Chapter 11
Iron and the Reticuloendothelial System

Günter Weiss

Keywords Anemia of chronic disease • Erythrophagocytosis • Heme oxygenase • Infection • Inflammation • Interferon-gamma • Interleukins • Macrophages • Monocyte

1 Introduction

Iron is an essential component for all cells and higher eukaryotes due to its central role for oxygen transport, electron transport during mitochondrial respiration, in forming a prosthetic group for central metabolic enzymes, and for the regulation of transcription via its role as the central component of ribonucleotide reductase [1–3]. Moreover, iron catalyzes the formation of hydroxyl radicals, which then modulate the binding affinity of critical transcription factors such as HIF-1 or NF-κB and thus affect the gene expression during inflammation [4]. Therefore, both iron overload and iron deficiency exert subtle effects on essential metabolic pathways and on the growth, proliferation, and differentiation of organisms. The tight control of iron homeostasis is thus a pre-requisite to maintain a sufficient supply of iron for essential metabolic pathways while avoiding the metals' detrimental effects on tissue damage via radical formation. In addition, iron is centrally involved in the regulation of cellular immune function, while on the other hand, it is an essential nutrient for invading microbes and tumor cells. Specifically, microorganisms have evoked multiple strategies to capture and ingest iron, which they need for proliferation, pathogenicity, and defense pathways including biofilm formation [5]. The divergent pathways by which microorganisms can acquire iron have been recently reviewed [6, 7].

While cells of the reticuloendothelial system (RES) play a key role in the control of body iron homeostasis by recycling iron and redistributing the metal to the circulation [8–10], the regulatory network between iron and immune function is of central importance for the pathophysiology and clinical course of diseases such as infections, cancer, or autoimmune disorders. The control over iron homeostasis under these circumstances is one of the most important determinants deciding about the fate of an infectious or malignant disease [11–13].

The close interaction between iron and immunity is underscored by observations that certain immunological proteins do alter cellular iron metabolism, as described for ß2-microglobulin, HFE, a non-classical MHC-I molecule linked to the majority of cases with human hemochromatosis,

G. Weiss, M.D. (✉)
Department of Internal Medicine I, Clinical Immunology and Infectious Diseases, Medical University, A-6020 Innsbruck, Austria
e-mail: guenter.weiss@i-med.ac.at

G.J. Anderson and G. McLaren (eds.), *Iron Physiology and Pathophysiology in Humans*, Nutrition and Health, DOI 10.1007/978-1-60327-485-2_11,

tumor necrosis factor receptor (TNFR), or the natural resistance-associated macrophage protein 1 (NRAMP1). Changes in immune function thus affect iron homeostasis and vice versa [14–18].

In being a central component for all proliferating cells, iron is also a central regulator of immune cell proliferation and function. Lymphocytes are central regulatory cells of specific immunity, which determine the functional activity of cells of the RES. Thus, a sufficient capacity of these cells to proliferate and differentiate is a pre-requisite for normal immune function [19–21]. Iron has turned out to be centrally involved in these processes, and thus, lymphocytes have evoked different mechanisms to acquire iron even under conditions when iron availability is limited. All lymphocyte subsets, which include B- and T-lymphocytes and natural killer (NK) cells, are dependent on transferrin/transferrin receptor (TfR)-mediated iron uptake, while a blockade of this pathway leads to diminished proliferation and differentiation of these cells [22]. Accordingly, mitogenic stimuli, such as phytohemagglutinin, increase TfR surface expression on B and T cells [20]. However, the lymphocyte subsets differ in their dependence on transferrin-mediated iron uptake. Accordingly, the induction of experimental iron overload in rats resulted in a shift in the ratio between T-helper (CD4+) and T-suppressor/cytotoxic T cells (CD8+), with a relative expansion of the latter [13]. Moreover, even T-helper (Th) subset responds differently to iron perturbations [23]. It is well established that there are several subsets of CD4+ T-helper cell that exist in man, termed type 1 (Th1), Th2, Th17, and Treg, each of which produces a typical set of cytokines that regulate different immune effector functions and that cross-react with each other. Th1-derived cytokines such as interferon (IFN)- or tumor necrosis factor (TNF)-ß activate macrophages, thus contributing to the formation of pro-inflammatory cytokines, such as TNF-α, IL-1, or IL-6, and the induction of cytotoxic immune effector mechanisms of macrophages including nitrogen and oxygen radical formation. By contrast, Th2 cells produce IL-4, IL-5, IL-9, and IL-13, which in part exert anti-inflammatory actions via inhibition of various macrophage functions and which activate immune cells involved in allergic reactions (e.g., IgE-secreting B cells). In addition, CD4+ cells with immune-suppressive properties have been described, consisting of T-regulatory $CD25^{+}CD4^{+}$ cells (T_{REG}). Treg inhibit T-cell activation in a cell contact-dependent manner, whereas Th3 cells produce cytokines with immune-deactivating effects such as transforming growth factor (TGF-ß) or IL-10, respectively [24]. In contrast, Th17 cells are major determinants of pro-inflammatory immune effector pathways, thus being centrally involved in many autoimmune diseases [25].

While Th1 clones are very sensitive to treatment with anti-TfR antibodies, resulting in inhibition of their DNA synthesis, Th2 cells are resistant to this procedure. This is partly attributed to the fact that Th2 clones exhibit larger chelatable iron storage pools than Th1 cells. Thus, Th1-mediated immune effector pathways are much more sensitive to changes in iron homeostasis *in vivo* [23]. The latter can partly be referred to a direct regulatory effect of iron on the activity of the central regulatory Th1 cytokine IFN-γ [26].

In contrast, circulating monocytes and tissue macrophages, being the central components of the RES, are differentiating cells and the major regulatory components of iron homeostasis and iron storage within the body.

2 Monocyte/Macrophage Iron Homeostasis

The body's daily demands for iron are estimated between 20 and 30 mg of the metal [1, 27, 28]. While only 10% of this need is compensated by iron absorption from the duodenum, the majority of iron originates from monocytes/macrophages pointing to the essential role of these cells for the recycling of iron from senescent erythrocytes, iron redistribution to the circulation, and thus for the maintenance of body iron homeostasis. In addition, in being cells of the RES monocytes/macrophages play important regulatory roles for iron homeostasis under inflammatory conditions.

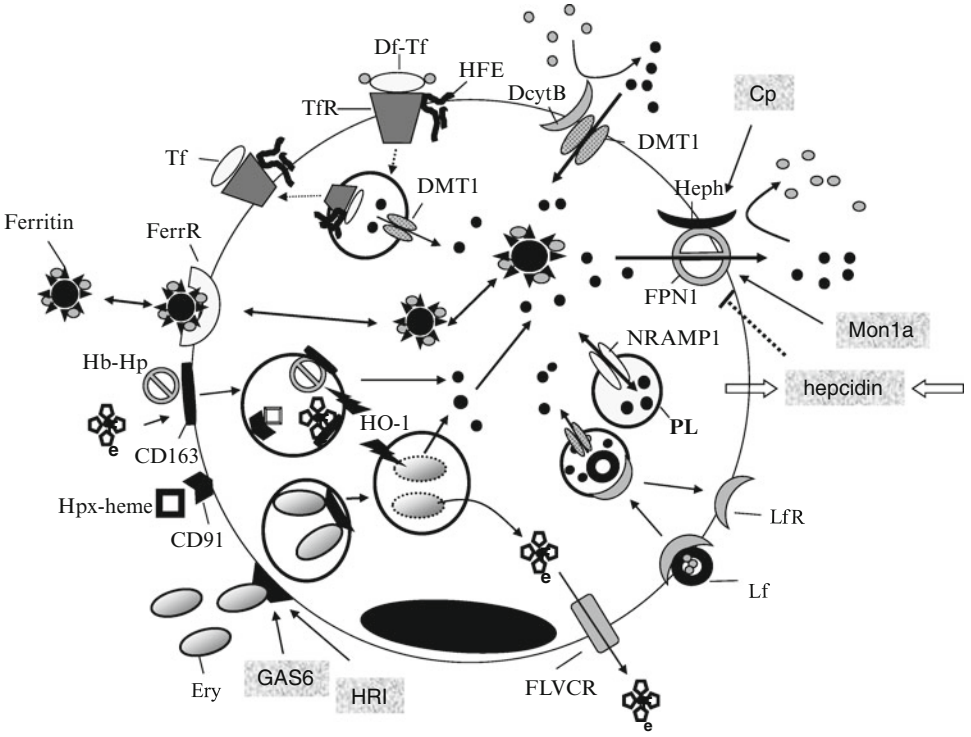

Fig. 11.1 Pathways for iron acquisition and iron release in moncoytes/macrophages and their exogenous modulation. Monocytes/macrophages can acquire iron via transferrin (*Tf*), transferrin receptor (*TfR*)-mediated endocytosis, directly via divalent metal transporter 1 (*DMT1*), by uptake of iron-loaden lactoferrin (*Lf*) via lactoferrin receptors (*LfR*), after binding of hemoglobin–haptoglobin (*Hb–Hp*) complexes or free heme to the CD163 surface receptor, binding of hemopexin–heme (*hpx–heme*) complexes to CD91, both with subsequent endocytosis, respectively, and finally via erythrophagocytosis which requires previous surface binding of erythrocytes and subsequent erythrocytosis. Erythrophagocytosis appears to be positively affected by the growth-arrest-specific gene 6 (*Gas6*) and heme-regulated eIF2alpha kinase (*HRI*), both of which are also produced and released by macrophages. Iron is recycled from erythrocytes and hemoglobin complexes following heme oxygenase-1 (*HO-1*)-mediated degradation of heme. However, heme may be directly released from degraded erythrocytes and then exported by the heme iron exporter, Vlfcr. In addition, the major iron secretory pathway from macrophages is controlled by the protein ferroportin (*FPN1*). The expression and transport capacity of this protein is negatively controlled by the acute-phase peptide hepcidin (from exogenous, liver, and endogenous, macrophage/moncoyte, sources; indicated by arrows) and positively affected by ceruloplasmin (*CP*) and Mon1a. Finally, macrophages express ferritin receptors which may mediate the uptake or release of iron-loaded ferritin from these cells. Ferric iron (*gray*) is reduced on the cell surface by duodenal cytochrome b oxidase (*DcytB*), while ferrous iron (*black*) is oxidized after ferroportin (*FP*)-mediated iron export by the membrane-bound ferroxidase hephaestin (*Heph*)

They are mainly responsible for a diversion of intercellular iron traffic leading to iron retention in the RES and a limited availability of the metal for erythropoiesis, thus leading to the development of anemia of chronic disease, also termed as anemia of inflammation [29–31].

