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Iron homeostasis in host defence and inflammation

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Abstract

Iron is an essential trace element for multicellular organisms and nearly all microorganisms. Although iron is abundant in the environment, common forms of iron are minimally soluble and therefore poorly accessible to biological organisms. Microorganisms entering a mammalian host face multiple mechanisms that further restrict their ability to obtain iron and thereby limit their pathogenicity. Iron levels also modulate host defence, as iron content in macrophages regulates their cytokine production. Here, we review recent advances that highlight the role of systemic and cellular iron-regulating mechanisms in protecting hosts from infection, emphasizing aspects that are applicable to human health and disease.

In vertebrates, iron is required as a functional component of many proteins that are involved in a broad range of vital biochemical functions, such as oxygen transport and energy production (TABLE 1). Iron is also essential for nearly all microorganisms, plants and invertebrate animals, in which it functions as a catalytic component of enzymes that mediate many redox reactions that are crucial for energy production and intermediary metabolism. Proteins may directly bind to iron or contain iron in the form of haem or iron–sulfur clusters.

Although the modern study of iron homeostasis began more than 80 years ago, a detailed understanding of its molecular basis emerged only in the twenty-first century and remains incomplete. Nevertheless, early investigators recognized a role for iron regulation in host defence. This led to the concept of iron-targeted nutritional immunity as a set of constitutive and inducible mechanisms that deny iron to invading pathogens and thereby limit their ability to harm the host[1].

In healthy organisms, iron is maintained at a stable concentration in the plasma, and it is stored in hepatocytes and splenic and hepatic macrophages at constant levels, despite a fluctuating supply of iron from the diet. This homeostasis is controlled by an endocrine system that resembles those that regulate glucose and calcium concentrations. Central to systemic iron regulation is the liver-derived hormone hepcidin (which is encoded by

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Competing interests statement

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HAMP), which regulates, and is in turn regulated by, systemic iron levels. The transcription of *HAMP* is induced by increasing levels of iron in the plasma and in hepatic cellular stores. In turn, by causing degradation of its receptor — the cellular iron exporter ferroportin (also known as SLC40A1) — hepcidin reduces the influx of iron into the plasma from stores and blocks further absorption of dietary iron (FIG. 1). When body iron levels decrease, hepcidin production is downregulated accordingly, allowing iron absorption to resume and levels of iron in the plasma to increase.

This feedback loop is disrupted by infection. Infection and inflammation induce hepcidin production, driving a decrease in plasma iron concentrations by inhibiting the absorption of iron and promoting the sequestration of iron in macrophages. Given the absolute dependence of most microorganisms on exogenous iron for their survival, it has been assumed that the hypoferraemia of infection and inflammation has a host defence function. Direct evidence of a role for hepcidin and hypoferraemia in host defence is finally being provided by recent studies.

In addition to systemic control by hepcidin, a distinct iron-regulatory system maintains iron homeostasis in individual cells, functioning largely independently of systemic regulation. Most cells obtain iron from the extracellular fluid by receptor-mediated endocytosis of the iron carrier protein transferrin. The levels of transferrin receptors and other proteins involved in cellular iron uptake, storage or use are controlled by iron-responsive element-binding protein 1 (IREBP1; also known as ACO1) and IREBP2 (also known as IREB2). The IREBPs are activated by cellular iron deficiency and bind to the iron-responsive elements (IREs) within mRNAs to regulate mRNA stability or translation. These mechanisms then function to correct cellular iron deficiency by increasing cellular iron uptake and releasing iron from cytoplasmic ferritin stores.

Macrophages take up large amounts of iron by phagocytosis and through degradation of senescent erythrocytes and other senescent or damaged cells. Within macrophages, phagocytosed iron enters the cytoplasm and can either be stored in ferritin, which is subject to translational regulation by the IREBPs, or be exported to the extracellular fluid through ferroportin. It has been suggested that IREBPs have a role in cellular iron regulation that is important for host defence and during inflammation[2]. However, the local and systemic responses to aseptic inflammation *in vivo* are preserved in the absence of either of the two IREBPs[3], which suggests that the role of the IREBPs in host defence and inflammation is minor or that the two IREBPs have mostly overlapping functions, an issue that needs to be examined further.

