Anemia of Inflammation



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KEYWORDS

- Anemia of chronic disease Hepcidin Ferroportin Cytokines
- Erythropoiesis-stimulating agents

KEY POINTS

- Anemia of inflammation results from hepcidin-induced hypoferremia combined with cytokine-mediated suppression of erythropoiesis and decreased lifespan of erythrocytes.
- Treatment of the cause of inflammation improves the anemia.
- Treatment with erythropoiesis-stimulating agents and/or intravenous iron is rarely necessary.

CLINICAL PRESENTATION

- Mild to moderate anemia (hemoglobin rarely <8 g/dL)
- Occurring in a setting of infection, inflammatory disease, or malignancy
- Low serum iron
- Systemic iron stores not depleted

Definitions

Anemia of inflammation (AI, formerly also called anemia of chronic disease or anemia of chronic disorders) is usually a mild to moderately severe anemia (hemoglobin rarely lower than 8 g/dL) that develops in the setting of infection, inflammatory disease, or malignancy.¹ The defining biochemical features of AI include low serum iron despite adequate systemic iron stores. The concentration of serum transferrin is also decreased during chronic inflammation but this is a lagging indicator because of the long half-life of transferrin (about 8 days) compared with iron (about 1.5 hours).² The erythrocytes are usually of normal size and have normal hemoglobin content but are reduced in number (normocytic, normochromic anemia). In some cases, particularly if the inflammatory disease is longstanding, the red cells are mildly decreased in size and hemoglobin content.

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Related Conditions

Anemia of critical illness presents with a similar pattern of findings but develops within days in patients who are hospitalized in intensive care units with infections, sepsis, or other inflammatory conditions.³ Anemia of critical illness may be exacerbated by frequent diagnostic phlebotomies or increased gastrointestinal blood loss as is common in such settings. Anemia of aging⁴ is a chronic anemia similar to Al but often occurring in the elderly without a specific diagnosis of a predisposing underlying disease. The prevalence of this anemia increases with age, and detailed studies often detect evidence of inflammation, including increased serum C-reactive protein or other biomarkers of inflammation. Anemia of chronic kidney disease is commonly attributed to erythropoietin deficiency but accumulating evidence favors a more complex pathogenesis with a large component of Al whose exacerbations may be manifested as "erythropoietin resistance".⁵

Diagnosis

The traditional gold standard for the diagnosis of AI was anemia with hypoferremia or with low transferrin saturation, despite the presence of Prussian blue stainable iron in bone marrow macrophages. The main confounding diagnostic entity that also presents with anemia and hypoferremia is iron deficiency anemia where there is no stainable iron in the marrow macrophages. This gold standard has been challenged not only because of the invasive nature of the marrow sampling procedure but also because of findings that bone marrow iron readings are qualitative and not always consistent between evaluators and in multiple specimens^{6,7} and that iron therapy may cause marrow iron deposition in a poorly bioavailable form, which cannot be used by iron-deficient patients.⁸ The marrow iron stain has largely been replaced by serum ferritin determinations. Low serum ferritin (less than 15 ng/mL for general population, with some laboratories using age and gender-specific norms) is highly specific for iron deficiency⁹ (genetic deficiency of L-ferritin is an extremely rare exception¹⁰) and effectively rules out AI. AI is diagnosed when anemia and hypoferremia are accompanied by serum ferritin that is not low. Serum ferritin is increased by inflammation, in part reflecting direct inflammatory regulation of ferroportin synthesis^{11,12} and in part because serum ferritin originates in macrophages where its synthesis is increased by iron sequestration¹³ that takes place during inflammation. Iron deficiency is presumed to coexist with AI when ferritin is insufficiently elevated for the intensity of inflammation. Serum ferritin is also increased by tissue injury, especially to the liver.

