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Iron sequestration and anemia of inflammation

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Abstract

Anemia of chronic disease, also called anemia of inflammation, is characterized by hypoferremia due to iron sequestration that eventually results in iron-restricted erythropoiesis. During the last decade, the molecular mechanisms of iron sequestration have been found to center on cytokine-stimulated overproduction of the iron-regulatory hormone hepcidin. The inflammatory cytokine IL-6 is a particularly prominent inducer of hepcidin but other cytokines are likely to contribute as well. Hepcidin excess causes the endocytosis and proteolysis of the sole known cellular iron exporter, ferroportin, trapping iron in macrophages and iron-absorbing enterocytes. The supply of iron to hemoglobin synthesis becomes limiting, eventually resulting in anemia. Depending on the details of the underlying disease, other inflammation-related mechanisms may also contribute to anemia.

Hypoferremia in infection and inflammation

As described more than sixty years ago, serum iron concentrations markedly decrease in humans[1] and in dogs[2] during the first few days of systemic infection or inflammation. More recent experimental studies in human volunteers injected with moderate doses of lipopolysaccharide [3] showed approximately a 50% decrease in serum iron by 24 hours. In another group of human subjects, a 3hr infusion of interleukin 6 (IL-6) was followed by an average 30% drop in serum iron 2 hrs later [4]. The rapid development of hypoferremia was also observed in mice with experimental meningococcal infection[5] or inflammation induced by turpentine[6] or LPS[7]. As suggested by the deleterious effects of iron supplementation during experimental infections[8,9], the hypoferremia probably contributes to host defense against infection, likely by decreasing the iron supply to invading microbes.

Hypoferremia of inflammation is caused by iron sequestration in macrophages

As discussed elsewhere in this issue, normally most of the iron delivered to plasma (about 20–25 mg/day) is provided by macrophages involved in recycling senescent erythrocytes, and only 1–2 mg/day comes from iron absorption in the duodenum, with additional variable amounts delivered from stored iron in hepatocytes. Studies with iron-radiolabeled damaged erythrocytes

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documented that inflammation or infection led to delayed appearance of radioactive iron in circulation and the accumulation of iron in macrophages ("reticuloendothelial system") both in humans[10] and in experimental animal models[11]. The inflammation-induced sequestration of iron in macrophages explained the hypoferremia of inflammation but the molecular pathways involved in this response were not known until a few years ago.

Anemia of chronic disease (anemia of inflammation)

Prolonged infection or inflammation often leads to the development of anemia (anemia of chronic disease, more recently called anemia of inflammation, AI). AI is usually a mild to moderate anemia (Hgb 7–12 g/dl) that develops in the setting of many infections and inflammatory disorders, and some malignancies [12]. The newer terminology is not only more reflective of the pathophysiology of this anemia but also includes an acute form of this disorder, anemia of critical illness [13], a condition that develops within days of the onset of illness.

AI is characterized by inadequate erythrocyte production in the setting of low serum iron and low iron-binding capacity (i.e. low transferrin) despite preserved or even increased macrophage iron stores in the bone marrow. Direct examination of the bone marrow for iron-containing macrophages has been superseded in medical practice by measurements of serum ferritin. In most patients with iron deficiency serum ferritin is below the normal range but it is normal or high in patients with AI, reflecting the stimulation of ferritin synthesis by both inflammation and macrophage iron loading. The erythrocytes are usually normocytic and normochromic but can be mildly hypochromic and microcytic, especially in AI of long duration or in children, who utilize additional iron for growth. In these settings hypochromia and microcytosis develop, presumably because iron restriction becomes more severe as iron stores are progressively depleted.

Most chronic bacterial, fungal, viral or parasitic infections with systemic manifestations can cause AI. AI is also common in rheumatologic disorders, systemic autoimmune disorders, inflammatory bowel diseases, and chronic kidney diseases. Among malignancies, ovarian cancer[14] and multiple myeloma[15] are often complicated by AI. Anemia of critical illness [13] may develop acutely (within days) in intensive care settings where the effects of infection or inflammation are exacerbated by disease-related or iatrogenic blood loss or red cell destruction, by themselves not sufficiently severe to cause anemia.

