Role of hepcidin-ferroportin axis in the pathophysiology, diagnosis, and treatment of anemia of chronic inflammation

Arielle L. LANGER, **Yelena Z. GINZBURG**

Division of Hematology and Oncology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Abstract

Anemia of chronic inflammation (ACI) is a frequently diagnosed anemia and portends an independently increased morbidity and poor outcome associated with multiple underlying diseases. The pathophysiology of ACI is multifactorial, resulting from the effects of inflammatory cytokines which both directly and indirectly suppress erythropoiesis. Recent advances in molecular understanding of iron metabolism provide strong evidence that immune mediators, such as IL-6, lead to hepcidin-induced hypoferremia, iron sequestration, and decreased iron availability for erythropoiesis. The role of hepcidin-ferroportin axis in the pathophysiology of ACI is stimulating the development of new diagnostics and targeted therapies. In this review, we present an overview of and rationale for inflammation-, iron-, and erythropoiesis-related strategies currently in development.

Keywords

Anemia; inflammation; iron metabolism

INTRODUCTION

Anemia of chronic inflammation (ACI) was previously termed anemia of chronic disease and is considered the second most frequent anemia in the world after iron deficiency anemia (IDA).[1,2] ACI is found in conditions associated with activation of the immune response, including chronic infections, autoimmune diseases, and malignancy. The presence of anemia in these conditions is considered evolutionarily advantageous in light of the iron dependence of pathogens and rapidly replicating cells. Thus, restricting iron availability serves to limit growth of pathogens and malignant cells at the expense of hemoglobin synthesis. Considered more broadly, the relative fatigue associated with illness and anemia serves to limit energy expenditure, better used to support the immune system, and prevents rapid spread of infectious pathogens, protecting species from ubiquitous exposure.

Anemia associated with chronic renal disease, congestive heart failure, and solid organ transplant is also categorized as ACI due to an inflammatory component in these conditions.

Correspondence to: Y. Z. Ginzburg, Division of Hematology and Oncology, Icahn School of Medicine at Mount Sinai, 1468 Madison Avenue, Annenberg Building, Floor 24, Room 64C, Box 1079, New York, NY 10029, USA. yelena.ginzburg@mssm.edu.

In contrast, idiopathic anemia of the elderly often presents with typical features of ACI although inflammation in this anemia remains presumptive. For the purposes of this manuscript, we offer a perspective on ACI in its broadest definition although the specific details may not apply identically to all diseases on this list (e.g., in chronic renal insufficiency, anemia is in large part driven by inadequate erythropoietin or "erythropoietin resistance" although therapeutic benefit in anemia management may be gained by suppressing hepcidin).

ACI is independently associated with poor outcome and a decrease in quality of life measures. Standard approaches, such as parenteral iron and erythropoiesis stimulating agents (ESAs), are not consistently effective.[3] Thus, despite its frequency and significant impact both on morbidity and mortality, the pathophysiology of ACI is incompletely understood and effective therapies are lacking. The goals of this manuscript are to

1.   describe the clinical presentation and diagnosis of ACI,

2.   elucidate our current understanding of iron metabolism and its mechanistic relationship with erythropoiesis, and

3.   discuss potential therapeutic modalities.

DEFINITION AND CLINICAL OVERVIEW OF ACI

ACI is characterized as a mild, normocytic normochromic anemia with hemoglobin ranging from 8 to 10 g/dL, with elevated inflammatory markers (e.g., CRP, ESR, IL-6) and decreased circulating serum iron concentration and transferrin saturation despite ample iron stores (e.g., serum ferritin > 100 ng/mL). In the setting of inflammation, ACI may be difficult to differentiate from IDA, and IDA may coexist with ACI. In the majority of cases, IDA in the setting of ACI is a consequence of blood loss, either gastrointestinal or genitourinary, and diagnosing site of and halting the source of bleeding are both required. Furthermore, iron absorption may be impaired (e.g., celiac disease). Thus, understanding when administration of iron is expected to at least in part improve anemia drives the need for identify IDA as a possible component of ACI.