Diagnostic Challenges

The determination of what constitutes "inappropriately low" ferritin may be difficult in practice because even patients with very high serum ferritin levels may respond to intravenous iron therapy by increasing hemoglobin.¹⁴ In principle, the limitations of serum ferritin could be circumvented by assaying additional markers of iron deficiency less affected by inflammation, most prominently soluble transferrin receptor.^{16–17} However, the relevant assays have not been standardized, the added value of such studies has not yet been convincingly demonstrated,¹⁸ and none have been widely adopted. When the anemia is clinically significant and a component of iron deficiency is suspected in a patient with AI, it may be reasonable to perform a therapeutic trial of intravenous iron. Current intravenous iron preparations are quite safe, but the very rare reactions to their administration and the possibility of exacerbating an existing or occult infectious process should be included in the risk-benefit analysis.¹⁹

Prevalence

Detailed statistics about the prevalence of AI are not available. It is estimated that the aging of the population and the high prevalence of chronic infections and inflammatory disorders worldwide combine to make AI the second most common cause of anemia worldwide, after iron deficiency. The order may eventually reverse as iron deficiency anemia is more effectively treated or prevented by dietary iron supplementation and by public health measures that curb intestinal parasitic infections.

PATHOPHYSIOLOGY

- Mildly shortened erythrocyte survival (increased destruction)
- Hypoferremia, iron-restricted erythropoiesis from cytokine-stimulated hepcidin increase
- Suppression of erythropoiesis by direct effects of cytokines on the marrow
- Variable effects of inflammation on erythropoietin production, renal excretion of hepcidin

Overview of the Causative Factors

Despite more than 50 years of investigation, our understanding of the pathophysiology of AI is incomplete. Already the earliest studies of AI indicated that the disorder is a consequence of a mild decrease in erythrocyte survival combined with impaired production of erythrocytes.^{1,20} The increased destruction of erythrocytes is predominantly attributable to macrophage activation by inflammatory cytokines but other hemolytic mechanisms may contribute in specific inflammatory diseases. The suppression of erythrocyte production has 2 major components, iron restriction and direct cytokine effects on erythropoietic progenitors. These effects combine to limit the erythropoietic response to erythropoietin, which becomes insufficient to compensate for the increased destruction of erythrocytes. In some situations, the production of erythropoietin may also be decreased, perhaps due to cytokine effects on the renal cells that produce the hormone. In severe inflammation, or when the primary pathology involves the kidneys, decreased renal excretion of hepcidin contributes to hepcidin accumulation and iron restriction.²¹ The complex pathogenesis of AI is summarized in Fig. 1 and discussed further.

Erythrocyte Destruction

Experiments with transfused erythrocytes showed that erythrocytes from AI patients and from normal controls survived longer in healthy recipients than in patients with AI.²⁰ The shortened survival of erythrocytes in AI has been attributed to macrophage activation by inflammatory cytokines that causes the macrophages to ingest and destroy erythrocytes prematurely. Anemia and excessive erythrophagocytosis are prominent features of macrophage activation syndromes, especially those associated with systemic juvenile rheumatoid arthritis.²² Here, treatment targeting interleukin 1 (IL-1) or IL-6 is proving effective, suggesting an important (although possibly indirect) role of these cytokines in the pathogenesis of excessive erythrophagocytosis. In mouse models, multiple cytokines, including interferon- γ and IL-4, have been implicated in activating macrophages for erythrophagocytosis.^{23,24} With the exception of fulminant hemophagocytic states, which are fortunately rare, erythrophagocytosis in AI is only mildly increased and could be readily compensated if the production of erythrocytes was not also impaired.^{1,20}

Hypoferremia

A recent review of mechanisms governing iron homeostasis is provided elsewhere.²⁵ Briefly, plasma iron concentrations are under homeostatic control of the hepatic iron



Fig. 1. The pathogenesis of AI is mediated by inflammatory cytokines and hepcidin, acting together to suppress erythropoiesis and shorten erythrocyte survival in blood. The effects of cytokines are denoted in light green, hepcidin effects in orange, and combined effects in red.

regulatory hormone hepcidin²⁶ and are normally maintained in the 10 to 30 µM range. Hepcidin acts by regulating the iron delivery to plasma from macrophages that recycle senescent erythrocytes, from duodenal enterocytes that absorb dietary iron, and from hepatocytes involved in iron storage. The molecular target of hepcidin is the sole known cellular iron exporter ferroportin,²⁷ expressed on cell membranes in tissues that deliver iron to plasma. The binding of hepcidin to ferroportin causes ferroportin endocytosis and its subsequent proteolysis in lysosomes. The loss of ferroportin from cell membranes causes a proportional reduction of iron export to plasma. The production of hepcidin by hepatocytes is in turn regulated by plasma and hepatic iron concentrations and inflammatory cytokines, chiefly IL-6.28,29 Inflammatory stimuli administered to humans³⁰ or experimental animals elicit a decrease in serum iron concentration within a few hours. The response depends on inflammation-induced increase in plasma concentrations of hepcidin.³¹ Increased hepcidin degrades cellular ferroportin and traps iron in macrophages, hepatocytes, and intestinal enterocytes so that less iron is delivered to plasma transferrin. The plasma iron compartment is then rapidly depleted of iron through continuing iron uptake by erythroid precursors.

