Anemia of Inflammation: A Review

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Abstract

Impaired iron homeostasis and the suppressive effects of proinflammatory cytokines on erythropoiesis, together with alterations of the erythrocyte membrane that impair its survival, cause the anemia of inflammation. Recent epidemiologic studies have connected inflammatory anemia with critical illness, obesity, aging, and kidney failure, as well as with cancer, chronic infection, and autoimmune disease. The proinflammatory cytokine, interleukin-6, the iron regulatory hormone, hepcidin, and the iron exporter, ferroportin, interact to cause iron sequestration in the setting of inflammation. While severe anemia is associated with adverse outcomes in critical illness, experimental models suggest that iron sequestration is part of a natural defense against pathogens. In animal models and human patients, experimental therapeutic approaches targeting interleukin-6 or the ferroportin-hepcidin axis have shown efficacy in reversing anemia, although these agents have not yet been approved for the treatment of the anemia of inflammation.

Keywords

EPIDEMIOLOGY

Inflammation is one of the most common causes of anemia in the elderly and chronically ill. In NHANES III, anemia of inflammation was defined as a low serum iron level (<10.74 μM or <60 μg/dL) without evidence of low iron stores, i.e. transferrin saturation >15%, serum ferritin >12 ng/mL, or erythrocyte protoporphyrin concentration >1.24 μM [1]. Other features of anemia of inflammation include inappropriately low levels of erythropoietin, and elevated measures of inflammatory markers, such as C-reactive protein [2]. In the National

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Health and Nutrition Examination Study (NHANES III), investigators discovered that about 1 million Americans over age 65 exhibit anemia related to inflammation.

Anemia in Critical Illness

The anemia of inflammation can occur in the setting of acute or chronic inflammation. The CRIT study of anemia and blood transfusion in the critically ill was an observational cohort analysis of 4892 patients in intensive care units across the United States [3]. The CRIT study revealed that anemia of inflammation develops in critically ill patients even within 30 days. The mean hemoglobin levels in critically ill individuals decreased over a 30-day period despite the administration of blood transfusions. The CRIT study also demonstrated that severe anemia, hemoglobin <9 g/dL, is an independent predictor of increased mortality and length of stay in critically ill patients.

Anemia in the elderly is frequently linked to inflammatory conditions. In a recent study of 191 consecutive hospitalized elderly patients with anemia, 70% of the patients were found to have anemia related to inflammation. Sixteen percent of the patients with anemia of inflammation had concomitant chronic renal failure [4]. Of the patients with inflammatory anemia, 71% were suffering from an acute infection, 12% had cancer, and 16% had a chronic infection, such as a pressure ulcer, or a chronic autoimmune inflammatory disease. [4]

Anemia in Obesity

As the prevalence of obesity is rising in the United States, increasing attention is being paid to the potential effect of obesity on erythropoiesis. Obese patients exhibit higher plasma levels of pro-inflammatory cytokines and acute phase reactants, as well as higher rates of iron-restricted erythropoiesis that can result in anemia [5]. Functional iron deficiency is defined as inappropriately low iron stores, despite the presence of inflammation, i.e. a normal serum ferritin (12–100 ng/mL for females or 15–100 ng/mL for males) and a serum C-reactive protein >3 mg/L [6][7] A recent cross-sectional study of 947 obese patients under evaluation for bariatric surgery revealed that 52.5% exhibited functional iron deficiency. The majority of obese patients with functional iron deficiency appear to have sequestration of iron as manifest by a serum transferrin saturation <20% [6]. Weight loss has been associated with an increase in transferrin saturation in overweight individuals [8], which supports the hypothesis that obesity causes iron sequestration.

Anemia in Cancer

Anemia of inflammation is a common manifestation of advanced cancer. A recent prospective, observational study of 888 patients with a variety of carcinomas revealed that 63.4% of the patients were anemic [9]. The prevalence and severity of anemia correlated with the stage of cancer [9]. The prevalence of increased mean plasma levels of markers of inflammation, including C-reactive protein, fibrinogen, IL-6, TNFα, IL-1β, ferritin, hepcidin, erythropoietin and reactive oxygen species, was significantly increased in advanced stage compared to early stage cancer patients. Supporting a role for inflammation in iron sequestration, serum iron levels were significantly reduced in advanced stage patients [9].