During infection, homeostatic mechanisms that store iron within macrophages, scavenge iron in tissues or remove iron-containing haem and haemoglobin from the circulation are controlled by cytokines. Enhanced iron scavenging and macrophage sequestration seem to have a dual function of denying iron to invading micro-organisms and protecting the host from the toxic effects of increased levels of iron, haem and haemoglobin that may be released as a result of tissue damage during infection and inflammation. Not surprisingly, many microorganisms have evolved mechanisms that evade or subvert iron-targeted nutritional immunity.

This Review describes the role of the systemic iron homeostatic system in host defence, as supported by human clinical and epidemiological studies, as well as by studies in animal models. We focus on the general mechanisms by which hosts and microorganisms modify host iron metabolism during infection and the effects of these changes on the outcome of infection. For more detailed perspectives, the reader is referred to other recent reviews[4,5].

Systemic iron homeostasis

Absorption and distribution of iron

Iron is one of the most abundant elements in the Earth's crust, but its oxidized (ferric) form is largely insoluble and therefore it is poorly absorbed by plants and animals. Accordingly, iron metabolism in vertebrates is characterized by the conservation and recycling of iron. The iron content of the body is maintained by regulating the absorption of iron in the proximal portion of the intestine, the duodenum, to compensate for unregulated iron losses. Obligatory small amounts of iron are lost through the shedding of intestinal and skin cells and minor physiological blood loss, and larger losses occur through menstruation, and bleeding after childbirth or injury. Iron demands are also increased during pregnancy and fetal growth, as well as during childhood growth.

The transfer of absorbed dietary iron into the plasma is mediated by ferroportin on enterocytes in the duodenum. Ferroportin also mediates the export of iron to the plasma from hepatocytes that store iron and from splenic, hepatic and bone marrow-derived macrophages that recycle iron from senescent erythrocytes (FIG. 1). Intestinal iron absorption and the iron-recycling pathway are regulated by hepcidin. Binding of hepcidin to ferroportin causes the endocytosis of ferroportin and diminishes iron export to the plasma from all of its major sources, trapping iron in duodenal enterocytes (which shed into the intestinal lumen after a few days) and in iron-recycling macrophages. Continued use of iron then rapidly depletes the plasma compartment, lowering plasma iron concentration[6].

More than half of the total iron in the body is bound by haemoglobin in erythrocytes, and as much as a quarter can be present in storage compartments of hepatocytes and macrophages (TABLE 2). Both ferrous and ferric forms of iron are highly reactive and so their biological forms are chaperoned by proteins or organic chelators. Most of the iron that enters the extracellular fluid is recycled from senescent erythrocytes and, to a much lesser extent, from other senescent cell types. By contrast, in humans dietary iron absorption normally accounts for only 5% of the influx of iron into the plasma. In the plasma and extracellular fluid, iron is bound to the transport protein transferrin, which has high-affinity iron-binding sites that are normally 20–40% saturated. The plasma contains only 0.1% of the iron content of the body and constitutes a transit compartment that turns over several times a day and functions to deliver iron predominantly to the erythropoietic bone marrow but also to every cell in the body.

Erythropoiesis is particularly sensitive to decreasing plasma iron concentrations and begins to be inhibited when the concentrations of transferrin-bound iron decrease to levels below its physiological range (10–30 μM). As erythrocytes contain much more iron than other cells and are relatively long-lived (120 days in humans), transient inhibition of erythropoiesis

during iron scarcity may serve to redirect iron to more essential processes. The apparent ability of erythroid precursors to export iron during iron deficiency lends support to this concept[7]. Compared with erythropoiesis, it seems that energy production, intermediary metabolism, neurobehavioural function and host defence (TABLE 1) are spared from the consequences of iron restriction, at least until it becomes very severe.

