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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 lysozomes, 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 models 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 IL1β stimulates hepcidin and induces hypoferremia 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]. IL1β 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 hepcdin 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 IFNγ. 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 IFNγ 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 hypoferremia 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, hepatits 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 ERRγ 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 lexaptepid (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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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.

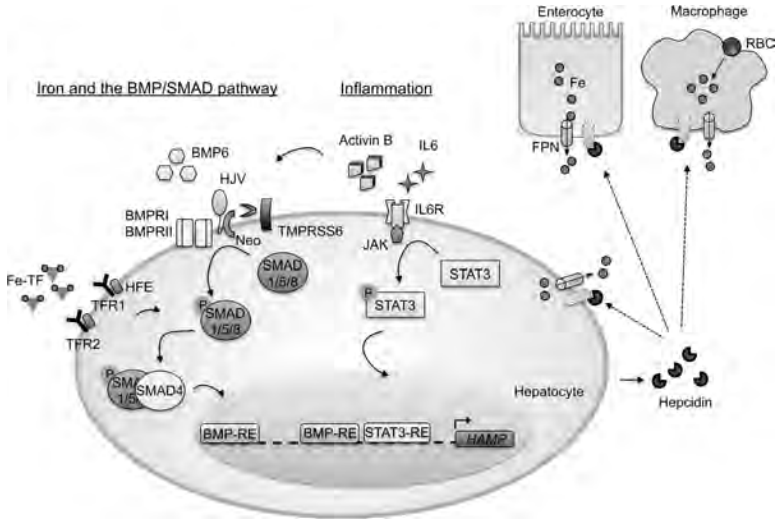

Figure 1. Current model of hepcidin regulation by iron and inflammation

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.

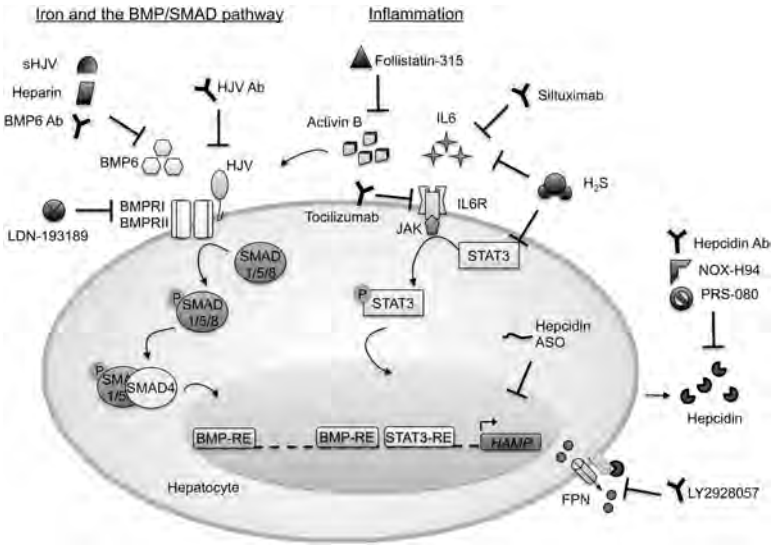

Figure 2. Experimental agents targeting the hepcidin-ferroportin axis as therapeutic strategies for AI

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 (FPN) 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.