PATHOPHYSIOLOGY

Recent discoveries indicate that both iron sequestration and impaired erythropoiesis cause the anemia of inflammation. Erythroid progenitors mature to erythrocytes through a series of stages that require co-ordination of iron acquisition and cell proliferation. As erythroid progenitors mature to the polychromatophilic stage, transferrin receptor 1 expression on the surface of the red cell membrane increases [10]. Macrophages export iron via ferroportin (fpn). The carrier protein, transferrin, binds free iron with high avidity. Transferrin-bound iron attaches to transferrin receptor 1. In an acidified vacuole, the iron is released from transferrin and exported to the cytoplasm by divalent metal transporter 1 (DMT1), while transferrin receptor is recycled to the cell surface (reviewed in [11]). The iron then enters the mitochondria where it is attached to protoporphyrin IX in the final step of heme synthesis.

Iron is effectively salvaged in the human body. Macrophages consume senescent erythrocytes, liberate iron from hemoglobin, and store the iron in ferritin for subsequent release of iron to developing erythrocytes via fpn.[10] Studies of the *fpn*-deficient zebrafish, *weissherbst* [12, 13], and the *fpn*-knockout mouse [14] revealed that fpn is required to export iron from macrophages to developing erythrocytes. In fpn-deficiency, iron remains sequestered in macrophages. This results in impaired iron delivery to developing erythrocytes and erythroid maturation arrest [12, 13].

Hepcidin is a short, cysteine-rich peptide hormone that regulates the intestinal absorption of iron and its release from macrophage iron stores. Human hepatocytes secrete the iron-regulatory peptide hormone, hepcidin [15–17] in response to iron overload or inflammation. Hepcidin binds fpn on the surface of macrophages resulting in the internalization and degradation of both proteins [18] and sequestration of iron in macrophages, away from the developing erythrocytes (Figure 1). Hepcidin appears to be the key regulator of iron homeostasis, because loss of function mutations in genes that regulate *Hepcidin* expression, e.g. *Transferrin receptor 2*, *HFE*, *Hemojuvelin*, or in *Hepcidin* itself have each been associated with hereditary iron overload syndromes [19].

Regulation of hepcidin

The BMP co-receptor, hemojuvelin, and TMPRSS6, [20–26] participate in regulating hepcidin transcription in the setting of iron overload. Iron overload enhances bone morphogenic protein (BMP) signaling and Smad protein phosphorylation that enhances hepcidin transcription. On the other hand, iron deficiency or the acute onset of anemia stimulates release of the hormone, erythroferrone, by erythroid progenitors, and erythropoietin by the kidney [11, 27] Erythroferrone suppresses *hepcidin* expression to promote intestinal iron absorption and macrophage iron release, while erythropoietin stimulates proliferation of committed erythroid progenitors [11, 27].

Interleukin-6 (IL-6) and interleukin-1β (IL-1β) [28] are key cytokines that mediate the effects of inflammation on the developing erythrocyte. The transcription factors that mediate the effects of inflammation include Stat3, C/EBPα, and p53 [29, 30] (Figure 2). IL-6 increases JAK/Stat signaling [31, 32], which promotes phosphorylation of Stat3 and Stat3 binding to the *Hepcidin* promoter [30, 33]. IL-1β induces *Hepcidin* expression via the C/

EBPα and BMP/SMAD signaling pathways. Hepatocyte damage, via endoplasmic reticulum stress or oxidation, enhances C/EBPα [34, 35] or Stat3 activity [36], respectively, leading to increased *Hepcidin* expression. Lipopolysaccharide (LPS), released by severe bacterial infection, activates toll-like receptor 4 (TLR4) signaling, which, in turn, enhances production of IL-6 [37] by macrophages that stimulates hepcidin expression. HMGB1, a proinflammatory cytokine that is produced in patients with critical illness, is associated with increased in-hospital mortality. HMGB1 has been shown to bind the MD2-TLR4 complex [38, 39] and to increase expression of TNF and IL-6, cytokines that impair erythropoiesis [40, 41]. Inhibition of HMGB1 activity ameliorates anemia of inflammation in mouse models [38]. Recent studies [42] indicate that inflammation in mouse models increases transcript levels of *Activin B*, a member of the TGF-β superfamily of signaling molecules. Activin B has been shown to increase *hepcidin* transcription in cultured hepatocytes in vitro via Smad signaling [42].