Increased Hepcidin Causes an Iron-Restricted Anemia Even in the Absence of Inflammation

An experiment of nature, the syndrome of iron-refractory iron deficiency anemia (IRIDA),³² provides an important insight into the role of hepcidin in the regulation of erythropoiesis and as a pathogenic component of AI. The otherwise healthy children with IRIDA suffer from a severely microcytic, hypochromic anemia and hypoferremia that respond poorly to treatment with oral iron and incompletely even to treatment

with intravenous (IV) iron.^{33,34} Guided by a mouse model of this condition,³⁵ the pathogenesis of IRIDA is now partially understood.³⁶ Most of the patients with IRIDA have homozygous or compound heterozygous mutations in the gene encoding the transmembrane serine protease TMPRSS6 (also called matriptase-2), leading to serum hepcidin levels that are high or inappropriately elevated considering that the patients are iron deficient. Hepcidin-mediated block in duodenal iron absorption is likely responsible for the ineffectiveness of oral iron therapy, and hepcidin-induced retention of iron in macrophages reduces the response to IV iron replacement therapy. Importantly, IRIDA patients continue to have microcytosis and hypochromia even after iron therapy, indicating that hemoglobin synthesis is impaired more than the production of erythrocytes. This is in contrast to AI, which is usually a normochromic normocytic anemia, indicating that in AI the impairment of hemoglobin synthesis is roughly balanced by decreased production of erythrocytes. Thus, direct suppression of erythrocyte production by inflammatory cytokines in AI may "compensate" for the effect of hypoferremia on hemoglobin synthesis, generating fewer erythrocytes but with normal size and hemoglobin content.

Suppression of Erythropoiesis by Inflammation

Inflammatory cytokines, including tumor necrosis factor (TNF) a, IL-1, and interferon-y, have been reported to suppress erythropoiesis in vitro³⁷⁻⁴¹ as well as in mouse models.^{24,42} Detailed understanding of the mechanisms involved has been hindered by the complexity of cytokine effects and the ability of each cytokine to regulate the production of many other cytokines.⁴¹ Nevertheless, several new and promising concepts about the effects of cytokines on erythropoiesis have recently emerged. Libregts and colleagues²⁴ developed a mouse model where overproduction of interferon- γ leads to the development of a mild-to-moderate normocytic, normochromic anemia. The model manifests a 50% decrease in erythrocyte survival attributable to interferon-y-mediated activation of macrophages in the splenic red pulp. The model also shows suppression of erythrocyte production affecting the erythroblast stages and the earliest erythroid-committed precursor burst-forming unit-erythrocyte but not proerythroblasts and colony-forming unit-erythrocyte (CFU-E). Importantly, myeloid CFU-granulocyte/macrophage colonies were increased. Microarray analysis of erythroblasts indicated that interferon- γ promotes the transcription of PU.1 and its target genes in an interferon regulatory factor 1-dependent manner but does not affect GATA-1 or its targets. PU.1 and GATA-1 antagonize each other's activity, so the increase in PU.1 would be expected to promote myelopoiesis at the expense of erythropoiesis. During infections with viruses or intracellular pathogens known to induce interferon-y, this mechanism may assure sufficient production of monocytes and macrophages, at the expense of temporary impairment of erythropoiesis. Whether other inflammatory cytokines use a similar or different mechanism remains to be determined.

Hepcidin-induced Hypoferremia and Interferon- γ Synergize to Suppress Erythropoiesis

Richardson and colleagues⁴³ examined how inflammatory cytokines and hypoferremia interact to affect erythropoiesis during Al. Using in vitro culture of human CD34+ primary progenitors, they documented that hypoferremia (transferrin saturation \leq 15%) potentiates the suppressive effects of TNF- α and interferon- γ on erythropoiesis. Surprisingly, erythropoietic suppression could be reversed by the addition of the Krebs cycle intermediate isocitrate, a product of the enzyme aconitase, which also functions as a cellular iron sensor. Isocitrate injections also reversed Al in a rat model of autoimmune arthritis induced by injection of streptococcal peptidoglycanpolysaccharide. The authors present evidence that hypoferremia activates PU.1 via a protein kinase C pathway, synergizing with the effect of interferon- γ . Isocitrate, acting on aconitase, reverses the effect of hypoferremia on PU.1 and relieves the suppression of erythropoiesis. It remains to be seen if these effects are important in other animal models and in human subjects.