The traditional gold standard approach to identify IDA in ACI is Prussian-blue staining for iron within macrophages on a bone marrow biopsy specimen. However, this approach has been challenged in light of its invasiveness, high inter-observer variability, and poor correlation between iron-loaded macrophages and iron bioavailability in high hepcidin states, e.g., ACI. Furthermore, because serum ferritin functions as an acute phase reactant, it alone is insufficiently specific to identify IDA in ACI, unless very low (i.e., serum ferritin < 10 ng/mL). Serum ferritin is in equilibrium with cellular ferritin, largely derived from macrophages where its expression is increased when cellular iron increases, as in ACI.[4] Thus, elevated ferritin concentration does not exclude a possible response to parenteral iron therapy.[5]

Additional parameters have been proposed to provide evidence of IDA in ACI. Soluble transferrin receptor 1 (sTfR1) concentration and sTfR1/log ferritin have been proposed as estimates of iron deficient erythropoiesis since circulating sTfR1 concentration is

proportional to cellular expression of the membrane-associated TfR1, increases with increased cellular iron requirements and cellular proliferation,[6–8] and is unaffected by inflammation. However, sTfR1 assays have not been standardized or widely adopted in light of the lack of convincing data of their utility.[9]

PATHOPHYSIOLOGY OF ACI

The pathophysiology of ACI is multifactorial, resulting from the effects of inflammatory cytokines such as TNF-α, interferon-γ, and IL-1 which suppress erythroid precursor differentiation and erythropoietin responsiveness,[10–12] impeding the bone marrow's ability to compensate for enhanced erythrophagocytosis by cytokine-activated splenic macrophages. In addition, inflammation may also influence erythropoietin production and renal excretion of hepcidin.[13–15] However, there is now some consensus that ACI is in large part a consequence of altered iron metabolism resulting from the immune modulation of an underlying chronic disease in which immune mediators, such as IL-6 and possibly other cytokines involved in host defense, lead to hepcidin-induced hypoferremia, resulting in iron sequestration within the reticuloendothelial system, decreasing iron availability for erythropoiesis (Table 1). Although treatment of the underlying disease remains at the center of therapy for ACI, recent advances in molecular understanding of the hepcidin-ferroportin axis are stimulating the development of new targeted therapies.

Cytokines, IL-6 in particular, stimulate the hepatic hormone hepcidin. IL-6 induction of hepcidin expression is mediated through STAT3 signaling in hepatocytes. More recent data suggests that IL-6 may have a secondary suppressive effect on erythroid precursors.[16] In addition, the loss of IL-6 and hepcidin in mice results in a milder anemia and more rapid recovery of hemoglobin in a well-established mouse model of ACI.[17] Furthermore, IL-6 knockout animals exhibited faster bone marrow recovery relative to hepcidin knockout animals.

IRON METABOLISM AND HEPCIDIN REGULATION

Iron must be tightly regulated, because iron deficiency results in anemia and when iron is in excess, free iron promotes generation of reactive oxygen species, causing tissue injury and organ failure. Dietary absorption, storage, and recycling of iron maintain its stable concentration in circulation. The majority of systemic iron is contained within hemoglobin in circulating red blood cells. Iron storage, in the form of cellular ferritin, is located within hepatocytes and splenic macrophages. Muscle contains iron in the form of myoglobin for oxygen storage, and all cells require small amounts of iron for synthesis of iron-containing proteins for cell metabolism. Iron movement between sites of absorption, storage, and utilization through circulation occurs bound to transferrin; of the 20 to 25 mg of iron delivered daily, only 2 to 4 mg exist in circulation as transferrin-bound iron at any one time. Most of the iron found in circulation is recycled after erythrophagocytosing red blood cells by splenic macrophages. As iron loss is not modulated during systemic iron deficiency or overload, total body iron control is mainly regulated during dietary iron absorption and recycling.

The peptide hormone hepcidin is the principal regulator of iron homeostasis,[18–20] including dietary iron absorption, iron recycling by macrophages, and the release of iron from hepatic stores. Hepcidin down-regulates iron release into plasma by binding to and functionally down-regulating ferroportin 1, the sole iron exporter.[21,22] Hepcidin expression is induced by circulating iron and tissue iron stores,[23,24] as well as inflammatory cytokines and suppressed by hypoxia,[25] irondeficiency, and ineffective erythropoiesis. The bone morphogenic protein (BMP) pathway is critical in iron regulation of hepcidin expression with a specific role for hemojuvelin as an iron-specific BMP co-receptor and stimulant of the BMP receptor in iron overload states.[26,27] HFE/TfR2 interacts with hemojuvelin and potentiates the BMP signaling pathway (e.g., BMP6 as the essential and specific BMP receptor ligand for iron-related signaling) and hepcidin transcription.[27,28]