Iron sequestration

Hepcidin is not the only protein causing iron sequestration during bacterial infection. Recent studies indicate that stimulation of toll-like receptors 2 and 6 (TLR2 and TLR6) in mouse models decreases expression of fpn in macrophages and causes iron sequestration without increasing macrophage *hepcidin* transcript levels [43]. LPS stimulates macrophages to produce lipocalin 2, which sequesters iron by binding bacterially produced siderophores [44]. Furthermore, infection or inflammation stimulates neutrophil release of the iron binding protein, Lactoferrin. Lactoferrin is internalized by bacteria, sequesters iron, and arrests microbial growth [45].

Obese individuals exhibit increased plasma levels of pro-inflammatory cytokines, including leptin, hepcidin, and the iron sequestering protein, lipocalin-2. There are two proposed mechanisms by which obesity may contribute to functional iron deficiency and anemia, based on experimental models: (1) Leptin and pro-inflammatory cytokines stimulate hepcidin production in adipocytes and hepatocytes [46]; (2) Adipocytes and peripheral blood mononuclear cells in obese patients produce lipocalin 2, which restricts iron availability to developing erythroid cells [47].

Changes in erythrocyte membrane

In addition to effects on iron metabolism, inflammatory cytokines diminish erythropoietin synthesis, impair the differentiation of erythroid progenitors, and shorten the lifespan of mature red blood cells [48]. Incubation of erythrocytes from healthy donors with plasma from patients with septic shock induced hydrolysis of red blood cell membrane phosphatidyl choline to lysophosphatidyl choline and increased erythrocyte phosphatidyl serine exposure [49], changes that may shorten erythrocyte survival. The presence of renal failure can exacerbate the effects of inflammation. Uremia is associated with reduced levels of albumin, increased levels of nitric oxide, and changes in the levels of erythrocyte membrane proteins that promote the accumulation of reactive oxygen species and oxidation of erythrocyte membrane proteins (reviewed in [50]). These alterations are thought to accelerate erythrocyte destruction and tissue injury.

NEW EXPERIMENTAL MODELS

In order to develop new therapies for the anemia of inflammation, animal models are needed that resemble the findings in human patients. In one recent model, killed *Brucella abortus* (KBA) bacteria are injected once by intraperitoneal injection. [40, 51] Fourteen days later, mean murine hemoglobin levels decline by 50%.. Erythropoiesis gradually recovers following the injection, just as human patients may recover from the insult of a critical illness, such as sepsis. Hepcidin deficiency blunted the severity of anemia following injection of KBA, while IL-6 deficiency protected against hypoferremia and anemia in murine models. *Hepcidin*-knockout mice exhibited significantly impaired survival compared to wild type controls following injection of KBA [51], which supports the long-held theory that anemia of inflammation is protective in critical illness.

EXPERIMENTAL TREATMENTS

The best treatment for the anemia of inflammation is to eradicate the underlying disease. When that is not possible, transfusions, intravenous iron supplementation, and erythropoiesis stimulating agents [52] may ameliorate the condition. Newer, experimental approaches target IL-6 activity and the hepcidin-ferroportin axis. In 1993, researchers observed that treating patients with metastatic renal cell carcinoma with a murine anti-IL-6 antibody improved paraneoplastic thrombocytosis and anemia [53, 54]. These observations led to the evaluation of antibodies against IL-6 in human patients with inflammatory anemia secondary to Multicentric Castleman Disease (MCD).

Patients with MCD exhibit generalized lymphadenopathy, systemic chronic inflammation, increased IL-6 activity and anemia of inflammation [55]. Siltuximab, a human-mouse chimeric anti-IL-6 antibody has been shown to improve anemia in patients with this condition [56]. Siltuximab was subsequently evaluated in a Phase II trial in patients with transfusion-dependent, low or intermediate risk myelodysplastic syndrome, whose levels of pro-inflammatory cytokines are often elevated, however siltuximab failed to reduce transfusion dependence in these patients [57]. Tocilizumab, a humanized monoclonal antibody against the IL-6 receptor that has been approved to treat rheumatoid arthritis, has been found to reduce serum hepcidin levels and improve anemia in patients with Castleman disease and rheumatoid arthritis [52, 55].