Regulation of hepcidin

Three major pathophysiological mechanisms influence hepcidin production: iron and inflammation are stimulatory, and erythropoietin-stimulated expansion of erythroid precursors is inhibitory. All pathways regulate hepcidin levels at the level of *HAMP* transcription, although those used by the erythroid regulator erythroferrone are not well understood.

Regulation of hepcidin by transferrin-bound iron concentrations and hepatic iron stores constitutes a feedback mechanism for iron homeostasis (FIG. 1). The identification of mutations causing genetic iron disorders in humans and studies examining the effects of disrupting these genes in mice have revealed the non-redundant function of the components of the homeostatic pathway. The regulatory loop is centred on the bone morphogenetic protein receptor (BMPR) pathway, which is adapted for iron regulation by several accessory proteins and iron sensors (FIG. 2 and TABLE 3; for more detail, see REFS 8–10). The BMPR is a heterodimer of type I and type II BMPRs that signals predominantly through the SMAD pathway to regulate *HAMP* transcription. In mice, BMP6 is the BMPR ligand that is required for normal hepcidin production and iron regulation *in vivo*, but other BMPs also have stimulatory activity in this system. As no genetic disorders that ablate *BMP6* in humans have been reported, it is not known whether BMP6 is required for iron regulation in humans. When hepatic iron overload develops, BMP6 production increases and this stimulates hepcidin production and limits further intestinal iron absorption and release of iron from macrophage stores. Although iron accumulates predominantly in hepatocytes, most iron-regulated BMP6 seems to be produced by other hepatic cell types (such as sinusoidal endothelial cells, stellate cells and macrophages). The iron sensors and signal transduction pathways by which intracellular iron regulates levels of BMP6 and hepcidin are not known, and their characterization is an important goal for future studies.

The glycosylphosphatidylinositol (GPI)-linked membrane protein haemojuvelin (also known as HFE2 and RGMC) is another iron-specific ligand that is required for baseline hepcidin production and for hepcidin and iron regulation. Haemojuvelin interacts with both BMPs and the BMPR, and it is negatively regulated through specific proteolytic cleavage by transmembrane protease serine 6 (TMPRSS6; also known as matriptase 2). Two transferrin receptors — TFR1 (also known as TFRC) and TFR2 — are the likely sensors of the concentration of extracellular transferrin-bound iron, and they interact with another iron-related membrane adaptor protein, HFE, and perhaps also with haemojuvelin to modulate BMPR signalling. Neogenin, a ubiquitous membrane protein, is also essential for iron-related BMPR signalling and may function by facilitating the interaction between BMPR, haemojuvelin and TMPRSS6 (REF. 11). The molecular interactions of the various

components of the iron-regulated BMPR complex remain incompletely characterized and are subject to active investigation.

Another set of regulatory factors conveys the iron requirements for erythropoiesis and includes the recently identified hormone erythroferrone[12], which is produced by erythroblasts in response to erythropoietic stimulation by endogenous or synthetic erythropoietin. Erythroferrone suppresses hepcidin synthesis, making more iron available for enhanced erythropoiesis.

Multiple endocrine stimuli — including testosterone[13], growth factors (such as epidermal growth factor, hepatocyte growth factor[14] and platelet-derived growth factor BB[15]) and gluconeogenesis[16] — also affect *HAMP* transcription in hepatocytes, as does endoplasmic reticulum stress[17,18], but the pathophysiological relevance of these pathways is poorly understood.

Another set of regulators of *HAMP* transcription mediates the effects of host defence and inflammation (FIGS 1,2). Interleukin-6 (IL-6) is the main cytokine that stimulates hepcidin synthesis during infection[19] and inflammation, but other factors can also contribute, including activin B[20], type I interferons (IFNs)[21,22] and BMP2 (REF. 23). Exogenous IL-22 increases hepcidin synthesis through an IL-6-independent mechanism[24,25], but the physiological role of IL-22 in iron regulation during inflammation has not been reported.