Iron absorption is greatly increased in response to hemorrhage or erythropoietin in conditions of ample iron stores. As a result, the effect of erythropoiesis on intestinal iron absorption is dominant over iron in regulating hepcidin. The mechanisms of this suppressive effect have not been fully elucidated but likely include factor(s) secreted by erythroid precursors.[29–33] Growth differentiation factor 15 and twisted gastrulation 1 are candidates identified in β-thalassemic humans and mice, respectively, but their roles have not been confirmed.[34,35] More recently, erythroferrone (ERFE) has been identified as a physiological erythroid-derived hepcidin suppresor, increased in bone marrow from β-thalassemic and phlebotomized wild type mice, suppressing hepcidin expression in vitro (Figure 1).[36] Furthermore, circulating ERFE concentrations are increased after erythropoietin administration and in β-thalassemic mice.[37] However, ERFE appears to play a minimal role in baseline erythropoiesis and iron regulation with ERFE knockout mice exhibiting only mild anemia during the postnatal period[36]; its main function is likely to facilitate iron mobilization during recovery from anemia.

PATHOPHYSIOLOGY OF IRON SEQUESTRATION

The resultant increase in circulating hepcidin concentration leads to increased binding, internalization, and degradation of ferroportin, expressed on cells involved in iron metabolism (e.g., duodenal enterocytes, macrophages, hepatocytes, and placental cells). As ferroportin exports iron into the circulation, its degradation traps iron within cells, leading to increased cellular ferritin and ultimately serum ferritin concentration (in equilibrium with ferritin inside cells). As a consequence, inflammation negatively affects erythropoiesis by sequestering iron away from erythroid precursors (i.e., within bone marrow macrophages), inducing iron restricted erythropoiesis (Figure 2).

ROLE OF IRON-, ERYTHROPOIESIS-, AND HYPOXIA-INDUCED REGULATION OF HEPCIDIN IN THE INFLAMMATORY RESPONSE

Some crosstalk between IL-6 induced STAT3 signaling and BMP/SMAD signaling to hepcidin has been documented. For example, the SMAD binding site on the hepcidin promoter remains essential for IL-6 mediated hepcidin expression,[38–40] and liver-specific Smad4 knockout mice demonstrate diminished hepcidin responsiveness to IL-6.[41] In vitro experiments reveal that methods blocking BMP receptor signaling inhibit IL-6-mediated

hepcidin expression,[27,42] providing a rationale for modulating the BMP pathway to alter hepcidin expression in the context of ACI.

Increased erythropoietic demand, e.g., after phlebotomy or ESA administration, results in the mobilization of iron for erythropoiesis. The term "functional iron deficiency" refers to insufficient iron availability at the site of erythroblast production, despite adequate body iron stores and typically applies to the high hepcidin state in patients with renal insufficiency. However, broadly speaking, ACI, another high hepcidin condition, can also conceptually be referred to as functionally iron deficient. As a consequence of this iron restricted erythropoiesis, ERFE increases and possibly provides some counter-regulation of elevated hepcidin. In fact, ERFE knockout mice treated with heat-killed *Brucella abortus*, a well-accepted model of ACI, exhibited delayed hemoglobin recovery as a consequence of inadequate hepcidin suppression.[43] Thus, suppressed erythropoiesis in ACI may enable further increase of hepcidin expression.

Iron homeostasis is also regulated by the hypoxia-mediated pathway, specifically, the involvement of hypoxia-inducible factors (HIFs) mediating hepcidin expression. Under normoxic conditions HIF1α is hydroxylated and marked for degradation by a complex of proteins containing von Hippel Landau (VHL). Hypoxia or mutations in VHL lead to HIF stabilization and transport to the nucleus where it functions as a transcription factor, affecting the expression of multiple genes in the erythropoietic cascade.[44] *Vhl* knockout mice exhibit persistent HIF1α hydroxylation with compensatory increases in serum erythropoietin, splenomegaly, and hepcidin suppression.[45] However, a concurrent *Vhl* and erythropoietin knockout results in hepcidin de-repression, suggesting that hepcidin suppression by hypoxia is mediated by erythropoiesis.[46]