Iron restriction is a major contributor to anemia of inflammation

The limitation of iron supply to erythropoiesis is a major factor in the development of AI. Other factors that variably contribute to AI include increased destruction of erythrocytes, diagnostic phlebotomy or other blood loss, suppression of the maturation of erythrocyte precursors by cytokines and cytokine-mediated interference with erythropoietin production or signaling. Several biochemical and histochemical observations support the importance of iron restriction as a key mechanism in AI. During the synthesis of heme, iron is incorporated into protoporphyrin IX with zinc as a minor alternative protoporphyrin ligand. As would be expected, the amount of zinc incorporated into protoporphyrin IX is increased in iron deficiency. In AI, zinc protoporphyrin is also increased [16], indicating that insufficient iron is reaching the sites of heme synthesis in the developing erythrocytes leading to the substitution of zinc. The number of sideroblasts, nucleated erythrocyte precursors that stain for iron with Prussian blue, is decreased in AI [12], again suggesting iron restriction. As a further indication of the limiting role of iron in patients with AI but no evidence of iron deficiency, the resistance of AI to erythropoietin therapy can sometimes be overcome by the co-administration of parenteral iron [17,18]. The mechanism by which high doses of intravenous iron preparations ameliorate resistance to pharmacologic doses of erythropoietin[19], even in situations where ferritin is elevated[20,21], is not yet clear. Nearly all iron from existing IV preparations is first

processed by macrophages, and its utilization for erythropoiesis is dependent on iron export through ferroportin to transferrin. Macrophage iron overload would be expected to increase membrane ferroportin[22] and increase iron availability for erythropoiesis. In parallel, high doses of erythropoietin may partially suppress hepcidin by as yet unknown mechanisms[23]. Previous attempts to treat AI with more moderate doses of iron alone (without added erythropoietin) have generally been unsuccessful, as iron became rapidly trapped in the macrophage compartment [12,24,25]. Although newer iron preparations appear to be well tolerated[26], the long term consequences of high dose iron therapy are not known and concerns have been raised about excess iron promoting infections and atherosclerosis[27–29] as well as carcinogenesis[30,31].

Molecular pathways of iron sequestration

Hepcidin and ferroportin

Hepcidin is a small peptide hormone secreted by hepatocytes, circulating in blood plasma and excreted in urine [32]. An injection of synthetic hepcidin into mice induces profound hypoferremia lasting 2–3 days (Figure 1)[33]. The endogenous peptide is essential for iron homeostasis as shown by the consequences of its absence (juvenile hemochromatosis[34,35]) or its excess (iron restricted anemia[36,37]). Hepcidin acts by binding to the cellular iron efflux channel ferroportin, and inducing its internalization and degradation[38]. Ferroportin is a 12-transmembrane segment protein found in all tissues that export iron to blood plasma[39–41]. It is the only known cellular exporter of elemental iron and it is also essential for iron homeostasis[42].

Hepcidin-induced ferroportin degradation inhibits iron release from macrophages

Macrophages recycle iron from senescent erythrocytes by a mechanism reviewed in detail in another chapter in this issue. The recycled iron is released to plasma through macrophage cell-membrane-associated ferroportin. During inflammation, hepcidin concentrations are high, which triggers increased endocytosis and proteolysis of ferroportin[38]. The efflux of ferrous iron from major iron transporting tissues (duodenal enterocytes, iron-recycling macrophages and iron-storing hepatocytes) into plasma is reduced (Figure 2) and the iron accumulates in their cytoplasmic ferritin. Continued consumption of iron by erythropoiesis then depletes the extracellular iron compartment leading to hypoferremia and iron-restricted erythropoiesis. The prominent effects of the loss of ferroportin on iron supply for erythropoiesis are illustrated by the consequences of moderate ferroportin deficiency or dysfunction due to autosomal dominant mutations in ferroportin (“ferroportin disease”). Despite iron-overloaded macrophages, the affected patients are susceptible to mild anemia, especially when phlebotomized[43].