Hepcidin and host defence

Stimulation of hepcidin synthesis during infection

The role of hepcidin as the iron-regulatory hormone that acts on ferroportin to modulate iron absorption and systemic iron distribution is now well accepted, but the strong connection between hepcidin and host defence is less well appreciated. Hepcidin was first isolated from human urine and plasma on the basis of its similarity to defensins, which are cationic cysteine-rich antimicrobial peptides of approximately 3 kDa in size. Hepcidin concentrations, similar to those of certain defensins, are greatly increased during infection and inflammation. Also similar to defensins, hepcidin has direct antimicrobial activity *in vitro*, but the concentrations and conditions required for this activity seem to be outside of the physiological range.

The increase in hepcidin secretion in response to infectious or inflammatory stimuli is responsible for the characteristic hypoferraemia of inflammation[26,27], which develops within a few hours of a systemic infection or following injection of microbial components or certain cytokines. High hepcidin concentrations cause iron sequestration in macrophages, which effectively redistributes iron not only from the plasma compartment but also from non-macrophage storage in hepatocytes. Prolonged activation of this mechanism during infection and inflammation imposes a biological cost in the form of anaemia of inflammation[27–29], a condition that is partly caused by the restriction of iron supply to the erythropoietic bone marrow.

Mice engineered to lack hepcidin fail to develop hypoferraemia after inflammatory stimulation, their serum iron concentrations may even increase during infection or

inflammation, and their anaemia of inflammation is milder[28,29]. Interestingly, although most bacterial and some viral infections (such as influenza virus and HIV) rapidly increase hepcidin production in humans and mouse models, hepatitis B and hepatitis C virus infections fail to elicit systemic inflammatory responses and hepcidin production[30]. The consequences of the altered inflammatory and iron responses for infections with these liver-tropic viruses remain to be determined.

Hepcidin-induced redistribution of iron is protective in malaria

Malaria infection causes inflammation and increases hepcidin levels, redistributing iron to macrophages and, in the long term, away from hepatocytes that normally support the initial stage of infection. This mechanism may function to prevent superinfection by additional malaria parasites during subsequent exposures to malaria-bearing mosquitoes, and it provides a form of innate immune protection in infants and children who have not yet developed an adaptive immune response to malarial parasites[31].

Hepcidin-induced hypoferraemia and host defence

It has long been assumed that the hypoferraemia that develops in response to infection or inflammation has a role in host defence against microorganisms that reside in extracellular fluids, but such a function has never been demonstrated and is by no means obvious. Specifically, many pathogenic microorganisms have systems that can effectively scavenge iron, even at low environmental concentrations. During infection, the iron concentration in the plasma may decrease from the normal range of 10–30 μM to as low as 1–3 μM. However, throughout its physiological range of concentrations, the iron remains tightly bound to transferrin, making the iron inaccessible to microbial ferric transporters. It is therefore not clear how changes in iron concentration would affect microbial iron transporters, which must function in environments with a wide range of iron concentrations.

We propose that the hypoferraemic response evolved to provide an increased capacity for transferrin to bind iron that would be released during acute infection and during the associated destruction of tissues and erythrocytes, and so limit the generation of non-transferrin-bound iron that can be readily used by many microorganisms. Moreover, non-transferrin-bound iron can amplify tissue injury either because it catalyses the extracellular production of injurious reactive oxygen species (ROS) or because it is rapidly taken up some cell types, causing cellular iron overload with the secondary generation of ROS. Exogenous or endogenous hepcidin would be expected to promote the clearance of non-transferrin-bound iron that is generated during tissue injury. Indeed, a recent study showed that exogenous hepcidin is protective against renal ischaemia–reperfusion injury and oxidative stress, and that hepcidin deficiency exacerbates these forms of injury[32]. Transferrin, a plasma protein with a half-life of 8–10 days, is recycled when it removes iron that is released from damaged tissues. By contrast, haemopexin and haptoglobin — which are acute-phase reactants that scavenge iron complexed in haem and haemoglobin, respectively — are rapidly consumed in the process and must be replaced through augmented synthesis. In chronic infection or inflammation, in which tissue destruction is generally slower, transferrin concentrations are somewhat decreased, which effectively raises the concentration of the diferric form, presumably to preserve its delivery to erythroid

precursors. The consequences of the malfunction of non-transferrin-bound iron scavenging are revealed in iron overload disorders.