USE OF HEPCIDIN AS A DIAGNOSTIC TOOL

In light of the central role hepcidin plays in the pathophysiology of ACI, the diagnostic potential of hepcidin has been studied extensively. Mass spectrometry, MALDITOF, and ELISA testing have all been used to determine hepcidin concentration in various clinical settings. This method has been useful in distinguishing an IDA component within ACI. Although hepcidin is elevated in patients with ACI relative to healthy controls, the coexistence of IDA and ACI results in a significant decrease in hepcidin relative to patients solely with ACI. This finding suggests that suppressive signaling as a consequence of iron restriction dominates stimulatory inflammatory signaling. However, standard cut-offs to enable clinical application of hepcidin in this clinical setting are not yet available. Despite this, relatively high hepcidin has been associated with lower mean corpuscular hemoglobin in anemic patients and impaired response to ESAs in an animal model of ACI.[47,48]

THE RATIONALE OF HEPCIDIN-LOWERING AGENTS

The goal of anemia management is to reverse patient symptoms attributable to decreased hemoglobin. Clinically, anemia has a broad and varied presentation including shortness of breath, lethargy, fatigue, skin pallor, palpitations, tachycardia, systolic flow murmurs, and angina, which can be difficult to distinguish from symptoms of the underlying disease,

complicating the decision regarding treating anemia in addition to treating the cause of inflammation. Overall, resolution of anemia in ACI parallels therapy and improvement of the underlying inflammatory condition although treatment of anemia is associated with improved quality of life. Many translational studies focus on the reversing hepcidin overexpression to mitigate anemia in ACI. In addition to ACI, other diseases in which hepcidin concentration is increased include iron refractory iron deficiency anemia due to an autosomal recessive mutation in TMPRSS6, malignancy-associated anemia, and chronic renal insufficiency. Thus, when treatment of the underlying disease is difficult or impractical, severe anemia is typically treated with ESAs and parenteral iron, but their limited efficacy has dampened enthusiasm even in the face of even mild risk. Hepcidin antagonists are being developed targeting either the hepcidin molecule of its regulators (e.g., BMP, TfR2) in the hope that the potential therapeutic efficacy of hepcidin antagonists may extend to even mild anemias.

METHODS FOR PHARMACOLOGICAL REDUCTION OF HEPCIDIN

Via the inflammatory signal

Several diseases in the ACI category (e.g., Castleman disease and rheumatoid arthritis) have benefited from Phase I and II clinical trials with hepcidin lowering agents (Table 2). Therapeutic monoclonal antibodies against IL-6 receptor (tocilizumab), IL-6 (siltuximab), and TNFa (golimumab or infliximab) resulted in decreased hepcidin and improved anemia. [49–53] In addition, in light of the STAT3-dependent signaling by IL-6, downstream inhibition of STAT3 signaling (e.g., curcumin, AG490, and PpYLKTK) is also expected to decrease hepcidin in humans, already demonstrated in vitro and in preclinical studies in vivo. [54–56] However, this class of agents is unlikely to come to market as a consequence of their non-specific effects (all), competing iron chelating properties (curcumin), and suboptimal pharmacokinetics (AG490 and PpYLKTK). Last, multiple approaches aimed at manipulating the BMP/SMAD signaling pathway to hepcidin have also been employed to reduce hepcidin expression.

Via the iron signal

Heparin, a glycosaminoglycan and frequently used agent in the treatment and prophylaxis of patients with existing or propensity toward blood clots, respectively, has a high affinity for BMP. Thus, heparin inhibits hepcidin expression by binding to and sequestering BMPs. [57] Furthermore, heparins without anticoagulating effects have been developed, demonstrating reversal of anemia in mouse models of ACI [58] and heparins with high sulfation grade and low anticoagulant activity are specifically able to block hepcidin in vitro and in preclinical studies in vivo. [59,60] These agents are poised for randomized clinical trials. Hemojuvulin (HJV), also known as HFE2, is a BMP co-receptor, enhancing hepcidin expression and soluble HJV, [42] resulting from furin-mediated cleavage of cellular HJV, [61] competes for binding of BMP to BMP receptor. [27,62] Administration of either neutralizing anti-HJV antibodies (AbbVie Pharmaceuticals) or sHJV fused with IgG Fc fragment result in hepcidin suppression [63] and correct anemia in murine ACI models. [64,65] Clinical studies using such agents in renal failure patients are underway.

