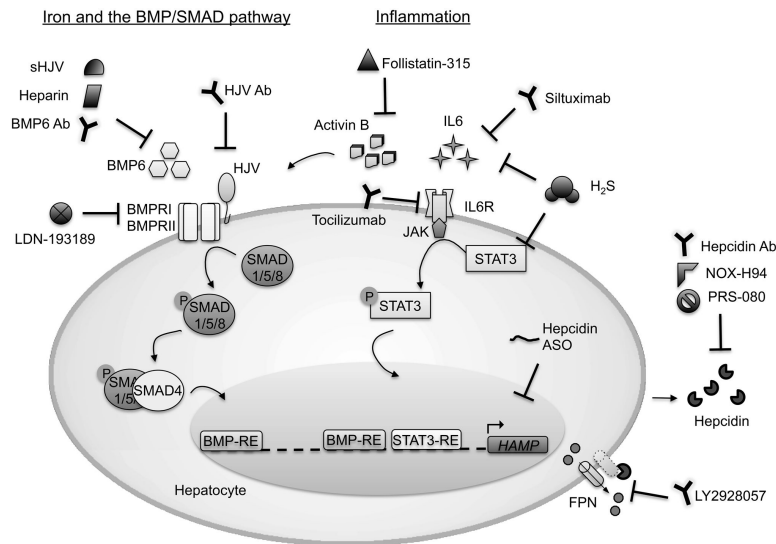


Figure 2. Experimental agents targeting the hepcidin-ferroportin axis as therapeutic strategies for AI

Agents targeting the BMP/SMAD pathway include: LDN-193189 inhibiting BMP type I receptor activity; soluble hemojuvelin fusion protein (sHJV), heparin, and BMP6 antibody (Ab) sequestering BMP ligands; hemojuvelin (HJV) Ab neutralizing HJV function; and follistatin-315 sequestering Activin B. Agents inhibiting IL6-STAT3 signaling include: Siltuximab neutralizing IL6, hydrogen sulfide suppressing IL6 and STAT3, and Tocilizumab targeting the IL6 receptor (IL6R). Agents targeting hepcidin and ferroportin include: hepcidin Ab, anticalins (PRS-080) and spiegelmers (NOX-H94) inhibiting hepcidin protein; hepcidin antisense oligonucleotides (ASO) targeting hepcidin mRNA; and FPN Ab (LY2928057) targeting the hepcidin binding site on FPN.