Animal Models of AI Show Partial Dependence on Hepcidin

A new mouse model of AI was generated by a single intraperitoneal injection of heatkilled *Brucella abortus*.⁴⁴ Like human AI, this model showed multifactorial pathogenesis including iron restriction from increased hepcidin, transient suppression of erythropoiesis, and shortened erythrocyte lifespan. Mice developed severe anemia with mild microcytosis and mild hypochromia, a hemoglobin nadir at 14 days and partial recovery by 28 days.^{45,46} After an early increase in inflammatory markers and hepcidin, the mice manifested hypoferremia despite iron accumulation in the liver. Erythropoiesis was suppressed between days 1 and 7, and erythrocyte destruction was increased as evidenced by shortened red blood cell lifespan and rare schistocytes on blood smears. Erythropoietic recovery began after 14 days but was ironrestricted, as documented by increased erythrocyte zinc protoporphyrin. In mice with ablated hepcidin-1 gene, anemia was milder, not iron-restricted, and with faster recovery, supporting the role of hepcidin in the development of AI.

In the same mouse model of AI, the therapeutic administration of antihepcidin monoclonal antibodies decreased the severity of anemia.^{44,47} Moreover, resistance to exogenous erythropoietin doses observed in this model was relieved by coadministration of the antibodies with erythropoietin. In the rat model of autoimmune arthritis induced by injection of streptococcal peptidoglycan-polysaccharide, suppressing hepcidin production by administration of the dorsomorphin derivative LDN-193189 or soluble hemojuvelin-Fc fusion protein, 2 agents that interfere with bone morphogenetic protein receptor signaling, also ameliorated anemia.⁴⁸

TREATMENT OF AI

- Treat the underlying disease
- Treat anemia specifically only if severe or limits activities of daily living
- Erythrocyte transfusion for acute symptoms
- Erythropoiesis-stimulating agents (ESAs) with or without IV iron (off-label treatment)
- Experimental therapies under development include new ESAs, anticytokine drugs, and agents targeting the hepcidin-ferroportin pathway

Current Therapy

Al is a secondary manifestation of inflammatory disorders, and treating the underlying disease will correct the anemia. Such treatment is not always possible. Direct treatment of anemia should be considered only if it is impairing the patient's performance, quality of life, or recovery from underlying illness. Inflammatory diseases sufficiently severe to cause AI may also cause fatigue or malaise through cytokine-dependent mechanisms, so these symptoms need not be caused by anemia. Potential therapies for AI include erythrocyte transfusions usually reserved for severe and acutely symptomatic anemia, and ESAs (erythropoietin and its derivatives, mimics or inducers, as they become available) with or without intravenous iron supplementation. Al is not a specifically approved indication for the use of ESAs but should be considered as an

Table 1 Experimental therapeutics for the treatment of AI (not including ESAs)				
Agent or Activity	Target	Chemistry	Development Status	Key Published Results
Tocilizumab	IL-6 receptor	Humanized monoclonal antibody	Approved to treat rheumatoid arthritis and juvenile rheumatoid arthritis (Genentech, Roche, Chugai)	Tocilizumab rapidly reduced hepcidin levels and improved anemia in patients with Castleman syndrome ⁵⁵ or rheumatoid arthritis ^{56,57}
Siltuximab	IL-6	Chimeric mouse-human monoclonal antibody	Submitted for FDA approval for multifocal Castleman disease (Janssen)	Siltuximab lowered hepcidin and improved anemia in patients with renal cell carcinoma ⁵⁸
Hepcidin binders	Hepcidin peptide	Monoclonal antibody	Preclinical to phase 1 (Lilly)	
		Anticalins	Preclinical (Pieris)	PRS-080 increased serum iron in monkeys ⁵⁹
		Spiegelmers	Phase 2 ^a (Noxxon)	NOX-H94 alleviated IL-6–induced anemia in a primate model ⁶⁰
Inhibitors of hepcidin production	Inhibit signaling by bone morphogenetic protein receptor type l	The kinase site of bone morphogenetic receptor l	Preclinical	LDN-193189 improved anemia in the mouse model of turpentine-induced AI ⁶¹
	Neutralize bone morphogenetic proteins	Soluble hemojuvelin-Fc fusion protein	Phase 2a ^a (FerruMax)	Hemojuvelin-Fc fusion protein alleviated anemia in a rat model of arthritis ⁴⁸ elicited by Group A streptococcal peptidoglycan-polysaccharide
		Heparin derivatives	Preclinical	Heparin reduced hepcidin expression ⁶² in mice and serum hepcidin concentrations in patients ⁶²
	Inactivate hepcidin mRNA	Antisense oligonucleotides (ASO)	Preclinical (Xenon/ISIS)	Antimouse hepcidin ASO improved anemia in a turpentine model of AI in mice ⁶³
	Inactivate transferrin receptor 2 mRNA	siRNA oligonucleotides	Preclinical (Alnylam)	Anti-TfR2 siRNA alleviated AI in rodent models ⁶⁴
Ferroportin blockers	Hepcidin binding site on ferroportin	Monoclonal antibody	Phase 1 ^a (Lilly)	