Antibodies and small molecule inhibitors of BMP/SMAD have demonstrated preclinical efficacy in decreasing hepcidin expression. For example, anti-BMP6 antibodies reduce hepcidin expression and increase serum iron concentration in mice.[66,67] In addition, dorsomorphin, an inhibitor of BMP/SMAD signaling, prevents hepcidin induction by IL-6 by blocking type I BMP receptors ALK2, ALK3, and ALK6, inducing hyperferremia in mice.[68] Furthermore, dorsomorphin derivatives, e.g., LDN-193189, prevent hepcidin induction and anemia in murine ACI and renal failure models.[65,69–71] However, because dorsomorphin and its derivatives do not specifically target BMP/SMAD signaling to hepcidin, they are considered suboptimal candidates for therapeutic development. TP-0184, a specific ALK2 inhibitor (Toledo Pharmaceuticals), has recently been shown to suppress hepcidin in mouse models of ACI and cancer,[72] but further studies are required to evaluate its full translational potential.

Via erythropoietic signal

Novel therapeutics targeting stabilization of HIFs provide promise in the treatment of ACI, enabling hepcidin blockade mediated via erythropoiesis, simultaneously stimulating erythropoiesis and iron availability. For example, propyl hydroxylase domain-2 (PDH2) inhibitors stabilize HIF-1 and HIF-2, stimulating endogenous erythropoietin production to suppress hepcidin and enable greater iron availability for erythropoiesis.[46,73,74] Other hypoxia inducible hormones such as platelet-derived growth factor BB interfere with endoplasmic reticulum stress-inducible signaling pathway involving CREB-H to suppress hepcidin.[75,76] In light of the recent discovery of erythroferrone and its negative regulation of hepcidin, modulation of this pathway is a clear area for future investigation.

Direct hepcidin inhibition

Direct hepcidin inhibitors, e.g., neutralizing antibodies, have demonstrated efficacy in increasing circulating and available iron for erythropoiesis, reversing anemia and increasing responsiveness to ESAs in mouse models of ACI[77] and increasing serum iron concentration and erythropoiesis in monkeys.[78] LY2787106, a monoclonal anti-hepcidin antibody (Eli Lily), has been developed and proposed as potential therapy in conditions of excess hepcidin,[79] recently demonstrating high tolerability, transient iron mobilization, and increased reticulocytosis in a Phase I study of cancer-related anemia patients.[80] PRS-080, a PEGylated anticalin with specific binding and neutralization of hepcidin (Pieris Pharmaceuticals), demonstrated suppression of hepcidin activity in vitro and in mice[81] with decreased hepcidin and transiently increased transferrin saturation at doses above 0.4 mg/kg in a Phase I study in healthy volunteers,[82] warranting further investigations in anemic patients. Last, NOX-H94, a spiegelmer (PEGylated nonnatural occurring mirror-image L-oligoribonucleotide) hepcidin antagonist (NOXXON Pharma AG), binds hepcidin with high affinity and specificity. NOX-H94 has been demonstrated to improve ACI in monkeys[83] and prevent hypoferremia in LSP-injected humans volunteers.[84] As a consequence, additional clinical trials in renal insufficiency, ACI, and cancer-related anemia are in the planning phases.

CONCLUSION

Anemia in cancer and inflammatory and autoimmune diseases is a common clinical condition often neglected by treating physicians. This challenge is a consequence of inadequate easy and standardized diagnostic tools, incomplete appreciation of variability in therapeutic targets for hemoglobin, insufficient guidelines to estimate the degree of functional iron deficit, and how to tailor therapy with parenteral iron, ESAs, and RBC transfusion for individual patients. Furthermore, it is only recently that we have enhanced our understanding of the complexity of regulation of iron metabolism and cross-talk with erythropoiesis. Based on this, novel therapeutics aiming to modulate hepcidin and increase iron availability for erythropoiesis are being explored from multiple directions. It is noteworthy to consider different therapeutic strategies in ACI associated with different underlying diseases and the potential of hepcidin-targeted therapeutics to enable some degree of physiological self-regulation to prevent inadequate or excessing dosing in individual patients. Many of these therapeutic approaches have been validated in preclinical and clinical settings, with some currently being further explored in randomized clinical trials and are poised to improve management of iron maldistribution and anemia in ACI.