Due to these multiple roles of iron homeostasis in health and disease, monocytes/macrophages have evoked multiple pathways to acquire, store, and recirculate iron (Fig. 11.1) [32, 33]. While the "classical" mammalian iron uptake mechanism via transferrin/TfR-mediated endocytosis is the preferred pathway in lymphocytes and is also used in monocytes/macrophages [34], in addition, the latter have the capacity to acquire the metal by different pathways, some of which are only found in these cells [35].

Specifically, the divalent metal transporter (DMT1) is highly expressed on monocytes and macrophages. DMT1 has initially been identified in rat duodenum where it pumps ferrous iron and other divalent metals by a hydrogen-coupled mechanism across the cell membrane [36]. DMT1 is also of importance for the transfer of iron from the endosome into the cytoplasm [37], and mutations of this transporter are associated with the development of iron deficiency anemia (for a review, see [38]). DMT1 cooperates with a membrane-bound ferric reductase, termed DcytB, which reduces ferric to ferrous iron at the outer membrane which is a pre-requisite to be transported by DMT1[39]. Accordingly, human macrophages take up iron chelates with a higher efficacy than diferric transferrin by a temperature-dependent but pH-independent process [40, 41].

Immune cells also express receptors for H-ferritin [42] which may be involved in the iron turnover and exchange between lymphocytes, hepatocytes, and macrophages [43]. However, the exact functions as well as the regulation of ferritin receptor expression are still elusive. Nonetheless, a recent study identified a novel receptor, Scara5, which mediates the endocytic uptake of ferritin into cells [44]. Opposite, monocytes and macrophages have been shown to act as the major source for serum ferritin [45]. Importantly, different cell types have contrasting preferences for L- or H-chain ferritin [46].

The iron-binding protein, lactoferrin (Lf), is a member of the transferrin family and is able to capture iron while at the same time it exerts distinct effects on immune function by regulating the proliferation and activation of lymphocytes, NK cells, and monocytes [47]. Lf is taken up after binding to specific receptors, Lf receptors (LfR), which are found at the cell surface of macrophages. The Lf/LfR complex is internalized most likely via an endocytotic process [48]. Once taken up, Lf plays regulatory roles within macrophages by modulating on the one hand iron-mediated cytotoxic effector mechanisms against intracellular pathogens via the formation of hydroxyl radicals, while on the other hand, apo-Lf protects macrophages from membrane peroxidation [47]. Evidence for the existence of Lf recirculation in macrophages was provided by experiments, demonstrating that Lf is released from macrophages which have been incubated in Lf-free media [47].

In addition, macrophages can acquire iron via phagocytosis of hemoglobin (Hb)–haptoglobin (Hp) complexes. The CD163 receptor is responsible for this endocytic process leading to removal of Hp–Hb complexes – but not of free Hp or Hb – from the circulation. CD163 is a member of the scavenger receptor cysteine-rich domain family. Interestingly, recent evidence also suggests that CD163 can take up hemoglobin even in the absence of Hp [49]. In addition, monocytes express CD91, the hemopexin receptor, which takes up heme captured by the heme-binding protein, hemopexin, resulting in induction of heme oxygenase-1 (HO-1) with subsequent iron accumulation in monocytes [50]. This receptor has been previously known as the low-density lipoprotein receptor-related protein (LRP)/CD91. Accordingly, endosomal uptake of the heme–hemopexin receptor resulted in lysosomal degradation of hemopexin [50].

Once iron enters the cells, it is either stored within ferritin, utilized upon incorporation into iron-containing enzymes, or exported and transferred to the circulation. An estimate of 10–20% percent remains in the labile iron pool which is important for macrophage effector functions and the regulation of cellular iron homeostasis [33, 51]. The latter is maintained at the posttranscriptional/translational level by interaction of cytoplasmic proteins, so-called iron regulatory proteins (IRP)-1 and 2, with RNA stem-loop structures, iron-responsive elements (IRE). IREs have been identified within the 5′ untranslated regions of the mRNAs coding for the central proteins for iron storage (H-chain and L-chain ferritin), iron consumption (erythroid aminolevulinic acid synthase, e-ALAS, the key enzyme in heme biosynthesis), and iron transport (ferroportin), while the mRNA coding for the major iron uptake protein, TfR, bears five IREs within its 3′ untranslated region (for a review, see [1, 52, 53]). Iron deficiency in cells stimulates the binding affinity of IRPs to IREs, thus resulting in inhibition of ferritin and e-ALAS expression by blocking the formation of the translation initiation complex [54]. Conversely, binding of IRPs to the IREs within the 3′ untranslated region of TfR mRNA increases the expression of this protein by prolonging TfR mRNA half-life and vice versa.

Apart from iron availability, the binding affinities of IRPs are further regulated by NO, hydrogen peroxide, superoxide anion, and hypoxia, conditions and compounds which are found during inflammatory processes [55–60].

Importantly, macrophages/monocytes release iron, which is essential for iron recirculation from degraded erythrocytes. HFE is a non-classical MHC class I molecule which is ubiquitously expressed on cells [61] and which has been found to be mutated in 80% of patients suffering from hereditary hemochromatosis [61–63]. This mutation results in loss of HFE function by disrupting its interaction with ß2-microglobulin. Evidence for the importance of such a condition on iron homeostasis and immune function has been provided earlier by the observation that ß2-microglobulin knockout mice not only develop parenchymal iron overload but also lack CD8+ cells [64, 65]. HFE interacts with transferrin-mediated iron uptake by forming a stoichiometric complex with TfR which lowers the affinity of TfR for iron-loaded transferrin, and thus, HFE affects cellular iron homeostasis [66–69]. Interestingly, HFE also serves as a ligand for the gamma–delta T-cell receptor which may be of importance for enterocyte differentiation [70]. In addition, HFE blocks the iron release from macrophages [71], as transfection of macrophages from hemochromatosis patients carrying the C282Y mutation with wild-type HFE resulted in an increased iron content of these cells [72].

The cellular mechanism underlying these observations may be traced back to modulation of iron release by ferroportin [71, 73]. Ferroportin (also called Ireg1 or SLC11A3) is a transmembrane iron exporter that is implicated in the basolateral transfer of ferrous iron to the circulation. Ferroportin is highly expressed in enterocytes, Kupffer cells, and spleen macrophages [74–76]. Mutations of ferroportin lead to iron overload disorders [77]. After being transported by ferroportin, ferrous iron undergoes oxidation which is maintained by the membrane-bound ferroxidase hephaestin, and ferric iron released from cells is the incorporation into transferrin for subsequent transport within the circulation [78]. Accordingly, monocytes from patients with hemochromatosis and from Hfe–/– mice have been found to be iron depleted [79, 80]. In addition, recent evidence demonstrates that monocytes and macrophages from Hfe–/– mice produce high amounts of the siderophore-capturing peptide lipocalin-2 (Lcn2), leading to macrophage/monocyte iron export [81]. As a consequence, such mice are protected from infection with the intracellular pathogen *Salmonella typhimurium* which has a high need for iron [81].

3 Erythrophagocytosis

Likewise, the major pathway by which monocytes and macrophages acquire iron is via erythrophagocytosis. After a mean half-life of 120 days, senescent erythrocytes express increased quantities of phosphatidylserine residues on their surfaces which are then recognized by macrophage/monocyte surface receptors, leading to attachment of erythrocytes and subsequent phagocytosis. Macrophages have been shown to phagocytose about three times as many erythrocytes as monocytes [82, 83]. Within the macrophage phagolysosome, the erythrocytes are degraded utilizing heme which then undergoes further degradation, a step which is controlled by the enzyme HO-1. This reaction yields iron, biliverdin, and carbon monoxide. Iron can be then shifted into the cytoplasm and incorporated into iron proteins or stored within ferritin [82–84].

Erythrophagocytosis is the most effective iron recycling system in the body, and 90% of the iron needed for erythropoiesis originates from this pathway. As ferroportin is the only known transmembrane iron exporter, the efficacy of iron recycling is linked to the expression of this protein [85]. Overexpression of ferroportin resulted in increased release and export of iron originating from phagocytosed erythrocytes [9]. Accordingly, ferroportin expression appears to be regulated by erythrophagocytosis. During early stages of erythrophagocytosis, an induction of HO-1 and ferroportin expression is observed which is associated with the release of heme into the cytoplasm [86].

At later stages, ferroportin expression is reduced which was linked to iron export and reduction of the cytoplasmic iron pool [8]. This can be also due to several alternative pathways. First, increased systemic iron availability induces the expression of the master iron regulatory peptide hepcidin in the liver [87–89]. Hepcidin is released into the circulation and binds on ferroportin when exposed on the cell surface which results in ferroportin internalization and ubiquitin-mediated degradation of this protein [90, 91]. This results in a blockage of iron export and monocyte/macrophage iron retention. Accordingly, exposure of macrophages to hepcidin reduced the recycling of iron following erythrophagocytosis and significantly decreased the release of non-heme iron from these cells [9, 92]. In addition, both DMT1 and NRAMP1 (see below) are important for iron recycling from senescent erythrocytes by shuttling iron from the phagolysosome to the cytoplasm [10, 93].

Monocytes and macrophages can also release heme via the surface receptor Flvcr (feline leukemia virus subgroup C receptor) [94]. Flvcr appears to be essential for erythropoiesis. In addition, Flvcr is highly expressed in duodenum, the kidney, and also in mononuclear cells and CD34+ progenitor cells. Macrophages, in which *Flvcr* had been deleted neonatally, did not differ from control macrophages in respect to their metabolic response to challenges with iron salts. However, when *Flvcr*-deficient cells were exposed to immunoglobulin-coated red blood cells, they presented with increased intracellular ferritin concentrations. This indicated first, a role of the heme export protein *Flvcr* in erythrophagocytosis, and secondly that heme derived from senescent erythrocytes is not fully degraded within the macrophages. Thus, a certain amount of this molecule is directly released into the circulation via this heme export protein (Fig. 11.1). This could be an important salvage pathway in case of increased erythrocyte degradation as it may occur during hemolytic anemia, malaria, or toxic/inflammatory erythrocyte damage, which exceeds the heme degradation capacity of macrophages. Alternatively, this mechanism could also prevent paralysis of the phagocytosis machinery and innate immune response by an overwhelming erythrophagocytosis and a subsequent drastic increase in intracellular iron concentration. However, heme released by macrophages will be captured by hemopexin in the circulation and redistributed to monocytes/macrophages which take up these complexes via the CD91 pathway. Nonetheless, this data provide evidence for another iron exit pathway from macrophages and monocytes which might be regulated by cytokines under inflammatory condition, thus contributing to macrophage iron retention, hypoferremia, and development of the anemia of chronic disease [29].