Iron sequestration and anemia in noninflammatory overproduction of hepcidin

Genetic disorders and noninflammatory diseases that give rise to excessive hepcidin production include iron-refractory iron deficiency anemia (IRIDA, discussed elsewhere in this issue) and hepatic adenomas that overproduce hepcidin[37,44]. Both disorders cause hypoferremia and anemia which is refractory to oral iron supplementation and is only partially corrected by parenteral iron. Moderate overproduction of hepcidin in transgenic mice or in mice bearing hepcidin-producing tumors also causes an iron-restricted anemia[45,46]. These diseases and models demonstrate that the essential features of AI are reproduced by overproduction of hepcidin. Unlike in most AI, some affected patients have severe microcytosis, probably a consequence of chronicity and severity of hepcidin overproduction.

Resistance to erythropoietin

Hyporesponsiveness to therapeutic erythropoietin has emerged as an important consequence of inflammation, especially in chronic kidney diseases[47]. This feature of inflammation is reproduced in the hepcidin-overexpressing mouse[46] suggesting that increased hepcidin and iron limitation contribute to erythropoietin resistance. Neutralization of hepcidin by monoclonal antibody has been shown to restore responsiveness to erythropoietin in a mouse model of AI[48].

Hepcidin and ferroportin regulation in inflammation

Limited studies in patients with inflammatory diseases document that serum hepcidin or its proxy urinary hepcidin are increased by inflammation (Figure 3). Specific mediators that can cause this increase have been analyzed in human volunteers, mouse models and in cell culture systems but the picture is far from complete.

Interleukin-6

Within hours after an inflammatory stimulus, hepcidin levels greatly increase[3,4,49], followed by the development of hypoferremia. As indicated by experiments with isolated human hepatocytes treated with macrophage supernatants and studies with IL-6-deficient mice, the acute induction of hepcidin is mediated in large part by IL-6[3,4,50] but other cytokines may also contribute, especially in the chronic setting. The regulation of hepcidin synthesis is predominantly transcriptional. The STAT3 pathway transduces the effects of IL-6 utilizing a canonical STAT3 binding site in the proximal hepcidin promoter[51–53].

In accordance with the role of IL-6 in hepcidin regulation, diseases of IL-6 excess are prominently associated with anemia. Castleman's syndrome, a lymphoproliferative disorder of uncertain etiology, manifests increased IL-6, increased hepcidin, hypoferremia and iron-restricted anemia[54,55], suggestive of a hepcidin-mediated anemia. Treatment with anti-IL6 receptor antibody rapidly decreases hepcidin and reverses the anemia[56–59]. IL-6 also has an important role in the pathogenesis of multiple myeloma, and IL-6 contributes to the hepcidin-inducing activity of sera from myeloma patients[60]. Higher hepcidin levels in myeloma correlate with lower hemoglobin[60], indicating a likely causal role of hepcidin overproduction in the pathogenesis of anemia in this disease. Another disease where IL-6 excess and iron-restricted anemia are prominent features is systemic onset juvenile chronic arthritis [61]. Finally, ovarian carcinomas complicated by pretreatment anemia are also associated with overproduction of IL-6.[14] Serial measurements of hepcidin, serum iron and erythrocyte kinetics in these diseases before and during treatment will be informative about the role of hepcidin in these disorders.

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMP-2, 4, 6 and 9 have been most studied in this regard) are potent inducers of hepcidin synthesis in cultured hepatocyte cell lines[62–64], primary hepatocytes[65], and in mouse models[66]. They regulate hepcidin transcription by binding to BMP receptors and activating the SMAD pathway. The essential role of this pathway for hepcidin and iron regulation was first demonstrated by studies of liver-specific SMAD4 knockout mice[67] which make very little hepcidin and develop parenchymal iron overload. The principal and specialized endogenous ligand of the iron-regulating BMP receptor in the liver appears to be BMP6 as shown by the development of iron overload in mice lacking BMP6[68,69] with the absence of significant skeletal or other abnormalities that are associated with the ablation of other BMPs. Whether BMPs contribute to inflammatory regulation of hepcidin and iron is not yet known.

Hemojuvelin

A very severe form of hepcidin deficiency leading to iron overload is found in patients[70] and mouse models[71,72] lacking the GPI-linked membrane protein hemojuvelin. This protein functions as a coreceptor for BMP2, 4 and 6[73] and the loss of hemojuvelin results in very low basal levels of hepcidin transcription. Unexpectedly, hemojuvelin mRNA levels are potently suppressed by inflammation[74], an effect mediated by tumor necrosis factor-alpha but not by IL-6[75]. It is not yet clear how this effect contributes to the regulation of hepcidin and iron during inflammation.