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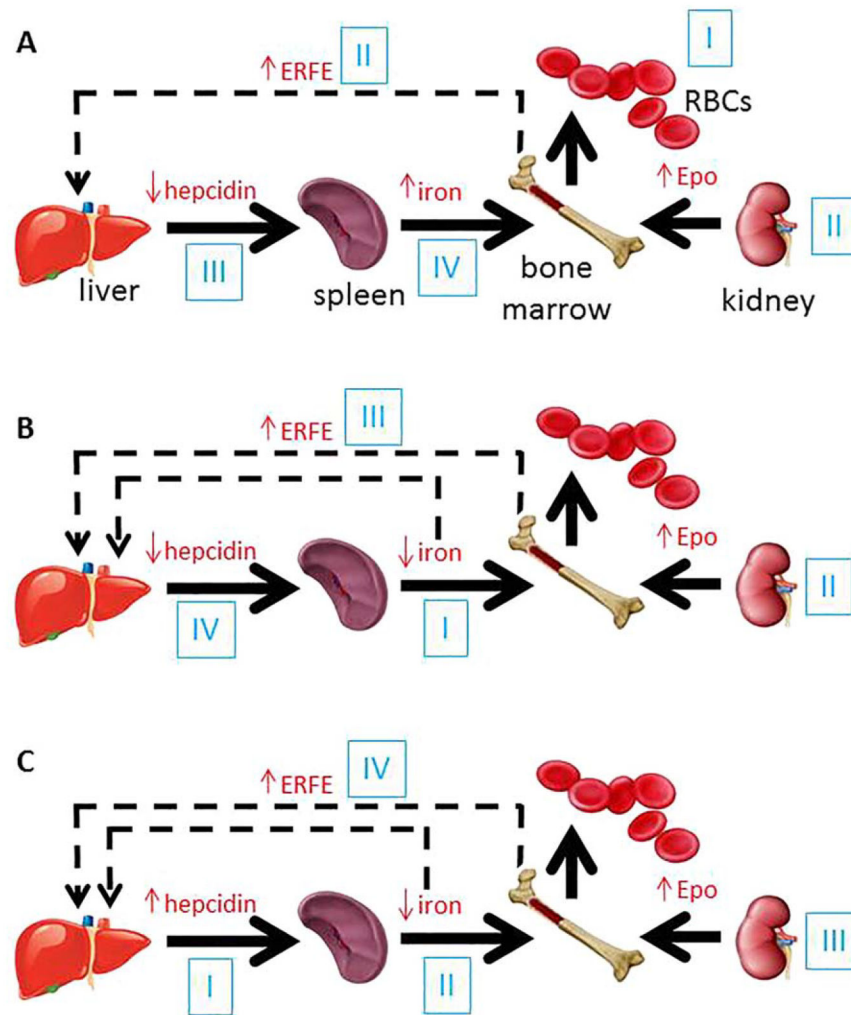
Figure 1.
Proposed steps in the cross-talk between erythropoiesis and iron metabolism in conditions associated with iron dysregulation. (A) In acute blood loss (I), resultant hypoxia leads to increased erythropoietin (II) and consequent increase in erythroferrone (II) which suppresses hepcidin (III) and enables an increase in iron absorption and iron recycling, resulting in increased circulating iron (IV) available for erythropoiesis. (B) In iron deficiency anemia, decreased circulation iron concentration (I) leads to anemia and resultant hypoxia which results in increased erythropoietin (II) and subsequently increased erythroferrone (III), suppressing hepcidin (IV) to increase iron absorption and recycling. (C) In anemia of chronic inflammation, IL-6 driven increase in hepcidin (I) results in the sequestration of iron in macrophages, enterocytes, and hepatocytes, decreasing iron release into the circulation (II). As a consequence, iron restricted erythropoiesis and anemia result in compensatory increase in erythropoietin (III) and erythroferrone (IV), the later providing negative feedback to suppress hepcidin (RBCs = red blood cells; Epo = erythropoietin; ERFE = erythroferrone).
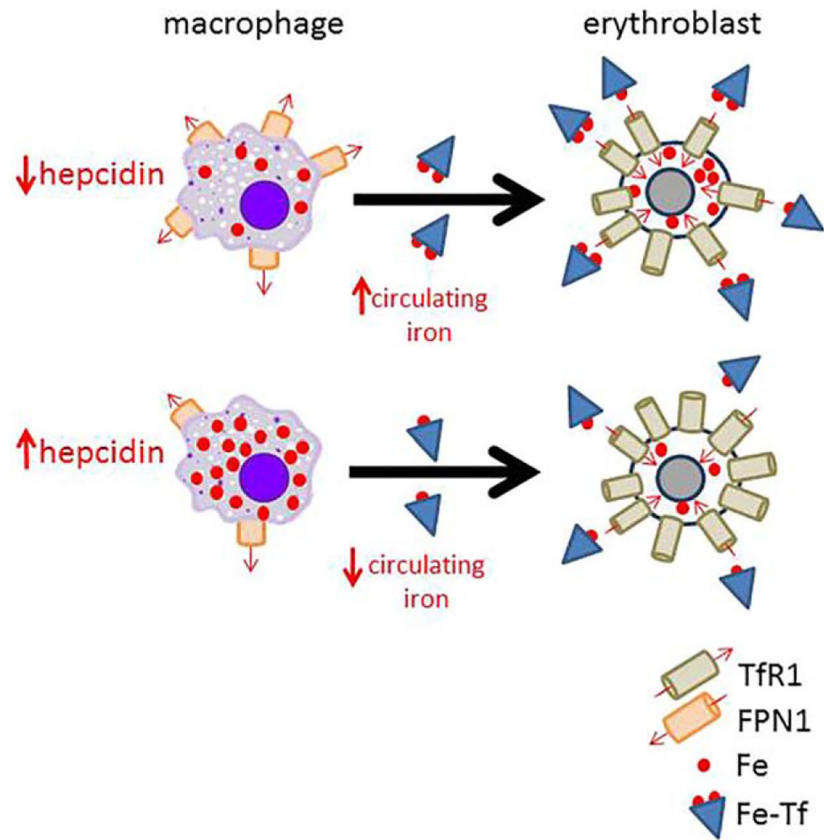
Figure 2.
Model of hepcidin's effect on iron availability for erythropoiesis. Because hepcidin functions post-translationally to influence iron efflux from cells by binding and internalizing ferroportin 1, the only known iron exporter, present on cells involved in iron metabolism (e.g., macrophages), decreased hepcidin leads to more ferroportin 1 present on cell membranes and consequently more iron efflux into circulation where it is available for uptake by erythroblasts via iron loaded transferrin binding to transferrin receptor 1. This happens in iron deficiency anemia. Alternatively, when hepcidin is high, its binding of ferroportin 1 prevents iron egress from cells and results in iron sequestration within macrophages (e.g., splenic macrophages, involved in iron recycling from senescent red blood cells), leading to iron restricted erythropoiesis. This happens in anemia of chronic inflammation (TfR1 = transferrin receptor 1; FPN1 = ferroportin 1; Fe = iron; Fe-Tf = iron loaded transferrin).