Finally, ferroportin expression and thus monocyte iron trafficking are controlled by additional pathways. First, the copper containing ferroxidase ceruloplasmin is not only responsible for the oxidation of ferrous iron but, in doing so, it stabilizes ferroportin [95]. Moreover, recent evidence suggests that a conserved gene, named *Mon1a*, coding for a protein with 556 amino acid residues, is involved in the trafficking of ferroportin within macrophages and thus in iron recycling from these cells [96]. Mice carrying a mutation in *Mon1a* present with increased spleen iron deposition, and accordingly, siRNA-mediated deletion of *Mon1a* resulted in decreased surface expression of ferroportin and macrophage iron retention. This may be referred to the importance of *Mon1a* in membrane trafficking. Accordingly, the expression of several cytokines, such as IL-6 and IL-12, was impaired in LPS-stimulated macrophages in which *Mon1a* expression had been reduced upon siRNA-mediated knockdown. This is of interest in respect to the putative mechanism underlying *Mon1a* action. Of note, two distinct splicing variants of ferroportin have recently been described which differ in their response to iron-mediated regulation [97]. Their tissue specific expression may thus impact on iron recirculation by monocytes/macrophages.

Finally, murine and human monocytes and macrophages produce considerable amounts of hepcidin [98–100]. In contrast to hepdicin expression in the liver, monocyte/macrophage hepcidin expression is not inducible by iron challenge at least in vitro. In contrast, hepcidin expression can by induced upon LPS stimulation which is referred to a TLR4-dependent mechanism [98] and independently from this by IL-6 [100] which results in induction of hepcidin transcription via STAT3-mediated activation [101–103]. Importantly, monocyte/macrophage-derived hepcidin can regulate ferroportin

expression in an autocrine fashion, thus resulting in ferroportin internalization and blockage of iron export [100]. Thus, via modulating the expression of cytokine inducers of hepcidin formation in the RES, regulatory molecules, such as Mon1a, may compromise iron homeostasis of these cells [104].

The hepcidin axis is also affected by the heme-regulated eIF2alpha kinase (HRI)[105]. HRI protein is mainly produced in erythroid precursors and is also present in murine macrophages [106]. Hri–/– mice exhibited impaired macrophage maturation and a weaker anti-inflammatory response with reduced cytokine production upon LPS challenge and thus a reduced production of hepcidin. In addition, macrophages from Hri–/– mice presented with an impairment of erythrophagocytosis, providing evidence for the role of HRI in recycling iron from senescent red blood cells [105].

A similar regulatory potential has also been demonstrated for another molecule, growth-arrest-specific gene 6 (Gas6), which is released by murine erythroblasts in response to erythropoietin treatment and which enhances erythropoietin signaling toward target cells [107]. Moreover, Gas6 and its cognate receptors Tyro3, Axl, and Mertk are also expressed by macrophages, and macrophages from Gas6–/– have a reduced capacity for erythrophagocytosis. In addition, loss of Gas6 resulted in increased release of cytokines such as IL-6 or IL-1ß from macrophages [107], cytokines which may also influence the expression of hepcidin, thus affecting monocyte iron homeostasis [41, 75, 108]. This provides another line of evidence that erythropoietic signals have a strong impact on the regulation of body iron homeostasis independent of iron availability [109]. In addition to erythropoietin [110], which downregulates hepcidin expression in the liver, and the putative role of Gas6, the growth differentiation factor-15 (GDF-15), a member of the TGF-ß superfamily which is induced upon erythroblast maturation, has recently been shown to inhibit hepcidin expression, thus contributing to iron overload in patients with thalassemia [111]. It is thus tempting to speculate that erythrophagocytosis may also impact on endogenous macrophage/monocyte expression of hepcidin, thereby ensuring increased ferroportin surface exposure and an efficient iron export and recirculation. However, patients with ACD had no significant differences in circulating GDF-15 levels as compared to controls or subjects with ACD and associated true iron deficiency [112].

4 Regulation of Macrophage/Monocyte Iron Metabolism During Inflammation

Monocytes and macrophage are the conductors that orchestrate iron homeostasis in health and disease and which are at the interface between iron and immunity [11, 33, 113]. This is due to the fact that macrophages need iron to produce highly toxic hydroxyl radicals by the enzyme phagocyte oxidase (phox) [4, 114], while at the same time, macrophages are major storage sites of iron under inflammatory conditions. Cytokines and radicals produced by macrophages as well as acute-phase proteins originating from the liver affect macrophage iron homeostasis by modulating iron uptake and iron release by these cells, leading to increased iron retention within macrophages under inflammatory conditions [34, 41, 115–119]. At the same time, iron modulates macrophage effector pathways by regulating cytokine activities, the induction of the antimicrobial machinery of macrophages, and indirectly via regulating lymphocyte proliferation and activities which then affect macrophage differentiation and activation [11, 21, 120–122]. Since iron is an essential compound for microbial growth and proliferation, the control over iron homeostasis is a central factor in infection. Thus, it is not surprising that phagolysosomal proteins, such as NRAMP1, which are associated with resistance toward infections with intracellular pathogens, also act as iron transporters. NRAMP1 has been identified as an innate immunity gene which was associated with resistance toward infections with intracellular pathogens such as *Leishmania*, *Salmonella*, or *Mycobacteria* species [17, 18]. Ectopic expression of NRAMP1 in COS-1 cells modulated intracellular levels of chelatable iron but did not influence iron uptake which suggested that NRAMP1 may be rather involved in intracellular iron

trafficking and mobilization of iron from intracellular vesicles [123, 124]. However, investigations of RAW264.7 macrophage cell line stably transfected with functional or non-functional NRAMP1 demonstrated that macrophages lacking functional NRAMP1 exhibited a significantly higher iron uptake via TfR and, as a consequence of this, an increased iron release mediated via increased ferroportin expression. Accordingly, as a net effect of the altered expression of iron transporters, the overall cellular iron content was lower in macrophages bearing functional NRAMP1 [124]. The attractive hypothesis that NRAMP1 expression may confer resistance toward intracellular pathogens either by limiting the availability of iron to the microbes or by supplying iron for the formation of toxic radicals by the Haber–Weiss reaction is supported by recent findings [125] and by the observation of different immune gene expression patterns along with changes in intracellular iron distribution in cells knocked out for NRAMP1 [126–128]. Moreover, NRAMP1 is able to transport Mn(II), Zn(II), and Fe(II) most likely by a proton gradient-dependent mechanism; however, there is discrepancy as to whether the direction of such a transport is from the cytoplasm to the phagosome or vice versa and if the underlying driving force is pH dependent [129, 130]. Interestingly, NRAMP1 expression appears to be regulated by iron perturbations, with increased NRAMP1 mRNA and protein levels being observed in macrophages loaded with iron [131], which would suggest that NRAMP1 and iron metabolism may regulate each other by a feedback loop.

Therefore, alterations in immune function affect iron homeostasis and vice versa [11, 14–16].

Under inflammatory conditions, iron accumulation and retention by macrophages are controlled by cytokines and acute-phase proteins, which affect the different iron accumulation and release pathways of these cells. These immune regulators act at different stages, thus modulating the expression of critical iron genes at the transcriptional and post-transcriptional levels by IRE/IRP-dependent and independent pathways.

The cause-effective role of cytokines for systemic iron regulation was first confirmed by the observation of sustained hypoferremia in mice injected with TNF-α or IL-1 [132]. Hypoferremia was paralleled by hyperferritinemia which was traced back to transcriptional induction of ferritin expression by cytokines in cells of the RES [133, 134]. In addition, the pro-inflammatory cytokines IL-1 and IL-6 regulate ferritin expression at the translational level by a mechanism being independent from the IRP system, namely, via stimulation of a so-called acute-phase box which is located within the 5 untranslated region of ferritin mRNA [134]. However, only limited information was available on how the induction of ferritin synthesis may lead to hypoferremia and increased iron storage within monocytes/macrophages since these pro-inflammatory cytokines downregulated TfR expression [11, 118]. One possibility was referred to stimulation of erythrophagocytosis by macrophages since TNF-α treatment stimulates phagocytosis of sialidase-treated erythrocytes due to enhanced expression of C3bi (CD11b/CD18) receptors. In addition, the enhanced formation of toxic radicals during inflammatory processes will cause damage of erythrocyte membranes which makes them more susceptible for erythrophagocytosis. Accordingly, the application of sublethal dosages of TNF-α to mice resulted in a shortening of erythrocyte half-life and a faster clearance of these cells from the circulation via erythrophagocytosis [135].

Importantly, pro-inflammatory stimuli can enhance the acquisition of non-transferrin-bound iron by macrophages. IFN-γ, LPS, or TNF-α upregulate DMT1 expression and increase iron influx into activated macrophages [41] (Table 11.1).

The intriguing relationship between immunity and iron homeostasis went into a new dimension upon identification of the acute-phase protein hepcidin [88, 89, 136–138]. The observation that hepcidin deficient mice injected with turpentine did not develop hypoferremia suggested that hepcidin may be involved in the pathogenesis of ACD [139, 140]. The underlying mechanisms appear to be the induction of hepcidin expression by LPS and IL-6 while TNF-α blocks hepcidin expression by hepatocytes in vitro [136, 141]. The cause-effective role of IL-6 and hepcidin for the development of hypoferremia was confirmed by experiments demonstrating that injection of IL-6 into volunteers resulted in increased hepcidin expression and induction of hypoferremia within 24 h. In addition,

Table 11.1 Pathways for the regulation of macrophage/monocyte iron homeostasis by cytokines, acute-phase proteins, and radicals on iron homeostasis (modified from [116])

Factors	Mechanisms
TNF-α	Induces ferritin transcription which promotes iron storage within cells of the RES
	Shortage of erythrocyte half-life (TNF-α) and stimulation of erythrophagocytosis
	Inhibits hepcidin formation
IL-1	Stimulates ferritin transcription and translation, the latter by activating an "acute-phase box" within ferritin mRNA
IL-6	Induces ferritin transcription/translation (see above)
	Stimulates hepcidin formation in monocytes/macrophages
	Stimulates CD163 and increases the uptake of hemoglobin–haptoglobin complexes by macrophages
	Induces heme oxygenase and heme degradation
IFN-γ/LPS	Stimulate DMT1 synthesis and increase uptake of ferrous iron into monocytes
	Downregulate FPN-1 expression, which inhibits iron export from macrophages
	Downregulate TfR via induction of a proximal inhibitory signal
	Induce nitric oxide (NO) formation
IL-4, -10, -13	Increase TfR expression and transferrin-mediated iron uptake into inflammatory macrophages
	Stimulate ferritin translation by inactivating IRP and decreasing NO expression
	IL-10 stimulates CD163 and increases the uptake of hemoglobin–haptoglobin complexes by macrophages
	Il-10 stimulates heme oxygenase expression and heme degradation
NO	Stimulates IRP-1 binding affinity, thus blocking ferritin translation and stabilizing TfR mRNA (feedback regulation with iron by affecting NO formation via modulating iNOS expression)
	Modulates IRP-2 expresison and stability
Oxygen radicals	H_2O_2 when applied extracellularly stimulates IRP-1 activity with blocking of ferritin translation and stabilizing TfR mRNA
	Superoxide anion formed intracellularily inhibits IRP binding affinity
Hepcidin	Formed upon stimulation of mice with LPS, IL-6, TGF-ß, and bone morphogenic proteins
	Blocks iron export from macrophages
	Inhibits duodenal iron absorption
	Exerts autocrine regulation of macrophage iron export
α1-AT	Limits iron uptake by erythroid progenitor cells by interfering with TfR

Abbreviations used: *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon, *DMT1* divalent metal transproter-1, *FPN1* ferroportin, *NO* nitric oxide, *H2O2* hydrogen peroxide, *α1-AT* alpha-1 antitrypsin

IL-6 knockout mice which were treated with turpentine in order to induce an inflammatory state did not develop hypoferremia [141]. Part of this may be referred to blockage of iron recirculation from macrophages thanks to the interaction of hepcidin with ferroportin with subsequent reduction of iron export from cells [90, 142, 143]. Accordingly, increased circulating hepcidin concentrations in serum of patients with ACD or a rat model of ACD were associated with decreased ferroportin expression along with reduced duodenal iron absorption and macrophage iron release [143]. However, the induction of hepcidin expression by cytokines is severely impaired in the presence of a concomitant iron deficiency [143–145] pointing to different hierarchies of hepcidin expression by signaling cascades induced by either iron, inflammation, hypoxia, or anemia [146].