Abbreviations: FDA, Food and Drug Administration; mRNA, messenger RNA; siRNA, small interfering RNA. ^a Clinicaltrials.gov.

alternative to chronic erythrocyte transfusion. The use of ESAs in AI is based on a small number of anecdotal reports^{49–53} that reported improvement of anemia, and similarities between AI and anemia of chronic kidney disease (CKD), the main indication for ESAs. In CKD, IV iron supplementation potentiates the effect of erythropoietin and its derivatives,⁵⁴ and it has been reported that IV iron may have a similar activity in AI.⁵³

Experimental Therapy

Experimental treatments of AI target cytokines or the hepcidin-ferroportin axis and its various regulators (Table 1). Most of these agents have proved effective in animal models and several are undergoing human trials. Anti-IL-6 agents and other anticytokine drugs that indirectly lower IL-6 levels are already approved for the treatment of severe inflammatory diseases. Some of these agents may prove to be very effective for the treatment of AI in other settings, reflecting the important role of IL-6 in its pathogenesis. Because AI affects symptoms and quality of life but has not been demonstrated to affect survival from the underlying disease, drugs specifically targeted for AI must not only demonstrate efficacy but also be well-tolerated and free of serious side effects.

REFERENCES

- 1. Cartwright GE, Lee GR. The anaemia of chronic disorders. Br J Haematol 1971; 21(2):147–52.
- 2. Katz JH. Iron and protein kinetics studied by means of doubly labeled human crystalline transferrin. J Clin Invest 1961;40:2143–52.
- 3. Corwin HL, Krantz SB. Anemia of the critically ill: "acute" anemia of chronic disease. Crit Care Med 2000;28(8):3098–9.
- 4. Beghe C, Wilson A, Ershler WB. Prevalence and outcomes of anemia in geriatrics: a systematic review of the literature. Am J Med 2004;116(7 Suppl 1):3–10.
- 5. Elliott J, Mishler D, Agarwal R. Hyporesponsiveness to erythropoietin: causes and management. Adv Chronic Kidney Dis 2009;16(2):94–100.
- 6. Barron BA, Hoyer JD, Tefferi A. A bone marrow report of absent stainable iron is not diagnostic of iron deficiency. Ann Hematol 2001;80(3):166–9.
- 7. Krause JR, Brubaker D, Kaplan S. Comparison of stainable iron in aspirated and needle-biopsy specimens of bone marrow. Am J Clin Pathol 1979;72(1):68–70.
- Thomason RW, Lavelle J, Nelson D, et al. Parenteral iron therapy is associated with a characteristic pattern of iron staining on bone marrow aspirate smears. Am J Clin Pathol 2007;128(4):590–3.
- 9. Ferraro S, Mozzi R, Panteghini M. Revaluating serum ferritin as a marker of body iron stores in the traceability era. Clin Chem Lab Med 2012;50(11):1911–6.
- Cozzi A, Santambrogio P, Privitera D, et al. Human L-ferritin deficiency is characterized by idiopathic generalized seizures and atypical restless leg syndrome. J Exp Med 2013;210(9):1779–91.
- Tran TN, Eubanks SK, Schaffer KJ, et al. Secretion of ferritin by rat hepatoma cells and its regulation by inflammatory cytokines and iron. Blood 1997; 90(12):4979–86.
- Thomson AM, Cahill CM, Cho HH, et al. The Acute Box cis-Element in Human Heavy Ferritin mRNA 5'-Untranslated Region Is a Unique Translation Enhancer That Binds Poly(C)-binding Proteins. J Biol Chem 2005;280(34):30032–45.
- Cohen LA, Gutierrez L, Weiss A, et al. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. Blood 2010;116(9): 1574–84.