Table 1

Pathophysiology of anemia of chronic inflammation

Increased inflammatory cytokines effect	Consequences on erythropoiesis	
Increased macrophage activation	•	Increased erythrophagocytosis
	•	Decreased RBC survival
Suppressed erythroid precursor differentiation	Fewer RBC	
Decreased erythropoietin responsiveness	Fewer erythroid precursors	
Decreased erythropoietin production	Fewer erythroid precursors	
Increased circulating hepcidin concentration	•	Macrophage iron sequestration
	•	Hypoferremia
	•	Iron-restricted erythropoiesis

Table 2

Hepcidin antagonists in development for ACI

Mechanism	Agents	Stage
Cytokine inhibition	Antibodies to IL–6 receptor, IL–6, or TNFα	Phase I and II trials; FDA approved for other indications
Intracellular inflammatory cascade	STAT3 inhibitors	Development halted
BMP inhibition	Heparins; antibodies to ALK subtypes, HJV or BMP6	Translational
EPO upregulation	PDH2 inhibitors	Translational
Direct hepcidin inhibition	Antibodies to hepcidin; hepcidin inhibitors	Phase I trials complete; further trials ongoing

ACI = anemia of chronic inflammation; IL–6 = interleukin 6; TNFα = tumor necrosis factor alpha; FDA = Food and Drug Administration; STAT3 = signal transducer and activator of transcription 3; BMP = bone morphogenic protein; ALK = activin-like kinase; HJV = hemojuvulin; EPO = erythropoietin; PDH2 = pyruvate dehydrogenase 2.