In addition, mammalian monocytes and macrophages produce small amounts of hepcidin in response to LPS or IL-6. While the basal expression is relatively low in comparison to the amount of hepcidin produced in the liver, microbial challenges such as group A streptococci and pseudomonas aeruginosa can induce a 20–80 fold increase of hepcidin expression in these cells by a TLR4-dependent pathway [98, 147], while IL-6-mediated induction of hepcidin is mediated via STAT3 activation [101–103]. Interestingly, hepcidin released by macrophages targets ferroportin in an autocrine

fashion, thereby promoting macrophage/monocyte iron accumulation during inflammatory processes [100]. This may be a fast-acting defense mechanism of the innate immune system against invading microbes [5, 12, 148]. Thereby, hepcidin targets ferroportin exposed on the cell surface, resulting in immediate blockage of iron release and thus to a reduced availability of the essential microbial nutrient iron in the circulation [5, 12].

However, effects of hepcidin on iron homeostasis appear to occur fast but only for a limited period of time. This has been confirmed by the observation that injection of LPS resulted in induction of hepcidin and development of hypoferremia, which lasted for several hours. Thereafter, serum iron concentrations returned to normal or were even higher than at baseline [149]. Thus, to ensure a sustained modulation of iron homeostasis under inflammatory conditions, a concerted action of different signals exerted by cytokines, acute-phase proteins, and hormones is mandatory [29].

A central regulatory factor which ensures a sustained iron retention in monocytes/macrophages and a reduced expression of ferroportin during inflammation is IFN-γ.

This Th1-derived cytokine IFN-γ not only induces ferritin transcription but also affects ferritin translation, which is based on activation of IRP binding affinity by the cytokine. This is in part due to stimulation of NO formation by IFN-γ [55, 56, 119] which then activates IRP-1 binding to the ferritin IRE, leading to inhibition of ferritin translation [1, 119]. However, NO exerts divergent effects on IRP-2, leading to either stabilization or degradation of this protein [150–152]. This may relate to contrasting effects of positively or negatively charged NO formulations and is determined by the iron status of the cells [153–158]. In addition, NO can also modulate ferritin expression by an IRP-independent mechanism [159]. Moreover, radicals formed during inflammatory processes such as hydrogen peroxide, superoxide anion, as well as hypoxia can modulate the binding affinities of IRPs to target IREs [57, 59, 152]. Specifically, hydrogen peroxide activates IRP-1 by a rapidly inducible process involving kinase/phosphatase signal transduction pathways resulting in post-transcriptional regulation of IRE-regulated target genes such as TfR and ferritin [1, 57, 160]. IFN-γ treatment of monocytes blocks the uptake of transferrin-bound iron via downregulation of TfR expression [11, 41, 118] which is most likely being due to induction of a proximal inhibitory factor by IFN-γ which inhibits TfR transcription. However, IFN-γ induces DMT1 expression and acts synergistically with LPS in this respect. This leads to stimulation of ferrous iron uptake into these cells and promotes their incorporation into ferritin [41]. At the same time, IFN-γ/LPS induce iron retention in macrophages by downregulating the transcriptional expression of ferroportin, thus blocking iron release from these cells [41, 75] which prolongs the blockage of ferroportin-mediated iron release initiated by monocyte/macrophage-derived hepcidin [100]. One might speculate that these two pathways act in a sequential line. Hepcidin mRNA expression in moncoytes/macrophages peaks 3 h after cytokine/LPS stimulation and then returns to baseline levels [100]. Thus, hepcidin may be part of a fast-acting innate immune effector arm aimed to prevent iron export from macrophages which is of relevance in the setting of microbial invasion, thereby reducing circulating iron concentrations and the availability of this nutrient for pathogens [12, 161]. IFN-γ/LPS then block ferroportin transcription, thus ensuring a prolonged blockage of iron export.

While anti-inflammatory cytokines such as IL-4, IL-10, or IL-13 do not affect the suppression of ferroportin mRNA expression by IFN-γ/LPS [41], treatment of murine macrophages with IL-4 and/or IL-13 prior to stimulation with IFN-γ suppresses NO formation and subsequently IRP activation which concomitantly enhances ferritin translation [162]. This has also been found to be true in human monocytic cells, THP-1, which do not express detectable amounts of iNOS [163]. Conversely, TfR mRNA levels increase following pre-treatment of IFN-γ-stimulated macrophages with the anti-inflammatory cytokines. This may be referred to IL-4/IL-13-mediated antagonization of the inhibitory signal which is induced by IFN-γ and which inhibits TfR expression by an IRP-independent pathway [162]. In addition, IL-10 and IL-6 may affect macrophage iron acquisition by stimulating the expression of hemoglobin scavenger receptor, CD163, thus promoting the uptake of hemoglobin–haptoglobin complexes into monocytic cells [164].

The role of Th2-derived cytokines for the development of hyperferritinemia under chronic inflammatory processes was confirmed by a clinical study in patients with Crohn's disease. Patients receiving therapy with human recombinant IL-10 as part of a placebo-controlled, double-blinded study developed a normocytic anemia which was preceded by a significant increase in serum ferritin levels while reticulocyte counts were not affected as compared to placebo treated controls [163]. Both anemia and hyperferritinemia resolved spontaneously within 2–4 weeks after stopping IL-10 therapy. Thus, Th2-derived cytokines may increase iron uptake via induction of TfR and CD163 but will also promote the iron storage within ferritin by activated macrophages. In addition, IL-10 stimulates HO-1 expression and activity, thus promoting iron re-utilization from phagocytosed erythrocytes, hemoglobin–haptoglobin complexes, and hemopexin-bound heme, respectively [165, 166].

In summary, pro- and anti-inflammatory cytokines and, most importantly, acute-phase proteins cooperate at multiple steps in increasing macrophage iron accumulation via stimulation of various iron acquisition pathways of these cells. At the same time, cytokines and hepcidin inhibit iron export from macrophages by downregulation of ferroportin expression, resulting in iron retention within cells of the RES and an iron-restricted erythropoiesis.

As a consequence of these processes, hypoferremia, hyperferritinema, and an iron-restricted anemia develop, termed as anemia of chronic disease (ACD) or anemia of inflammation [29, 31, 167, 168]. The iron restriction to erythroid cells is further aggravated by the action of other acute-phase proteins, such as alpha-1 antitrypsin (α1-AT), which interferes with cellular iron homeostasis in erythroid progenitor cells by competitively blocking the binding of transferrin to TfR, thus reducing TfR-mediated iron uptake [169, 170]. This points to the additional pathophysiological factors playing a role for the development of anemia of chronic disease, namely, a reduced differentiation and proliferation rate of erythroid progenitor cells and an impaired biological activity of erythropoietin. These latter mechanisms relate to the negative effects of cytokines on erythropoietin formation and activity as well as to the induction of toxic or apoptotic pathways in erythroid progenitors by these immune modulators, which are further aggravated by the reduced availability of iron [29, 133, 142, 171–173].

Thus, under conditions of chronic immune activation, the described diversion of iron occurs, resulting in hypoferremia and hyperferritinemia, the main diagnostic hallmarks for the identification of ACD [31, 168, 174, 175]. ACD is the most frequent anemia in hospitalized patients, occurring frequently in subjects suffering from chronic inflammatory disorders, such as autoimmune diseases, chronic infections, or malignancies.

Although the development of anemia is associated with detrimental effects especially in relation to cardiac function, quality of life, growth, and mental development [176], the underlying hypoferremia and the diversion of iron from the circulation may also harbor some potentially positive effects, especially when cancer or infections underlie chronic immune activation.

First, the withdrawal of iron from the circulation and its storage within the RES reduces the availability of this essential nutrient for microorganisms and tumor cells, which need the metal for their growth and proliferation. Thus, limitation of iron availability is a very effective defense strategy of the body to control the growth of pathogens [5, 12]. Moreover, the expression of iron uptake and acquisition systems of microbes or fungi has been linked to their pathogenicity [177, 178]. Accordingly, limitation of iron availability or blockade of iron uptake or recruiting pathways (siderophore systems) affects microbe survival [6, 177, 179–181]. In line with this, macrophages challenged with bacterial pathogens produce and secrete the protein lipocalin-2 which sequesters iron-loaded bacterial siderophores thus limiting their growth [81, 182–184]. Lipocalin-2 (Lcn2, 24p3) captures iron-laden microbial siderophores, thus interfering with the acquisition of siderophore-bound iron by bacteria [182]. Moreover, Lcn2 delivers siderophore-bound iron to mammalian cells, which are able to import the complex via lipocalin-2 receptor (LcnR, 24p3R) [185]. Most interestingly, recent data provide evidence for the existence of mammalian siderophores which are captured by lipocalin-2, thus indicating that lipocalin-2 may be involved in transcellular and transmembrane iron trafficking in mammals [186, 187].

Nonetheless, macrophages challenged with intracellular pathogens, such as *Salmonella* or *Mycobacteria* spp., induce regulatory pathways to limit the availability of iron for these pathogens, which can in part refer to modulation of iron transport by NRAMP1 or ferroportin [123, 125, 181, 188]. Mutations in NRAMP1 have been associated with a reduction of IFN-γ-triggered immune effector pathways such as release of nitrate from macrophages, a mechanism being indicative for endogenous NO formation [189], while overexpression of wild type but not mutant FPN1 reduces the intracellular growth of *S. typhimurium* in J774 macrophages [190], as modulation of ferroportin expression can also control the growth of other bacteria [161, 191].

Accordingly, the development of anemia limits the oxygen transport capacity in general, but rapidly proliferating tissues are more affected since oxygen is an essential compound for energy metabolism and thus for the proliferation of cells.