Direct regulation of ferroportin during inflammation

The reduction of ferroportin protein expression during inflammation[76] was noted even before the discovery of its posttranslational regulation by hepcidin. Although hepcidin is probably the principal systemic regulator of ferroportin, the production and trafficking of ferroportin may also be regulated by mechanisms independent of hepcidin. Ferroportin mRNA levels in the liver and the spleen were shown to be suppressed after the administration of lipopolysaccharide to mice[76]. Moreover, ferroportin transcripts contain a 5′ iron regulatory element (IRE)[41] that could mediate translational repression of ferroportin synthesis by IRP1 during inflammation and oxidative stress[77].

Therapeutic perspectives

Anemia of inflammation can add substantially to the morbidity of the underlying disease and is often a predictor of adverse outcome[78]. Increased awareness of the key role of iron sequestration in AI has led to the empiric use of high dose IV iron to ameliorate inflammation-induced resistance to erythropoietin[19,20,47]. Systematic studies of pathways that mediate iron sequestration in AI have pinpointed molecular targets for the treatment of this anemia in those situations when the underlying disease cannot be reversed. Anti-IL-6 treatments, which should alleviate AI along with other IL-6-mediated pathology in a variety of inflammatory disorders, are already undergoing clinical trials[59]. Because of the specialized involvement of these molecules in the iron pathway, the therapeutic antagonism of hepcidin, membrane hemojuvelin, or BMP6 could have a highly selective beneficial effect in AI.

Conclusion

Anemia of chronic disease develops in a great variety of disease settings but is commonly characterized by inflammation, hypoferremia, iron sequestration in macrophages, and iron-restricted erythropoiesis. At the molecular level, cytokine-stimulated overproduction of hepcidin causes the endocytosis and degradation of the iron efflux channel ferroportin, decreasing the delivery of iron from macrophages and enterocytes to plasma. Hypoferremia ensues, and restricts the supply of iron to hemoglobin synthesis, eventually resulting in anemia. Similar mechanisms may also account for erythropoietin resistance in the setting of chronic kidney diseases. Evolving understanding of its pathogenetic pathways should improve the treatment of this anemia.

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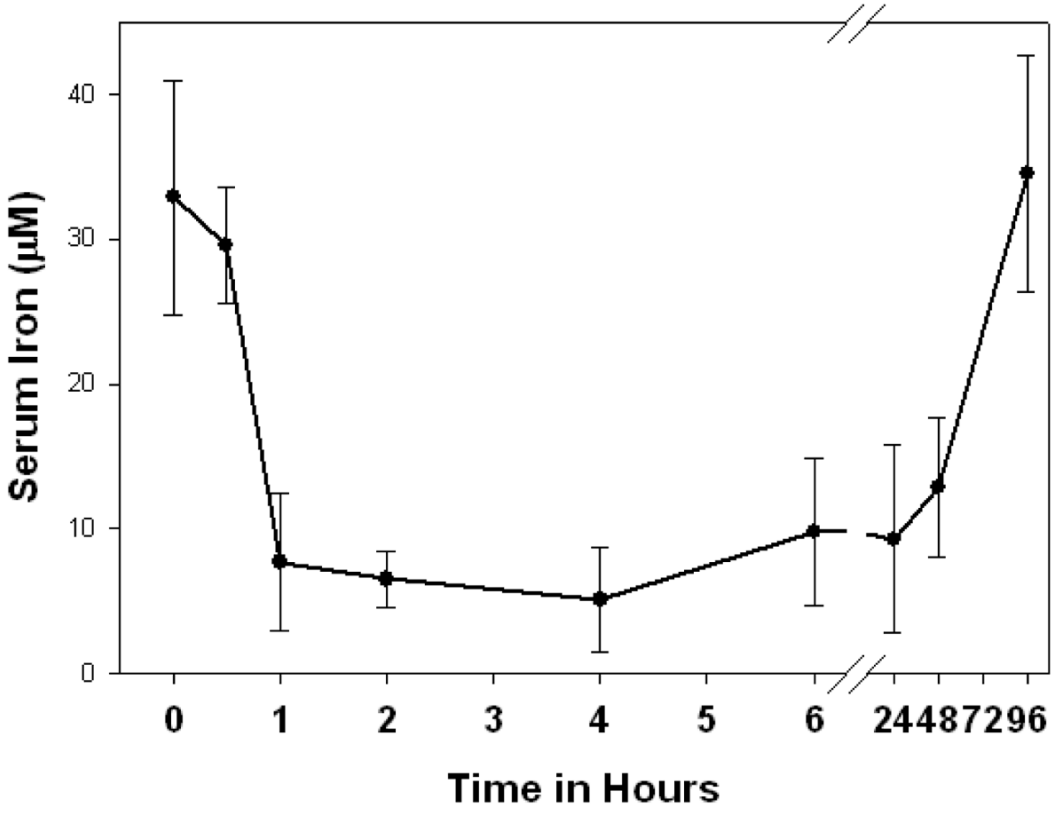