- 14. Cazzola M, Ponchio L, de Benedetti F, et al. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood 1996;87(11): 4824–30.
- 15. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood 2003;101(9):3359–63.
- Pettersson T, Kivivuori SM, Siimes MA. Is serum transferrin receptor useful for detecting iron-deficiency in anaemic patients with chronic inflammatory diseases? Br J Rheumatol 1994;33(8):740–4.
- 17. Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990;75(9):1870–6.
- Infusino I, Braga F, Dolci A, et al. Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anemia a meta-analysis. Am J Clin Pathol 2012;138(5):642–9.
- Litton E, Xiao J, Ho KM. Safety and efficacy of intravenous iron therapy in reducing requirement for allogeneic blood transfusion: systematic review and meta-analysis of randomised clinical trials. BMJ 2013;347:f4822.
- 20. Freireich EJ, Ross JF, Bayles TB, et al. Radioactive iron metabolism and erythrocyte survival studies of the mechanism of the anemia associated with rheumatoid arthritis. J Clin Invest 1957;36(7):1043–58.
- Troutt JS, Butterfield AM, Konrad RJ. Hepcidin-25 concentrations are markedly increased in patients with chronic kidney disease and are inversely correlated with estimated glomerular filtration rates. J Clin Lab Anal 2013;27(6):504–10.
- 22. Correll CK, Binstadt BA. Advances in the pathogenesis and treatment of systemic juvenile idiopathic arthritis. Pediatr Res 2014;75(1–2):176–83.
- 23. Milner JD, Orekov T, Ward JM, et al. Sustained IL-4 exposure leads to a novel pathway for hemophagocytosis, inflammation, and tissue macrophage accumulation. Blood 2010;116(14):2476–83.
- 24. Libregts SF, Gutierrez L, de Bruin AM, et al. Chronic IFN-gamma production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. Blood 2011;118(9):2578–88.
- 25. Ganz T. Systemic iron homeostasis. Physiol Rev 2013;93(4):1721-41.
- 26. Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012; 1823(9):1434–43.
- 27. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306(5704): 2090–3.
- 28. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113(9):1271–6.
- 29. 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(2):745–52.
- **30.** Kemna E, Pickkers P, Nemeth E, et al. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. Blood 2005; 106(5):1864–6.
- **31.** Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest 2002;110(7):1037–44.
- 32. Finberg KE, Heeney MM, Campagna DR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet 2008;40(5):569–71.