Inhibitors of hepcidin production, blockers of hepcidin peptide activity, and antibodies to fpn have also been used to modulate the anemia of inflammation. A potent, orally available BMP receptor antagonist, LDN-193189, has been shown to decrease BMP signaling and reduce *hepcidin* mRNA levels in mice in a dose-dependent manner. Furthermore, LDN-193189 ameliorated the anemia of inflammation in mice that had been injected with turpentine to generate sterile abscesses [58]. Other efforts have focused on agents that are administered parenterally. NOX-H94, a structured L-oligoribonucleotide that binds human hepcidin with a high affinity, blocked IL-6 induced hypoferremia and moderated the development of anemia in cynomolgus monkey models [59]. In human trials, NOX-H94 prevented LPS-induced hypoferremia [60]. The human anti-hepcidin antibody, 12B9M, increased serum iron levels in cynomolgus monkeys, but has not been evaluated in a primate

model of anemia of inflammation. [61] A Phase I trial to evaluate the effect of an antibody against ferroportin, LY2928057, has been completed, but the results are not yet available (clinicaltrials.gov). Table 1 summarizes other experimental therapies in development. Conversely, small molecules that increase hepcidin expression may be developed into treatments for iron overload [62–64].

Our knowledge of the interaction between inflammation, iron metabolism, and erythropoiesis has improved our ability to understand the anemia of inflammation. While drugs have not yet been approved to treat this condition, several agents are under investigation, and some agents improve anemia of inflammation in patients with Castleman disease or rheumatoid arthritis. In some cases, the anemia of inflammation may be protective, for instance in animal models of the anemia of critical illness, *hepcidin*-deficient mice exhibited significantly lower rates of survival than wild type animals [51]. Thus the best course of action continues to be to identify and treat the underlying causes of the anemia of chronic disease.

Acknowledgments

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KEY POINTS

- In the anemia of inflammation, proinflammatory cytokines suppress erythropoiesis, cause iron sequestration via effects on the iron regulatory hormone, hepcidin, and promote alteration in the erythrocyte cell membrane leading to shortened red blood cell survival.

- In the setting of critical illness, severe anemia is associated with increased mortality.

- Advanced age, cancer, rheumatologic diseases, chronic infection, and kidney failure are also associated with the anemia of inflammation.