Third, the reduction of circulating iron strengthens the immune response directed against invading pathogens and tumor cells by stimulating Th1-mediated immune effector pathways of macrophages and by affecting the differentiation of lymphocyte [192].

Specifically, iron loading of monocytes/macrophages results in an inhibition of IFN-γ-mediated pathways such as formation of TNF-α, reduced expression of MHC class II antigens and ICAM-1, decreased formation of neopterin, and impaired tryptophan degradation via IFN-γ-mediated induction of indoleamine 2,3-dioxygenase [26, 115, 122]. As a consequence of this, iron-loaded macrophages have an impaired potential to kill various bacteria, parasites, and fungi (such as *Legionella*, *Listeria*, *Ehrlichia, Mycobacteria,* Salmonella, Leishamania, Plasmodia, *Candida*, *Mucor*, and also viruses, *in vitro* and *in vivo)* by IFN-γ-mediated pathways [11, 179, 193–195]. Part of this can be attributed to the reduced formation of NO in the presence of iron since NO is an essential effector molecule of macrophages to fight infectious pathogens and tumor cells [196]. Iron blocks the transcription of inducible NO synthase (iNOS or NOSII), the enzyme being responsible for cytokine-inducible high-output formation of NO by hepatocytes or macrophages [197], and by inhibiting the binding affinity of the transcription factors NF-IL6 and of hypoxia-inducible factor-1 to the iNOS promoter, iron impairs iNOS inducibility by cytokines [120, 198, 199]. According to the regulatory feedback loop, NO produced by activated macrophages activates the IRE-binding function of IRP-1, leading to inhibition of ferritin translation [55, 56], thus linking maintenance of iron homeostasis to NO formation for host defense. In line with this, recent data provided evidence that injection of hepcidin into mice increased their survival following endotoxin injection which was paralleled by reduced formation of cytokines such as TNF-α or IL-6[200]. According to the known interaction of iron with pro-inflammatory immune effector pathways [11, 33, 122, 193, 201], one may speculate that this interesting observation may be due to hepcidin-mediated macrophage iron retention with subsequent inhibition of this pro-inflammatory immune effector pathways.

Via its deactivating effect toward IFN-γ function, iron also affects the Th1/Th2 balance, with Th1 effector functions being weakened while Th2-mediated cytokine production, such as IL-4 activity, is increased, a condition which is a rather unfavorable in case of a tumor disease or an infection [193, 202]. Iron overload also has negative effects on neutrophil function as iron therapy of chronic hemodialysis patients impaired the potential of neutrophils to kill bacteria and reduced their capacity to phagocyte foreign particles [203].

Thus, both iron overload and iron deficiency have unfavorable immunological effects in vivo. Accordingly, mice kept on an iron-rich diet presented with a reduced production of IFN- γ as compared to mice fed with a normal diet, while animals receiving an iron-deficient diet presented with a decreased T-cell proliferation [204]. Both iron-overloaded and iron-deficient mice had an increased mortality when receiving a sublethal dose of LPS as compared to animals with a normal iron status. While a minimum amount of iron is required for the generation of toxic oxygen species by phox [205], macrophage iron overload inhibits the transcription of iNOS and thus the generation of NO [120, 199], the formation of TNF-α, and antigen presentation via MHC II [115, 122], as discussed above.

Thus, investigations of the net effects of disturbances of iron homeostasis on immune function and the course of disease being associated with an activated immune system such as infections, autoimmune disorders, or cancer are of great clinical interest.

Several studies investigated the effects of iron homeostasis on the course or incidence of infections [12]. Interestingly, in one study, iron-deficient children had a reduced incidence of infection as compared to children with a balanced iron status [12, 33]. Accordingly, iron deficiency was associated with a higher percentage of CD8+ cells producing IL-6, a more pronounced expression of T-cell activation markers on lymphocytes, and an increased formation of IFN-γ as compared to Malawian children with a normal iron status [33]. In line with this, oral iron supplementation in children was associated with an increased incidence of malaria in endemic areas and increased odds for a complicated clinical course of the infection [206]. Moreover, children suffering from cerebral malaria due to *Plasmodium falciparum* infection, and receiving iron chelator therapy, desferrioxamine, in addition to a standard antimalarial treatment, presented with an improved clinical course as reflected by a shorter duration of coma and fever and an increased clearance of *Plasmodia* from the circulation [207]. Children receiving desferrioxamine had higher levels of Th1 cytokines and NO, while serum concentration of Th2 cytokines (IL-4) tended to be lower [203, 208] which indicated that withdrawal of iron increases Th1-mediated immune function also in vivo [194]. However, no survival benefit was obtained which may be referred to the poor intracellular penetration of the drug [209].

In Africa, an endemic form of secondary iron overload traced back to the consumption of traditional iron-containing beer linked to a mutation in the ferroportin gene [210] is associated with an increased incidence and mortality from tuberculosis [211]. These data are supported by in vitro findings showing that changes in intramacrophage iron availability stimulate the proliferation of mycobacteria and weaken antimycobacterial defense mechanisms of macrophages [188, 212, 213].

Other infections ranging from bacterial, viral, fungal to parasitic disease where iron overload is associated with a unfavorable course of the infection and/or an impaired immune response have been well summarized in an excellent review [12].

Subsequent studies of macrophage/monocyte iron metabolism under iron-deficient/overload and inflammatory conditions will further extend our knowledge of body iron homeostasis, the impact of erythroid factors on iron recruitment and recirculation, and toward the regulatory pathways in host–pathogen interactions. This may hold the key for future therapeutic developments for the treatment of iron metabolism diseases as well as for anemia of chronic disease. Notably, monocyte/macrophage iron retention seen in inflammation can most effectively be overcome by correcting the underlying disease. However, being aware of the multiple pathways and regulatory factors which control iron homeostasis during inflammation, it is questionable whether modification of one single pathway, e.g., antagonizing of the hepcidin/ferroportin interaction, may have a significant therapeutic effect and whether or not this will improve iron recirculation from macrophages. In addition, we have to acquire new information on adaptive changes of iron homeostasis during therapeutic procedures (e.g., iron supplementation therapy) as well as on the consequences of such interventions on the course of the underlying disease, both of which will be helpful and necessary to optimize therapeutic approaches for correction of disturbances in body iron homeostasis during inflammation.

Acknowledgment Grant support by the Austrian Research Funds-FWF P-19664 is gratefully acknowledged.

References

1. Hentze MW, Muckenthaler MU, Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. Cell. 2004;117:285–97.
2. Nairz M, Weiss G. Molecular and clinical aspects of iron homeostasis: from anemia to hemochromatosis. Wien Klin Wochenschr. 2006;118:442–62.

3. Andrews NC, Schmidt PJ. Iron homeostasis. Annu Rev Physiol. 2007;69:69–85.
4. Rosen GM, Pou S, Ramos CL, Cohen MS, Britigan BE. Free radicals and phagocytic cells. FASEB J. 1995;9:200–9.
5. Schaible UE, Kaufmann SH. Iron and microbial infection. Nat Rev Microbiol. 2004;2:946–53.
6. Hantke K. Iron and metal regulation in bacteria. Curr Opin Microbiol. 2001;4:172–7.
7. Winkelmann G. Microbial siderophore-mediated transport. Biochem Soc Trans. 2002;30:691–6.
8. Delaby C, Pilard N, Puy H, Canonne-Hergaux F. Sequential regulation of ferroportin expression after erythrophagocytosis in murine macrophages: early mRNA induction by haem, followed by iron-dependent protein expression. Biochem J. 2008;411:123–31.
9. Knutson MD, Oukka M, Koss LM, Aydemir F, Wessling-Resnick M. Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. Proc Natl Acad Sci USA. 2005;102:1324–8.
10. Soe-Lin S, Apte SS, Andriopoulos Jr B, Andrews MC, Schranzhofer M, Kahawita T, et al. Nramp1 promotes efficient macrophage recycling of iron following erythrophagocytosis in vivo. Proc Natl Acad Sci USA. 2009;106:5960–5.
11. Weiss G. Iron and immunity: a double-edged sword. Eur J Clin Invest. 2002;32:70–8.
12. Weinberg ED. Iron loading and disease surveillance. Emerg Infect Dis. 1999;5:346–52.
13. de Sousa M, Reimao R, Porto G, Grady RW, Hilgartner MW, Giardina P. Iron and lymphocytes: reciprocal regulatory interactions. Curr Stud Hematol Blood Transfus. 1991;58:171–7.
14. Porto G, Reimao R, Goncalves C, Vicente C, Justica B, de Sousa M. Haemochromatosis as a window into the study of the immunological system: a novel correlation between CD8+ lymphocytes and iron overload. Eur J Haematol. 1994;52:283–90.
15. Meyer PN, Gerhard GS, Yoshida Y, Yoshida M, Chorney KA, Beard J, et al. Hemochromatosis protein (HFE) and tumor necrosis factor receptor 2 (TNFR2) influence tissue iron levels: elements of a common gut pathway? Blood Cells Mol Dis. 2002;29:274–85.
16. Roy CN, Andrews NC. Recent advances in disorders of iron metabolism: mutations, mechanisms and modifiers. Hum Mol Genet. 2001;10:2181–6.
17. Forbes JR, Gros P. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. Trends Microbiol. 2001;9:397–403.
18. Blackwell JM, Searle S, Goswami T, Miller EN. Understanding the multiple functions of Nramp1. Microbes Infect. 2000;2:317–21.
19. Latunde-Dada GO, Young SP. Iron deficiency and immune responses. Scand J Immunol Suppl. 1992;11:207–9.
20. Brock JH. The effect of iron and transferrin on the response of serum-free cultures of mouse lymphocytes to concanavalin A and lipopolysaccharide. Immunology. 1981;43:387–92.
21. Porto G, De Sousa M. Iron overload and immunity. World J Gastroenterol. 2007;13:4707–15.
22. Seligman PA, Kovar J, Gelfand EW. Lymphocyte proliferation is controlled by both iron availability and regulation of iron uptake pathways. Pathobiology. 1992;60:19–26.
23. Thorson JA, Smith KM, Gomez F, Naumann PW, Kemp JD. Role of iron in T cell activation: TH1 clones differ from TH2 clones in their sensitivity to inhibition of DNA synthesis caused by IgG Mabs against the transferrin receptor and the iron chelator deferoxamine. Cell Immunol. 1991;134:126–37.
24. Farrar JD, Asnagli H, Murphy KM. T helper subset development: roles of instruction, selection, and transcription. J Clin Invest. 2002;109:431–5.
25. Awasthi A, Kuchroo VK. Th17 cells: from precursors to players in inflammation and infection. Int Immunol. 2009;21:489–98.
26. Weiss G, Fuchs D, Hausen A, Reibnegger G, Werner ER, Werner-Felmayer G, et al. Iron modulates interferon-gamma effects in the human myelomonocytic cell line THP-1. Exp Hematol. 1992;20:605–10.
27. Frazer DM, Anderson GJ. The orchestration of body iron intake: how and where do enterocytes receive their cues? Blood Cells Mol Dis. 2003;30:288–97.
28. Brissot P, Troadec MB, Loreal O. The clinical relevance of new insights in iron transport and metabolism. Curr Hematol Rep. 2004;3:107–15.
29. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005;352:1011–23.
30. Spivak JL. Iron and the anemia of chronic disease. Oncology (Williston Park). 2002;16:25–33.
31. Andrews NC. Anemia of inflammation: the cytokine-hepcidin link. J Clin Invest. 2004;113:1251–3.
32. Weiss G. Iron acquisiton by the reticuloendothelial system. In: Templeton D, editor. Molecular and cellular iron transport. New York: Marcel Dekker Inc.; 2002. p. 467–88.
33. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr. 2001;131:616S–35.
34. Fahmy M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. Biochem J. 1993;296:175–81.
35. Kaplan J. Mechanisms of cellular iron acquisition: another iron in the fire. Cell. 2002;111:603–6.

36. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997;388:482–8.
37. Fleming MD, Trenor 3rd CC, Su MA, Foernzler D, Beier DR, Dietrich WF, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet. 1997;16:383–6.
38. Andrews NC. The iron transporter DMT1. Int J Biochem Cell Biol. 1999;31:991–4.
39. McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291:1755–9.
40. Olakanmi O, Stokes JB, Britigan BE. Acquisition of iron bound to low molecular weight chelates by human monocyte-derived macrophages. J Immunol. 1994;153:2691–703.
41. Ludwiczek S, Aigner E, Theurl I, Weiss G. Cytokine-mediated regulation of iron transport in human monocytic cells. Blood. 2003;101:4148–54.
42. Moss D, Fargion S, Fracanzani AL, Levi S, Cappellini MD, Arosio P, et al. Functional roles of the ferritin receptors of human liver, hepatoma, lymphoid and erythroid cells. J Inorg Biochem. 1992;47:219–27.
43. Ramm GA, Ruddell RG, Subramaniam VN. Identification of ferritin receptors: their role in iron homeostasis, hepatic injury, and inflammation. Gastroenterology. 2009;137:1849–51.
44. Li JY, Paragas N, Ned RM, Qiu A, Viltard M, Leete T, et al. Scara5 is a ferritin receptor mediating non-transferrin iron delivery. Dev Cell. 2009;16:35–46.
45. Cohen LA, Gutierrez L, Weiss A, Leichtmann-Bardoogo Y, Zhang DL, Crooks D, et al. Serum ferritin is derived primarily from macrophages through a non-classical secretory pathway. Blood. 2010;116:1574–84.
46. Fisher J, Devraj K, Ingram J, Slagle-Webb B, Madhankumar AB, Liu X, et al. Ferritin: a novel mechanism for delivery of iron to the brain and other organs. Am J Physiol Cell Physiol. 2007;293:C641–9.
47. Brock JH. The physiology of lactoferrin. Biochem Cell Biol. 2002;80:1–6.
48. Crouch SP, Slater KJ, Fletcher J. Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin. Blood. 1992;80:235–40.
49. Schaer DJ, Schaer CA, Buehler PW, Boykins RA, Schoedon G, Alayash AI, et al. CD163 is the macrophage scavenger receptor for native and chemically modified hemoglobins in the absence of haptoglobin. Blood. 2006;107:373–80.
50. Hvidberg V, Maniecki MB, Jacobsen C, Hojrup P, Moller HJ, Moestrup SK. Identification of the receptor scavenging hemopexin-heme complexes. Blood. 2005;106:2572–9.
51. Kakhlon O, Cabantchik ZI. The labile iron pool: characterization, measurement, and participation in cellular processes(1). Free Radic Biol Med. 2002;33:1037–46.
52. Eisenstein RS, Ross KL. Novel roles for iron regulatory proteins in the adaptive response to iron deficiency. J Nutr. 2003;133:1510S–6.
53. Rouault T, Klausner R. Regulation of iron metabolism in eukaryotes. Curr Top Cell Regul. 1997;35:1–19.
54. Muckenthaler M, Gray NK, Hentze MW. IRP-1 binding to ferritin mRNA prevents the recruitment of the small ribosomal subunit by the cap-binding complex eIF4F. Mol Cell. 1998;2:383–8.
55. Weiss G, Goossen B, Doppler W, Fuchs D, Pantopoulos K, Werner-Felmayer G, et al. Translational regulation via iron-responsive elements by the nitric oxide/NO-synthase pathway. EMBO J. 1993;12:3651–7.
56. Drapier JC, Hirling H, Wietzerbin J, Kaldy P, Kuhn LC. Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. EMBO J. 1993;12:3643–9.
57. Pantopoulos K, Hentze MW. Rapid responses to oxidative stress mediated by iron regulatory protein. EMBO J. 1995;14:2917–24.
58. Schneider BD, Leibold EA. Effects of iron regulatory protein regulation on iron homeostasis during hypoxia. Blood. 2003;102:3404–11.
59. Cairo G, Recalcati S, Pietrangelo A, Minotti G. The iron regulatory proteins: targets and modulators of free radical reactions and oxidative damage. Free Radic Biol Med. 2002;32:1237–43.
60. Leipuviene R, Theil EC. The family of iron responsive RNA structures regulated by changes in cellular iron and oxygen. Cell Mol Life Sci. 2007;64:2945–55.
61. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13:399–408.
62. Anderson GJ, Powell LW. HFE and non-HFE hemochromatosis. Int J Hematol. 2002;76:203–7.
63. Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med. 2004;350:2383–97.
64. Rothenberg BE, Voland JR. beta2 knockout mice develop parenchymal iron overload: a putative role for class I genes of the major histocompatibility complex in iron metabolism. Proc Natl Acad Sci USA. 1996;93:1529–34.
65. Santos M, Schilham MW, Rademakers LH, Marx JJ, de Sousa M, Clevers H. Defective iron homeostasis in beta 2-microglobulin knockout mice recapitulates hereditary hemochromatosis in man. J Exp Med. 1996;184:1975–85.
66. Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell. 1998;93:111–23.

67. Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, et al. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci USA. 1997;94:13198–202.
68. Ludwiczek S, Theurl I, Artner-Dworzak E, Chorney M, Weiss G. Duodenal HFE expression and hepcidin levels determine body iron homeostasis: modulation by genetic diversity and dietary iron availability. J Mol Med. 2004;82:373–82.
69. Fleming RE, Sly WS. Mechanisms of iron accumulation in hereditary hemochromatosis. Annu Rev Physiol. 2002;64:663–80.
70. Ten Elshof AE, Brittenham GM, Chorney KA, Page MJ, Gerhard G, Cable EE, et al. Gamma delta intraepithelial lymphocytes drive tumor necrosis factor-alpha responsiveness to intestinal iron challenge: relevance to hemochromatosis. Immunol Rev. 1999;167:223–32.
71. Drakesmith H, Sweetland E, Schimanski L, Edwards J, Cowley D, Ashraf M, et al. The hemochromatosis protein HFE inhibits iron export from macrophages. Proc Natl Acad Sci USA. 2002;99:15602–7.
72. Montosi G, Paglia P, Garuti C, Guzman CA, Bastin JM, Colombo MP, et al. Wild-type HFE protein normalizes transferrin iron accumulation in macrophages from subjects with hereditary hemochromatosis. Blood. 2000;96:1125–9.
73. Pietrangelo A. Physiology of iron transport and the hemochromatosis gene. Am J Physiol Gastrointest Liver Physiol. 2002;282:G403–14.
74. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell. 2000;5:299–309.
75. Yang F, Liu XB, Quinones M, Melby PC, Ghio A, Haile DJ. Regulation of reticuloendothelial iron transporter MTP1 (Slc11a3) by inflammation. J Biol Chem. 2002;277:39786–91.
76. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature. 2000;403:776–81.
77. Pietrangelo A. Hemochromatosis: an endocrine liver disease. Hepatology. 2007;46:1291–301.
78. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, et al. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat Genet. 1999;21:195–9.
79. Cairo G, Recalcati S, Montosi G, Castrusini E, Conte D, Pietrangelo A. Inappropriately high iron regulatory protein activity in monocytes of patients with genetic hemochromatosis. Blood. 1997;89:2546–53.
80. Ludwiczek S, Theurl I, Bahram S, Schumann K, Weiss G. Regulatory networks for the control of body iron homeostasis and their dysregulation in HFE mediated hemochromatosis. J Cell Physiol. 2005;204:489–99.
81. Nairz M, Theurl I, Schroll A, Theurl M, Fritsche G, Lindner E, et al. Absence of functional Hfe protects mice from invasive Salmonella enterica serovar Typhimurium infection via induction of lipocalin-2. Blood. 2009;114:3642–51.
82. Moura E, Noordermeer MA, Verhoeven N, Verheul AF, Marx JJ. Iron release from human monocytes after erythrophagocytosis in vitro: an investigation in normal subjects and hereditary hemochromatosis patients. Blood. 1998;92:2511–9.
83. Kitagawa S, Yuo A, Yagisawa M, Azuma E, Yoshida M, Furukawa Y, et al. Activation of human monocyte functions by tumor necrosis factor: rapid priming for enhanced release of superoxide and erythrophagocytosis, but no direct triggering of superoxide release. Exp Hematol. 1996;24:559–67.
84. Zuckerbraun BS, Billiar TR. Heme oxygenase-1: a cellular Hercules. Hepatology. 2003;37:742–4.
85. Knutson MD, Vafa MR, Haile DJ, Wessling-Resnick M. Iron loading and erythrophagocytosis increase ferroportin 1 (FPN1) expression in J774 macrophages. Blood. 2003;102:4191–7.
86. Delaby C, Pilard N, Hetet G, Driss F, Grandchamp B, Beaumont C, et al. A physiological model to study iron recycling in macrophages. Exp Cell Res. 2005;310:43–53.
87. Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol. 2006;290:G199–203.
88. Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, et al. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc Natl Acad Sci USA. 2002;99:4596–601.
89. Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem. 2001;276:7811–9.
90. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306:2090–3.
91. De Domenico I, Ward DM, Langelier C, Vaughn MB, Nemeth E, Sundquist WI, et al. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell. 2007;18:2569–78.
92. Delaby C, Pilard N, Goncalves AS, Beaumont C, Canonne-Hergaux F. Presence of the iron exporter ferroportin at the plasma membrane of macrophages is enhanced by iron loading and down-regulated by hepcidin. Blood. 2005;106:3979–84.
93. Soe-Lin S, Apte SS, Mikhael MR, Kayembe LK, Nie G, Ponka P. Both Nramp1 and DMT1 are necessary for efficient macrophage iron recycling. Exp Hematol. 2010;38:609–17.