- **33.** Finberg KE. Iron-refractory iron deficiency anemia. Semin Hematol 2009;46(4): 378–86.
- 34. Camaschella C, Poggiali E. Inherited disorders of iron metabolism. Curr Opin Pediatr 2011;23(1):14–20.
- **35.** Du X, She E, Gelbart T, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science 2008;320(5879):1088–92.
- **36.** Silvestri L, Pagani A, Nai A, et al. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab 2008;8(6):502–11.
- **37.** Means RT Jr, Krantz SB. Inhibition of human erythroid colony-forming units by tumor necrosis factor requires beta interferon. J Clin Invest 1993;91(2):416–9.
- Means RT Jr, Dessypris EN, Krantz SB. Inhibition of human erythroid colonyforming units by interleukin-1 is mediated by gamma interferon. J Cell Physiol 1992;150(1):59–64.
- Means RT Jr, Krantz SB. Inhibition of human erythroid colony-forming units by gamma interferon can be corrected by recombinant human erythropoietin. Blood 1991;78(10):2564–7.
- Broxmeyer HE, Williams DE, Lu L, et al. The suppressive influences of human tumor necrosis factors on bone marrow hematopoietic progenitor cells from normal donors and patients with leukemia: synergism of tumor necrosis factor and interferon-gamma. J Immunol 1986;136(12):4487–95.
- Felli N, Pedini F, Zeuner A, et al. Multiple members of the TNF superfamily contribute to IFN-γmediated inhibition of erythropoiesis. J Immunol 2005; 175(3):1464–72.
- Johnson RA, Waddelow TA, Caro J, et al. Chronic exposure to tumor necrosis factor in vivo preferentially inhibits erythropoiesis in nude mice. Blood 1989; 74(1):130–8.
- Richardson CL, Delehanty LL, Bullock GC, et al. Isocitrate ameliorates anemia by suppressing the erythroid iron restriction response. J Clin Invest 2013; 123(8):3614–23.
- 44. Sasu BJ, Cooke KS, Arvedson TL, et al. Antihepcidin antibody treatment modulates iron metabolism and is effective in a mouse model of inflammationinduced anemia. Blood 2010;115(17):3616–24.
- 45. 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(8):1137–45.
- 46. 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(8):1129–36.
- 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(17):3054–61.
- Theurl I, Schroll A, Sonnweber T, et al. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. Blood 2011; 118(18):4977–84.
- Kato Y, Takagi C, Tanaka J, et al. Effect of daily subcutaneous administration of recombinant erythropoietin on chronic anemia in rheumatoid arthritis. Intern Med 1994;33(4):193–7.
- 50. Peeters HR, Jongen-Lavrencic M, Bakker CH, et al. Recombinant human erythropoietin improves health-related quality of life in patients with rheumatoid arthritis and anaemia of chronic disease; utility measures correlate strongly with disease activity measures. Rheumatol Int 1999;18(5–6):201–6.

- **51.** Peeters HR, Jongen-Lavrencic M, Vreugdenhil G, et al. Effect of recombinant human erythropoietin on anaemia and disease activity in patients with rheumatoid arthritis and anaemia of chronic disease: a randomised placebo controlled double blind 52 weeks clinical trial. Ann Rheum Dis 1996;55(10):739–44.
- 52. Pettersson T, Rosenlof K, Friman C, et al. Successful treatment of the anemia of rheumatoid arthritis with subcutaneously administered recombinant human erythropoietin. Slower response in patients with more severe inflammation. Scand J Rheumatol 1993;22(4):188–93.
- **53.** Arndt U, Kaltwasser JP, Gottschalk R, et al. Correction of iron-deficient erythropoiesis in the treatment of anemia of chronic disease with recombinant human erythropoietin. Ann Hematol 2005;84(3):159–66.
- 54. Singh A. Hemoglobin control, ESA resistance, and regular low-dose IV iron therapy: a review of the evidence. Semin Dial 2009;22(1):64–9.
- 55. Song SN, Tomosugi N, Kawabata H, et al. Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood 2010; 116(18):3627–34.
- 56. Song SN, Iwahashi M, Tomosugi N, et al. Comparative evaluation of the effects of treatment with tocilizumab and TNF-alpha inhibitors on serum hepcidin, anemia response and disease activity in rheumatoid arthritis patients. Arthritis Res Ther 2013;15(5):R141.
- 57. 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(6):R204.
- **58.** Schipperus M, Rijnbeek B, Reddy M, et al. CNTO328 (Anti-IL-6 mAb) treatment is associated with an increase in hemoglobin (Hb) and decrease in hepcidin levels in renal cell carcinoma (RCC) [abstract]. Blood 2009;22(114):4045.
- 59. Hohlbaum A, Gille H, Christian J, et al. Iron mobilization and pharmacodynic marker measurements in non-human primates following administration of PRS-080, a novel and highly specific anti-hepcidin therapeutic [abstract]. Am J Hematol 2013;88(5):E41.
- **60.** 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(12):2311–5.
- **61.** Steinbicker AU, Sachidanandan C, Vonner AJ, et al. Inhibition of bone morphogenetic protein signaling attenuates anemia associated with inflammation. Blood 2011;117(18):4915–23.
- 62. Poli M, Girelli D, Campostrini N, et al. Heparin: a potent inhibitor of hepcidin expression in vitro and in vivo. Blood 2011;117(3):997–1004.
- **63.** Crosby JR, Gaarde WA, Egerston J, et al. Targeting hepcidin with antisense oligonucletides improves anemia endpoints in mice. Blood 2006;108(11 Pt 1): 83A–4A.
- 64. Akinc A, Chan-Daniels A, Sehgal A, et al. Targeting the Hepcidin Pathway with RNAi Therapeutics for the Treatment of Anemia. Blood 2011;118(21):315.