Figure 1.
Mice injected with synthetic hepcidin (50 µg) develop prolonged and severe hypoferremia (from Reference 33).

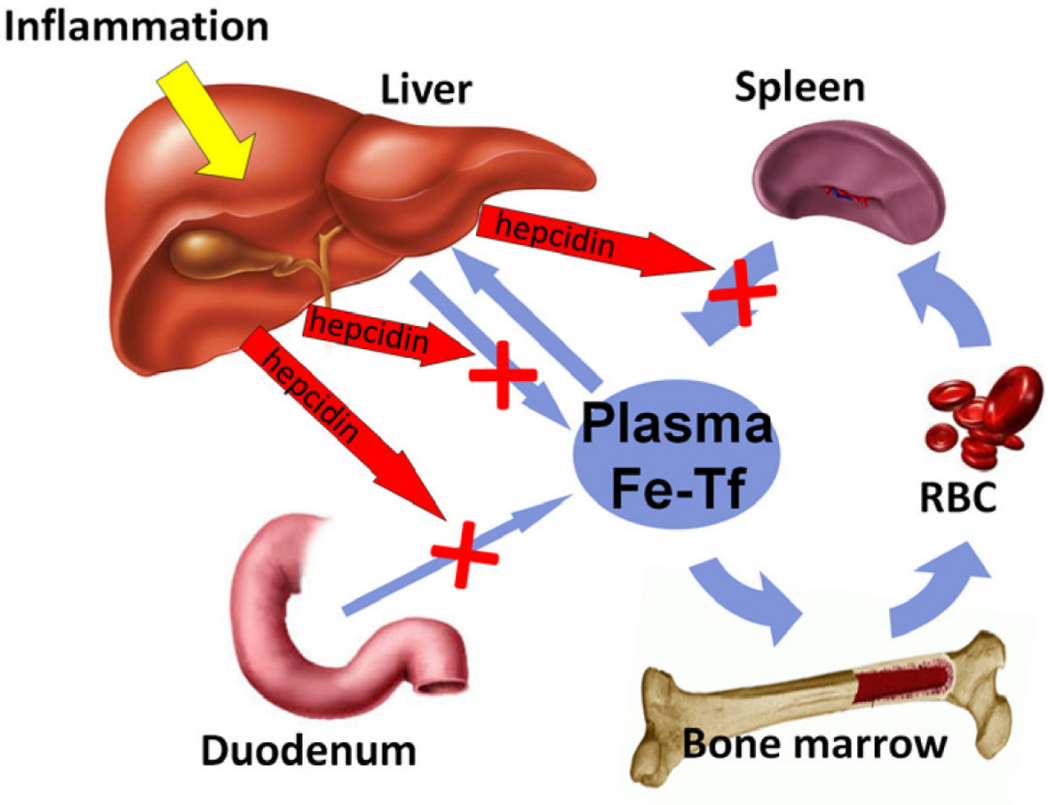

Figure 2.
Inflammation-induced hepcidin causes hypoferremia by inhibiting the major iron flows into plasma (mainly recycled iron from splenic and hepatic macrophages, but also dietary iron from the duodenum and stored iron from hepatocytes). Prolonged hypoferremia limits the availability of iron for hemoglobin synthesis and erythropoiesis, causing AI.