- Experimental therapies reverse the effects of the anemia of inflammation in animal models, but have not been approved for human use.
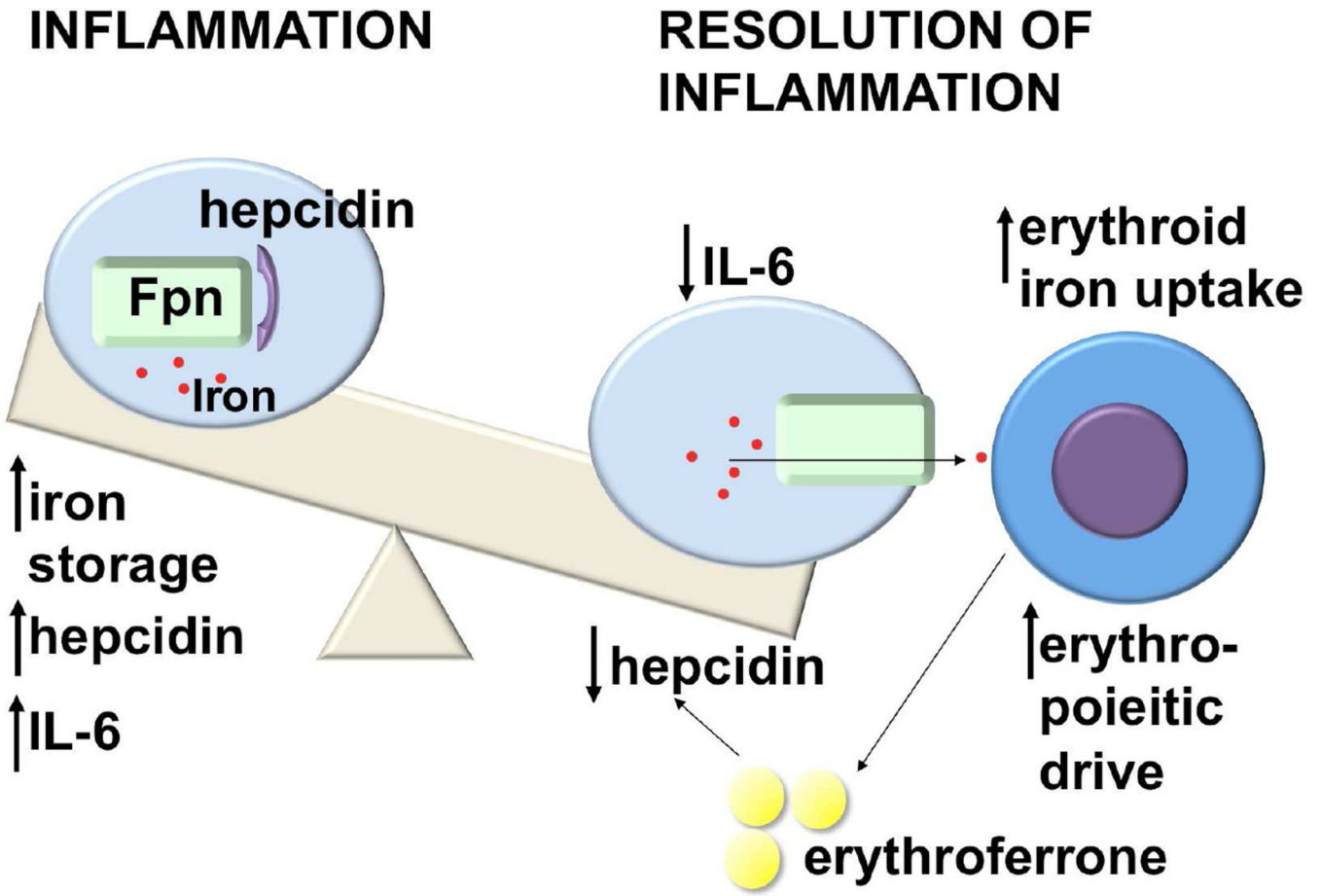
Figure 1.
Inflammation stimulates increased production of the iron-regulatory peptide, hepcidin, by hepatocytes and the pro-inflammatory cytokine, IL-6, which suppresses erythropoiesis. Hepcidin binds the iron exporter, ferroportin (fpn), causing internalization and degradation of both proteins and decreasing delivery of iron from macrophages to developing erythrocytes. This impairs erythroid development and leads to anemia. Increased erythropoietic drive stimulates erythroid progenitors to release erythroferrone, a hormone that suppresses hepcidin expression. When inflammation resolves, hepcidin and IL-6 levels decline, allowing iron to be exported to from macrophages to erythrocytes and promoting erythropoiesis.
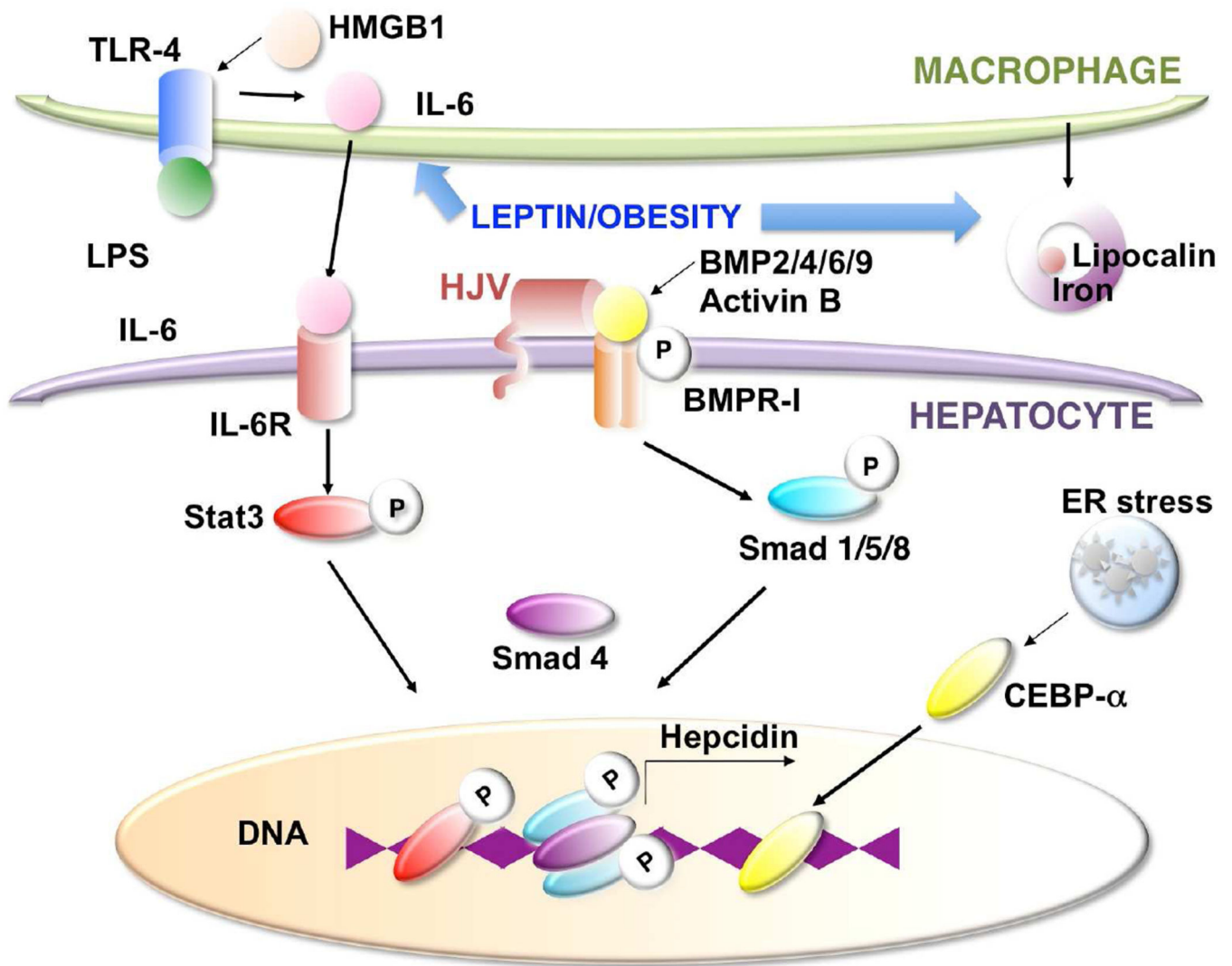
Figure 2.
Inflammation and hepatocyte damage augment *Hepcidin* transcription and iron sequestration via several pathways. Lipopolysaccharide (LPS), released by bacterial infection, and the proinflammatory cytokine, HMGB1, activate toll-like receptor 4 (TLR-4) signaling, which increases IL-6 release by macrophages, while leptin and obesity also promote IL-6 release. IL-6 signaling leads to phosphorylation of Stat3 and increased Stat3 binding to the *Hepcidin* promoter, while endoplasmic reticulum (ER) stress in hepatocytes promotes CEBP-α binding to the *Hepcidin* promoter. Bone morphogenic protein (BMP) or activin signaling via ligands, including BMP2,4,6, and 9 and Activin B, activate receptors, such as BMP receptor-I, causing Smad phosphorylation and Smad binding to the *Hepcidin* promoter, which is required for *Hepcidin* transcription. The BMP co-receptor, hemojuvelin (HJV) interacts with the BMP receptor to enhance BMP signaling. Inflammation or obesity also promotes macrophage release of lipocalin, which can interact with bacterial siderophores to sequester iron.