Iron overload disorders and infections

Hereditary haemochromatosis

Hereditary haemochromatosis is a disease characterized by partial or complete hepcidin deficiency that is caused by genetic lesions in the regulators of *HAMP* transcription or in the *HAMP* gene itself[33]. Affected individuals hyper-absorb dietary iron and release excessive amounts of iron into the plasma from macrophages, causing transferrin saturation to levels that are above its physiological range (20–45%). Transferrin saturation levels of greater than 80% are prominently associated with the formation of non-transferrin-bound iron, which is then rapidly taken up by vulnerable tissues (such as hepatocytes and cardiac myocytes) through alternative iron transporters (such as zinc transporter ZIP14 (also known as SLC39A14) in the liver[34]). Cellular iron overload increases the production of ROS, causing cellular injury. Tissue iron overload and resulting toxicity is proportional to the severity and duration of hepcidin deficiency, and results in progressive iron-mediated damage to the liver, endocrine glands and cardiac myocytes. Environmental and genetic factors— most notably including alcohol consumption and gender — affect the amount of iron overload, especially in the most common form of the disease, which is caused by mutations in *HFE* that partially preserve hepcidin synthesis[35].

Iron-loading anaemias

Anaemia owing to ineffective erythropoiesis, as occurs most prominently in patients with β-thalassaemia, is associated with severe iron overload that develops even in the absence of blood transfusions but is exacerbated in patients who receive repeated transfusions[36,37]. Despite high concentrations of iron and deposition of iron in the liver and other vital organs, the blood level of hepcidin in untransfused patients with β-thalassaemia is very low[38–40], owing to suppression by mediators originating from the expanded erythroblast population in the bone marrow[12,41]. Candidate hepcidin suppressors include erythroferrone and growth and differentiation factor 15 (GDF15), but the pathophysiological role of these and potentially other relevant mediators remains an active area of study.

Infections associated with genetic iron overload

Although the relative risk has not yet been estimated, it is clear that patients with hereditary haemochromatosis are predisposed to otherwise rare infections with two Gram-negative bacterial species: the marine organism *Vibrio vulnificus*[42] (and related *Vibrio* species) and the largely zoonotic bacterium *Yersinia enterocolitica*[43,44]. In several reported cases, lethal infections with siderophilic bacteria developed before haemochromatosis was diagnosed[44–46]. In a particularly poignant case, a researcher with latent hereditary haemochromatosis died rapidly after exposure to a laboratory strain of *Yersinia pestis*[45] that was thought to be attenuated because it lacked yersinia-bactin, which is a siderophore required for efficient iron uptake. The pathogenicity of similar strains was greatly enhanced in haemochromatotic mice that were generated by disruption of the gene encoding either hepcidin or its essential regulator haemojuvelin[47]. Patients with iron overload associated

with β-thalassaemia are also at increased risk of infection with siderophilic bacteria[48,49], as well as other infections, although the evidence for these associations is less conclusive[48,50,51].

Effects of hepcidin expression and hypoferraemia in infection

Recent studies in hepcidin-deficient mice have identified a role for hepcidin in protection from infection with siderophilic bacteria. Hepcidin-deficient mice die of sepsis in less than 16 hours after subcutaneous infection with *V. vulnificus* at doses that are otherwise well tolerated by wild-type mice[52]. Hepcidin-deficient mice are also much more susceptible than wild-type mice to death from infection with *Y. enterocolitica* (D. Stefanova, Y. Bulut, T.G. and E.N., unpublished observations) and certain attenuated strains of *Y. pestis*[47]. Interestingly, mice infected with siderophilic bacteria were rescued by treatment with potent hepcidin agonists (known as minihepcidins) that induced hypoferraemia. These treatments had little or no effect on iron stores but redistributed iron from the extracellular space into macrophages. By contrast, systemic iron depletion of hepcidin-deficient mice offered only partial protection against *V. vulnificus*, as serum iron concentrations remained high. Susceptibility to death from infection with *V. vulnificus* correlated with the presence of non-transferrin-bound iron in the mouse sera and with the ability of sera from these mice to support rapid *in vitro* growth of *V. vulnificus*. *Ex vivo* growth of *V. vulnificus* in mouse sera was greatly accelerated by the addition of ferric iron in amounts that exceeded the binding capacity of transferrin[52].