94. Keel SB, Doty RT, Yang Z, Quigley JG, Chen J, Knoblaugh S, et al. A heme export protein is required for red blood cell differentiation and iron homeostasis. Science. 2008;319:825–8.
95. De Domenico I, Ward DM, di Patti MC, Jeong SY, David S, Musci G, et al. Ferroxidase activity is required for the stability of cell surface ferroportin in cells expressing GPI-ceruloplasmin. EMBO J. 2007;26:2823–31.
96. Wang F, Paradkar PN, Custodio AO, McVey Ward D, Fleming MD, Campagna D, et al. Genetic variation in Mon1a affects protein trafficking and modifies macrophage iron loading in mice. Nat Genet. 2007;39:1025–32.
97. Zhang DL, Hughes RM, Ollivierre-Wilson H, Ghosh MC, Rouault TA. A ferroportin transcript that lacks an iron-responsive element enables duodenal and erythroid precursor cells to evade translational repression. Cell Metab. 2009;9:461–73.
98. Peyssonnaux C, Zinkernagel AS, Datta V, Lauth X, Johnson RS, Nizet V. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood. 2006;107:3727–32.
99. Nguyen NB, Callaghan KD, Ghio AJ, Haile DJ, Yang F. Hepcidin expression and iron transport in alveolar macrophages. Am J Physiol Lung Cell Mol Physiol. 2006;291:L417–25.
100. Theurl I, Theurl M, Seifert M, Mair S, Nairz M, Rumpold H, et al. Autocrine formation of hepcidin induces iron retention in human monocytes. Blood. 2008;111:2392–9.
101. Pietrangelo A, Dierssen U, Valli L, Garuti C, Rump A, Corradini E, et al. STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. Gastroenterology. 2007;132:294–300.
102. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood. 2007;109:353–8.
103. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood. 2006;108:3204–9.
104. Fraenkel PG, Traver D, Donovan A, Zahrieh D, Zon LI. Ferroportin1 is required for normal iron cycling in zebrafish. J Clin Invest. 2005;115:1532–41.
105. Liu S, Suragani RN, Wang F, Han A, Zhao W, Andrews NC, et al. The function of heme-regulated eIF2alpha kinase in murine iron homeostasis and macrophage maturation. J Clin Invest. 2007;117:3296–305.
106. Chen JJ. Regulation of protein synthesis by the heme-regulated eIF2alpha kinase: relevance to anemias. Blood. 2007;109:2693–9.
107. Angelillo-Scherrer A, Burnier L, Lambrechts D, Fish RJ, Tjwa M, Plaisance S, et al. Role of Gas6 in erythropoiesis and anemia in mice. J Clin Invest. 2008;118:583–96.
108. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood. 2003;101:2461–3.
109. Finch C. Regulators of iron balance in humans. Blood. 1994;84:1697–702.
110. Nicolas G, Viatte L, Bennoun M, Beaumont C, Kahn A, Vaulont S. Hepcidin, a new iron regulatory peptide. Blood Cells Mol Dis. 2002;29:327–35.
111. Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med. 2007;13:1096–101.
112. Theurl I, Finkenstedt A, Schroll A, Nairz M, Sonnweber T, Bellmann-Weiler R, et al. Growth differentiation factor 15 in anaemia of chronic disease, iron deficiency anaemia and mixed type anaemia. Br J Haematol. 2010;148:449–55.
113. Knutson M, Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. Crit Rev Biochem Mol Biol. 2003;38:61–88.
114. Mastroeni P, Vazquez-Torres A, Fang FC, Xu Y, Khan S, Hormaeche CE, et al. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival in vivo. J Exp Med. 2000;192:237–48.
115. Recalcati S, Pometta R, Levi S, Conte D, Cairo G. Response of monocyte iron regulatory protein activity to inflammation: abnormal behavior in genetic hemochromatosis. Blood. 1998;91:2565–72.
116. Weiss G. Modification of iron regulation by the inflammatory response. Best Pract Res Clin Haematol. 2005;18:183–201.
117. Nairz M, Fritsche G, Brunner P, Talasz H, Hantke K, Weiss G. Interferon-gamma limits the availability of iron for intramacrophage Salmonella typhimurium. Eur J Immunol. 2008;38:1923–36.
118. Byrd TF, Horwitz MA. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. J Clin Invest. 1993;91:969–76.
119. Mulero V, Brock JH. Regulation of iron metabolism in murine J774 macrophages: role of nitric oxide-dependent and -independent pathways following activation with gamma interferon and lipopolysaccharide. Blood. 1999;94:2383–9.
120. Weiss G, Werner-Felmayer G, Werner ER, Grunewald K, Wachter H, Hentze MW. Iron regulates nitric oxide synthase activity by controlling nuclear transcription. J Exp Med. 1994;180:969–76.
121. Recalcati S, Locati M, Marini A, Santambrogio P, Zaninotto F, De Pizzol M, et al. Differential regulation of iron homeostasis during human macrophage polarized activation. Eur J Immunol. 2010;40:824–35.

122. Oexle H, Kaser A, Most J, Bellmann-Weiler R, Werner ER, Werner-Felmayer G, et al. Pathways for the regulation of interferon-gamma-inducible genes by iron in human monocytic cells. J Leukoc Biol. 2003;74:287–94.
123. Kuhn DE, Lafuse WP, Zwilling BS. Iron transport into mycobacterium avium-containing phagosomes from an Nramp1(Gly169)-transfected RAW264.7 macrophage cell line. J Leukoc Biol. 2001;69:43–9.
124. Fritsche G, Nairz M, Theurl I, Mair S, Bellmann-Weiler R, Barton HC, et al. Modulation of macrophage iron transport by Nramp1 (Slc11a1). Immunobiology. 2007;212:751–7.
125. Nairz M, Fritsche G, Crouch ML, Barton HC, Fang FC, Weiss G. Slc11a1 limits intracellular growth of Salmonella enterica sv. Typhimurium by promoting macrophage immune effector functions and impairing bacterial iron acquisition. Cell Microbiol. 2009;11:1365–81.
126. Alter-Koltunoff M, Ehrlich S, Dror N, Azriel A, Eilers M, Hauser H, et al. Nramp1-mediated innate resistance to intraphagosomal pathogens is regulated by IRF-8, PU.1, and Miz-1. J Biol Chem. 2003;278:44025–32.
127. Fritsche G, Dlaska M, Barton H, Theurl I, Garimorth K, Weiss G. Nramp1 functionality increases inducible nitric oxide synthase transcription via stimulation of IFN regulatory factor 1 expression. J Immunol. 2003;171:1994–8.
128. Mulero V, Searle S, Blackwell JM, Brock JH. Solute carrier 11a1 (Slc11a1; formerly Nramp1) regulates metabolism and release of iron acquired by phagocytic, but not transferrin-receptor-mediated, iron uptake. Biochem J. 2002;363:89–94.
129. Canonne-Hergaux F, Gruenheid S, Govoni G, Gros P. The Nramp1 protein and its role in resistance to infection and macrophage function. Proc Assoc Am Physicians. 1999;111:283–9.
130. Jabado N, Jankowski A, Dougaparsad S, Picard V, Grinstein S, Gros P. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. J Exp Med. 2000;192:1237–48.
131. Baker ST, Barton CH, Biggs TE. A negative autoregulatory link between Nramp1 function and expression. J Leukoc Biol. 2000;67:501–7.
132. Alvarez-Hernandez X, Liceaga J, McKay IC, Brock JH. Induction of hypoferremia and modulation of macrophage iron metabolism by tumor necrosis factor. Lab Invest. 1989;61:319–22.
133. Konijn AM, Carmel N, Levy R, Hershko C. Ferritin synthesis in inflammation. II. Mechanism of increased ferritin synthesis. Br J Haematol. 1981;49:361–70.
134. Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood. 2002;99:3505–16.
135. Moldawer LL, Marano MA, Wei H, Fong Y, Silen ML, Kuo G, et al. Cachectin/tumor necrosis factor-alpha alters red blood cell kinetics and induces anemia in vivo. FASEB J. 1989;3:1637–43.
136. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood. 2003;102:783–8.
137. Loreal O, Brissot P. Hepcidin: small molecule, large future. Rev Med Interne. 2003;24:213–5.
138. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood. 2002;100:3776–81.
139. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest. 2002;110:1037–44.
140. Fleming RE, Sly WS. Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. Proc Natl Acad Sci USA. 2001;98:8160–2.
141. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004;113:1271–6.
142. Theurl I, Mattle V, Seifert M, Mariani M, Marth C, Weiss G. Dysregulated monocyte iron homeostasis and erythropoietin formation in patients with anemia of chronic disease. Blood. 2006;107:4142–8.
143. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood. 2009;113:5277–86.
144. Darshan D, Frazer DM, Wilkins SJ, Anderson GJ. Severe iron deficiency blunts the response of the iron regulatory gene Hamp and proinflammatory cytokines to lipopolysaccharide. Haematologica. 2010;95:1660–7.
145. Lasocki S, Millot S, Andrieu V, Letteron P, Pilard N, Muzeau F, et al. Phlebotomies or erythropoietin injections allow mobilization of iron stores in a mouse model mimicking intensive care anemia. Crit Care Med. 2008;36:2388–94.
146. Camaschella C, Silvestri L. New and old players in the hepcidin pathway. Haematologica. 2008;93:1441–4.
147. Liu XB, Nguyen NB, Marquess KD, Yang F, Haile DJ. Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. Blood Cells Mol Dis. 2005;35:47–56.
148. Weiss G. Iron, infection and anemia – a classical triad. Wien Klin Wochenschr. 2002;114:357–67.
149. Constante M, Wang D, Raymond VA, Bilodeau M, Santos MM. Repression of repulsive guidance molecule C during inflammation is independent of Hfe and involves tumor necrosis factor-alpha. Am J Pathol. 2007;170:497–504.
150. Bouton C, Drapier JC. Iron regulatory proteins as NO signal transducers. Sci STKE. 2003;2003:pe17.