Table 1

Agents with potential activity against the anemia of inflammation

Class of Agent	Name	Stage of development	Route of administration	Citation
Anti-IL-6	Elsilimomab or BE-8	Decreased thrombocytosis and anemia in patients with metastatic renal cell carcinoma.	Intravenous	[53, 54]
Anti-IL-6	Siltuximab	Approved to treat Castleman disease; Decreased anemia in patients with Castleman disease; Not effective in patients with early stage myelodysplastic syndrome.	Intravenous	[56, 57]
Anti-IL-6 Receptor	Tocilizumab	Approved to treat rheumatoid arthritis; Decreased anemia in patients with Castleman disease	Intravenous	[55]
BMP receptor antagonist	LDN-193189	Evaluated in mouse models.	Oral	[58]
Anti-hepcidin oligoribonucleotide	Spiegelmer NOX-H94	Evaluated in cynomolgus monkeys and Phase I trial in humans published.	Intravenous	[59, 60]
Anti-hepcidin antibody	12B9M	Evaluated in cynomolgus monkeys.	Intravenous	[61]
Anti-ferroportin antibody	LY2928057	Phase I trial in humans completed. Results unpublished.	Intravenous	Clinicaltrials.gov
Anti-hemojuvelin antibody	ABT-207 and h5F9-AM8	Preclinical study in rats.	Intravenous	[66]
Soluble hemojuvelin extracellular domain fused with immunoglobulin Fc	HJV.fc	Preclinical study in rats.	Intravenous	[67]
siRNA against hepatic EglN prolyl hydroxylases	EglN1+2+3 siRNA lipid nanoparticles	Preclinical study in mice.	Intravenous	[68]
Suppressor of erythroid iron restriction response, bypasses effect of aconitase inactivation	Isocitrate	Preclinical study in rats.	Intravenous	[69]
Inhibitor of activin signaling, decreases LPS or KBA-induced hepcidin expression in mice	Follistatin-315	Preclinical study in mice	Intraperitoneal	[42]

Adapted from Fraenkel PG. Understanding anemia of chronic disease. Hematology Am Soc Hematol Educ Program 2015;14-8; with permission.