Therefore, we propose that hepcidin-induced hypoferraemia functions to limit the availability of non-transferrin-bound iron during infections. This mechanism is particularly effective against bacterial strains that lack siderophore-mediated iron uptake (for example, marine *Vibrio* species). The paucity of well-documented examples of protective effects of reactive hypoferraemia is at odds with the evolutionary conservation of this mechanism and its obvious biological cost in the form of anaemia of inflammation. It is therefore likely that the protective impact of this mechanism against extracellular microorganisms is much broader than we currently realize.

Paradoxically, studies in mouse models have shown that infections with intracellular microorganisms that reside in macrophages may be enhanced by hepcidin-induced iron sequestration, and conversely, hepcidin deficiency in mouse models of hereditary haemochromatosis may inhibit the growth of macrophage-resident bacteria, including *Mycobacteria tuberculosis*[53] or *Salmonella enterica* serovar *enterica* Typhimurium[54]. It has been suggested[55] that increased resistance to intracellular bacteria in individuals homozygous for *HFE*, and possibly also in those that are heterozygous, led to the high prevalence of *HFE* mutations in northern European populations. Although these concepts are supported by experiments with isolated human macrophages and with mouse models, genetic epidemiological studies showing the impact of hereditary haemochromatosis on human infections with intracellular microorganisms are lacking.

Iron deficiency and host defence

Tropical infections

Iron deficiency is widespread in developing countries and is a major cause of chronic illness and loss of economic productivity[56]. In an attempt to address this problem, various means of dietary iron supplementation have been tested. Unfortunately, evidence is accumulating that indiscriminate iron supplementation may increase morbidity and mortality from malaria, diarrhoeal illness and tuberculosis[57–59]. These effects have been attributed to multiple mechanisms: residual iron in the faeces causing changes in the intestinal flora; stimulation of pathogen growth by increased iron concentrations in plasma or specific organs (as discussed in connection with iron overload disorders); or the effects of iron on host defence. The possibility that iron deficiency, which is endemic in tropical regions, may be protective against certain infections has also been raised.

Intestinal flora

Lactic acid bacteria (also known as Lactobacillales) are normal components of the intestinal flora and have little or no requirement for iron; they thrive in the relatively iron-poor environment of the human intestine, but they may be displaced by other microorganisms when iron is administered[60]. Motivated to understand the connection between iron supplementation and diarrhoeal illness in African children, some but not all studies found an altered composition of the intestinal flora and increased inflammation in children who were treated to prevent or reverse iron deficiency[61–63].

Protective effects of iron deficiency in malaria

Following infection, malarial parasites undergo extensive replication in hepatocytes, the main cellular site of iron storage. The merozoites then infect erythrocytes — the largest iron compartment in the body — where they continue to multiply. The parasites require iron at both the liver and the erythrocyte stage. During iron deficiency, the liver becomes depleted of stored iron (ferritin), and the erythrocytes become smaller and contain less haemoglobin. Epidemiological evidence suggests that iron deficiency is remarkably protective against high-grade parasitaemia and severe malaria[64,65], perhaps explaining why more effective iron absorption and storage mechanisms conferred by haemochromatosis mutations are less common in the tropical regions than in northern Europe. In *ex vivo* erythrocytes from iron-deficient donors, the malarial parasite *Plasmodium falciparum* invades fewer cells and shows slower intracellular growth than in erythrocytes from iron-replete donors[66]. The protective effect of iron deficiency is reversed by iron supplementation.