151. Bouton C, Oliveira L, Drapier JC. Converse modulation of IRP1 and IRP2 by immunological stimuli in murine RAW 264.7 macrophages. J Biol Chem. 1998;273:9403–8.
152. Hanson ES, Leibold EA. Regulation of the iron regulatory proteins by reactive nitrogen and oxygen species. Gene Expr. 1999;7:367–76.
153. Kim S, Wing SS, Ponka P. S-nitrosylation of IRP2 regulates its stability via the ubiquitin-proteasome pathway. Mol Cell Biol. 2004;24:330–7.
154. Caltagirone A, Weiss G, Pantopoulos K. Modulation of cellular iron metabolism by hydrogen peroxide. Effects of H2O2 on the expression and function of iron-responsive element-containing mRNAs in B6 fibroblasts. J Biol Chem. 2001;276:19738–45.
155. Kim S, Ponka P. Nitric oxide-mediated modulation of iron regulatory proteins: implication for cellular iron homeostasis. Blood Cells Mol Dis. 2002;29:400–10.
156. Hanson ES, Rawlins ML, Leibold EA. Oxygen and iron regulation of iron regulatory protein 2. J Biol Chem. 2003;278:40337–42.
157. Wang J, Chen G, Pantopoulos K. Nitric oxide inhibits the degradation of IRP2. Mol Cell Biol. 2005;25:1347–53.
158. Wang J, Fillebeen C, Chen G, Andriopoulos B, Pantopoulos K. Sodium nitroprusside promotes IRP2 degradation via an increase in intracellular iron and in the absence of S nitrosylation at C178. Mol Cell Biol. 2006;26:1948–54.
159. Mikhael M, Kim SF, Schranzhofer M, Soe-Lin S, Sheftel AD, Mullner EW, et al. Iron regulatory protein-independent regulation of ferritin synthesis by nitrogen monoxide. FEBS J. 2006;273:3828–36.
160. Tacchini L, Gammella E, De Ponti C, Recalcati S, Cairo G. Role of HIF-1 and NF-kappaB transcription factors in the modulation of transferrin receptor by inflammatory and anti-inflammatory signals. J Biol Chem. 2008;283:20674–86.
161. Sow FB, Florence WC, Satoskar AR, Schlesinger LS, Zwilling BS, Lafuse WP. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. J Leukoc Biol. 2007;82:934–45.
162. Weiss G, Bogdan C, Hentze MW. Pathways for the regulation of macrophage iron metabolism by the anti-inflammatory cytokines IL-4 and IL-13. J Immunol. 1997;158:420–5.
163. Tilg H, Ulmer H, Kaser A, Weiss G. Role of IL-10 for induction of anemia during inflammation. J Immunol. 2002;169:2204–9.
164. Graversen JH, Madsen M, Moestrup SK. CD163: a signal receptor scavenging haptoglobin–hemoglobin complexes from plasma. Int J Biochem Cell Biol. 2002;34:309–14.
165. Bach FH. Heme oxygenase-1: a therapeutic amplification funnel. FASEB J. 2005;19:1216–9.
166. Lee TS, Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med. 2002;8:240–6.
167. Cartwright GE. The anemia of chronic disorders. Semin Hematol. 1966;3:351–75.
168. Means Jr RT. Recent developments in the anemia of chronic disease. Curr Hematol Rep. 2003;2:116–21.
169. Graziadei I, Gaggl S, Kaserbacher R, Braunsteiner H, Vogel W. The acute-phase protein alpha 1-antitrypsin inhibits growth and proliferation of human early erythroid progenitor cells (burst-forming units-erythroid) and of human erythroleukemic cells (K562) in vitro by interfering with transferrin iron uptake. Blood. 1994;83:260–8.
170. Weiss G, Graziadel I, Urbanek M, Grunewald K, Vogel W. Divergent effects of alpha 1-antitrypsin on the regulation of iron metabolism in human erythroleukaemic (K562) and myelomonocytic (THP-1) cells. Biochem J. 1996;319:897–902.
171. Jelkmann W. Proinflammatory cytokines lowering erythropoietin production. J Interferon Cytokine Res. 1998;18:555–9.
172. Means Jr RT, Krantz SB. Inhibition of human erythroid colony-forming units by gamma interferon can be corrected by recombinant human erythropoietin. Blood. 1991;78:2564–7.
173. Goodnough LT. Red cell growth factors in patients with chronic anemias. Curr Hematol Rep. 2002;1:119–23.
174. Matzner Y, Levy S, Grossowicz N, Izak G, Hershko C. Prevalence and causes of anemia in elderly hospitalized patients. Gerontology. 1979;25:113–9.
175. Weiss G. Pathogenesis and treatment of anaemia of chronic disease. Blood Rev. 2002;16:87–96.
176. Collins AJ, Ma JZ, Ebben J. Impact of hematocrit on morbidity and mortality. Semin Nephrol. 2000;20:345–9.
177. Schrettl M, Bignell E, Kragl C, Joechl C, Rogers T, Arst Jr HN, et al. Siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigatus virulence. J Exp Med. 2004;200:1213–9.
178. Boyer E, Bergevin I, Malo D, Gros P, Cellier MF. Acquisition of Mn(II) in addition to Fe(II) is required for full virulence of Salmonella enterica serovar Typhimurium. Infect Immun. 2002;70:6032–42.
179. Kontoghiorghes GJ, Weinberg ED. Iron: mammalian defense systems, mechanisms of disease, and chelation therapy approaches. Blood Rev. 1995;9:33–45.
180. Walter T, Olivares M, Pizarro F, Munoz C. Iron, anemia, and infection. Nutr Rev. 1997;55:111–24.

181. Nairz M, Theurl I, Ludwiczek S, Theurl M, Mair SM, Fritsche G, et al. The co-ordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular Salmonella typhimurium. Cell Microbiol. 2007;9:2126–40.
182. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004;432:917–21.
183. Fluckinger M, Haas H, Merschak P, Glasgow BJ, Redl B. Human tear lipocalin exhibits antimicrobial activity by scavenging microbial siderophores. Antimicrob Agents Chemother. 2004;48:3367–72.
184. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to *Escherichia coli* infection but not to ischemia–reperfusion injury. Proc Natl Acad Sci USA. 2006;103:1834–9.
185. Devireddy LR, Gazin C, Zhu X, Green MR. A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell. 2005;123:1293–305.
186. Bao G, Clifton M, Hoette TM, Mori K, Deng SX, Qiu A, et al. Iron traffics in circulation bound to a siderocalin (Ngal)-catechol complex. Nat Chem Biol. 2010;6:602–9.
187. Devireddy LR, Hart DO, Goetz DH, Green MR. A mammalian siderophore synthesized by an enzyme with a bacterial homolog involved in enterobactin production. Cell. 2010;141:1006–17.
188. Olakanmi O, Schlesinger LS, Ahmed A, Britigan BE. Intraphagosomal Mycobacterium tuberculosis acquires iron from both extracellular transferrin and intracellular iron pools. Impact of interferon-gamma and hemochromatosis. J Biol Chem. 2002;277:49727–34.
189. Barton CH, Whitehead SH, Blackwell JM. Nramp transfection transfers Ity/Lsh/Bcg-related pleiotropic effects on macrophage activation: influence on oxidative burst and nitric oxide pathways. Mol Med. 1995;1:267–79.
190. Chlosta S, Fishman DS, Harrington L, Johnson EE, Knutson MD, Wessling-Resnick M, et al. The iron efflux protein ferroportin regulates the intracellular growth of Salmonella enterica. Infect Immun. 2006;74:3065–7.
191. Paradkar P, De Domenico I, Durchfort N, Zohn I, Kaplan J, Ward DM. Iron-depletion limits intracellular bacterial growth in macrophages. Blood. 2008;112:866–74.
192. Weiss G, Wachter H, Fuchs D. Linkage of cell-mediated immunity to iron metabolism. Immunol Today. 1995;16:495–500.
193. Mencacci A, Cenci E, Boelaert JR, Bucci P, Mosci P, Fe d'Ostiani C, et al. Iron overload alters innate and T helper cell responses to Candida albicans in mice. J Infect Dis. 1997;175:1467–76.
194. Fritsche G, Larcher C, Schennach H, Weiss G. Regulatory interactions between iron and nitric oxide metabolism for immune defense against *Plasmodium falciparum* infection. J Infect Dis. 2001;183:1388–94.
195. Boelaert JR, Vandecasteele SJ, Appelberg R, Gordeuk VR. The effect of the host's iron status on tuberculosis. J Infect Dis. 2007;195:1745–53.
196. Bogdan C. Nitric oxide and the immune response. Nat Immunol. 2001;2:907–16.
197. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol. 1997;15:323–50.
198. Melillo G, Taylor LS, Brooks A, Musso T, Cox GW, Varesio L. Functional requirement of the hypoxia-responsive element in the activation of the inducible nitric oxide synthase promoter by the iron chelator desferrioxamine. J Biol Chem. 1997;272:12236–43.
199. Dlaska M, Weiss G. Central role of transcription factor NF-IL6 for cytokine and iron-mediated regulation of murine inducible nitric oxide synthase expression. J Immunol. 1999;162:6171–7.
200. De Domenico I, Zhang TY, Koening CL, Branch RW, London N, Lo E, et al. Hepcidin mediates transcriptional changes that modulate acute cytokine-induced inflammatory responses in mice. J Clin Invest. 2010;120:2395–405.
201. Wessling-Resnick M. Iron homeostasis and the inflammatory response. Annu Rev Nutr. 2010;30:105–22.
202. Weiss G, Thuma PE, Mabeza G, Werner ER, Herold M, Gordeuk VR. Modulatory potential of iron chelation therapy on nitric oxide formation in cerebral malaria. J Infect Dis. 1997;175:226–30.
203. Patruta SI, Edlinger R, Sunder-Plassmann G, Horl WH. Neutrophil impairment associated with iron therapy in hemodialysis patients with functional iron deficiency. J Am Soc Nephrol. 1998;9:655–63.
204. Omara FO, Blakley BR. The effects of iron deficiency and iron overload on cell-mediated immunity in the mouse. Br J Nutr. 1994;72:899–909.
205. Collins HL, Kaufmann SH, Schaible UE. Iron chelation via deferoxamine exacerbates experimental salmonellosis via inhibition of the nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst. J Immunol. 2002;168:3458–63.
206. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr. 2006;84:1261–76.
207. Gordeuk V, Thuma P, Brittenham G, McLaren C, Parry D, Backenstose A, et al. Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. N Engl J Med. 1992;327:1473–7.

208. Thuma PE, Weiss G, Herold M, Gordeuk VR. Serum neopterin, interleukin-4, and interleukin-6 concentrations in cerebral malaria patients and the effect of iron chelation therapy. Am J Trop Med Hyg. 1996;54:164–8.
209. Thuma PE, Mabeza GF, Biemba G, Bhat GJ, McLaren CE, Moyo VM, et al. Effect of iron chelation therapy on mortality in Zambian children with cerebral malaria. Trans R Soc Trop Med Hyg. 1998;92:214–8.
210. Gordeuk VR, Caleffi A, Corradini E, Ferrara F, Jones RA, Castro O, et al. Iron overload in Africans and African-Americans and a common mutation in the SCL40A1 (ferroportin 1) gene small star, filled. Blood Cells Mol Dis. 2003;31:299–304.
211. Gordeuk VR. African iron overload. Semin Hematol. 2002;39:263–9.
212. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, et al. Transcriptional adaptation of mycobacterium tuberculosis within macrophages: insights into the phagosomal environment. J Exp Med. 2003;198:693–704.
213. Appelberg R. Macrophage nutriprive antimicrobial mechanisms. J Leukoc Biol. 2006;79:1117–28.