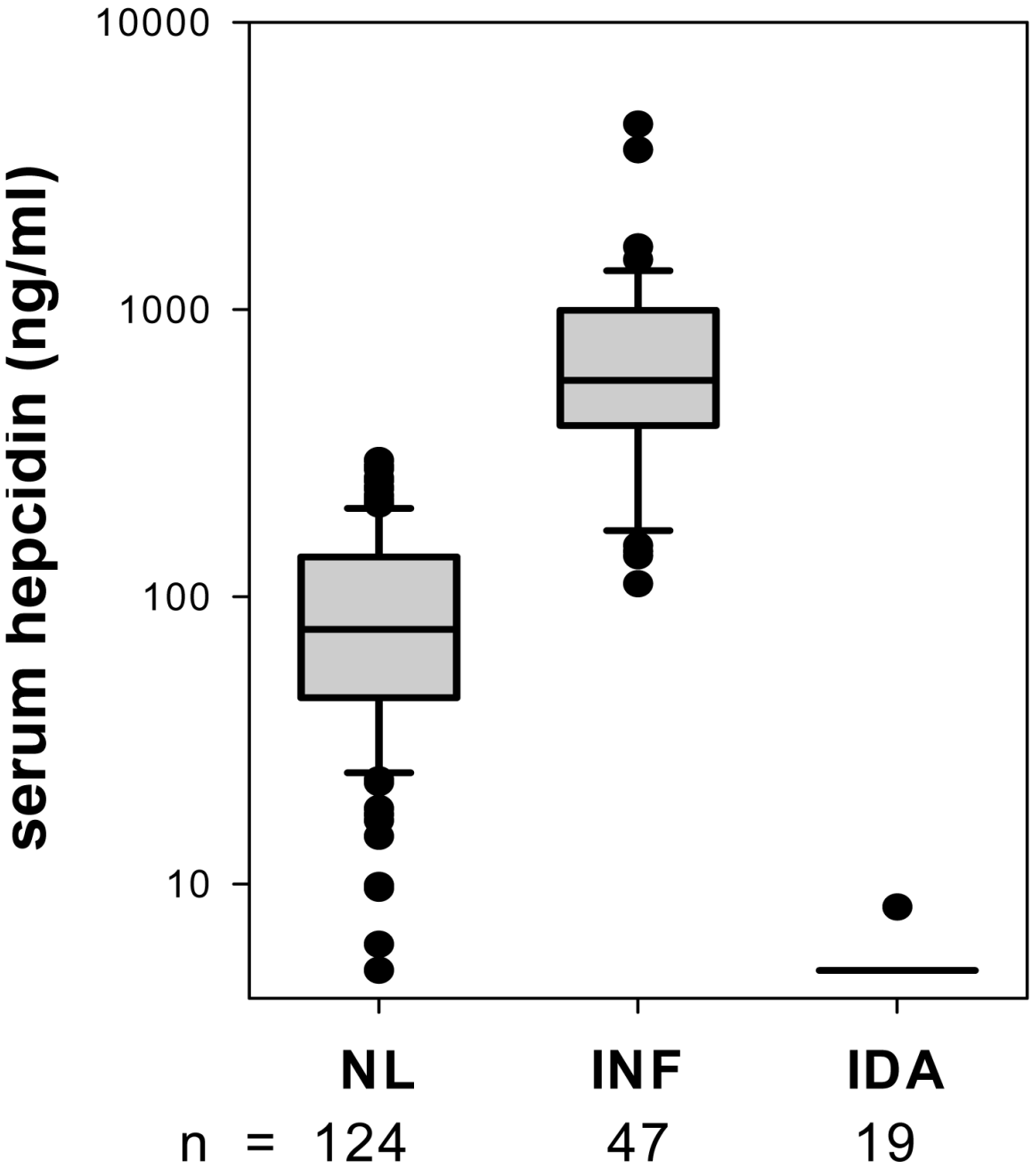

Figure 3.
Patients with inflammation (defined by CRP greater than 10 mg/dl) have elevated serum hepcidin, in contrast to patients with iron deficiency (defined as ferritin less than 10 ng/ml) in whom hepcidin is low or unmeasurable (from reference [79]).