Iron deficiency and inflammation

There is increasing evidence from mouse models that the macrophage iron depletion seen in iron deficiency, and paradoxically also in hereditary haemochromatosis, may have pro-inflammatory effects[67]. Iron is a required cofactor for enzymes that mediate oxygen-dependent prolyl and asparaginyl hydroxylation of hypoxia-inducible factor 1α (HIF1α), which leads to proteasomal degradation of this transcription factor. Iron depletion of macrophages could therefore mimic the effects of hypoxia, stabilizing HIF1α and promoting

the transcription and synthesis of IL-1β[68] and perhaps other cytokines. IL-1β mediates systemic inflammation and activates important host defence mechanisms[69]. A direct hepcidin-mediated anti-inflammatory effect mediated by ferroportin and the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway was proposed[70], but JAK–STAT-mediated signalling by ferroportin was not confirmed in subsequent more-detailed studies[71]. Although the induction of IL-1β is not primarily modulated by hepcidin, hepcidin causes iron sequestration in macrophages (FIG. 3) and would be expected to have an anti-inflammatory effect[67].

Hepcidin-independent iron sequestration

Iron-sequestering proteins

In addition to hepcidin — the effects of which on ferroportin cause redistribution of iron into macrophages — the tissues and cells of body surfaces and innate immune cells express other molecules that relocate and sequester iron in its various forms and that may function in host defence (TABLE 4). These include extracellular proteins, such as lactoferrin, siderocalin (also known as NGAL or lipocalin 2), haptoglobin and haemopexin, and membrane-associated proteins, such as natural resistance-associated macrophage protein 1 (NRAMP1; also known as SLC11A1) and TFR1. Lactoferrin is a homologue of the iron carrier protein transferrin but differs from transferrin by continuing to bind iron even at low pH, which is common in inflamed and infected tissues. Siderocalin, a member of a family of proteins that contain a pocket (calyx) for small mostly hydrophobic molecules, binds iron when it is complexed with small natural organic chelators. Haptoglobin and haemopexin are carrier proteins that transport free haemoglobin and haem, respectively, for degradation by macrophages and hepatocytes. NRAMP1 is a ferrous iron transporter found in the membranes of phagocytic vacuoles of macrophages and neutrophils. The concentrations of these iron-binding and iron-transporting molecules are greatly increased during infection and inflammation, owing to cytokine-driven synthesis (siderocalin, haptoglobin and haemopexin are induced by inflammatory cytokines; NRAMP1 is induced by lipopolysaccharide and IFNγ[72]) and to degranulation of neutrophils (lactoferrin and siderocalin). By contrast, macrophage expression of TFR1, which mediates iron delivery to endosomes, is decreased by IFNγ exposure during intracellular infections[73].

Iron sequestration and host defence

Studies in mutant mice have revealed that siderocalin and NRAMP1 have demonstrable non-redundant roles in host defence. The loss of siderocalin in mice causes increased mortality from septicaemia following infection with Gram-negative bacteria[74]. Mice with a loss-of-function mutation in the divalent metal transporter NRAMP1 show increased susceptibility to specific intracellular infections that target macrophages[72] (TABLE 4). *NRAMP1* variants are associated with an increased risk of tuberculosis in humans[75], supporting an important contribution of this mechanism to resistance against intracellular infections. The specific function of NRAMP1 in phagocytic vacuoles is not definitively understood, but most studies suggest that NRAMP1 transports iron from phagosomes and other vacuoles that contain microorganisms to the cytoplasm of macrophages[76–78], thereby starving vacuolar microorganisms of iron.

Microbial countermeasures

Rapid microbial multiplication and clonal selection allow microorganisms to evade conditions that restrict their growth, including iron sequestration by their hosts[79].

Extracellular bacteria and host ferroproteins

Pathogenic Gram-negative bacteria (such as *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Haemophilus influenzae)*, which are highly adapted to their human hosts, have evolved specific mechanisms for hijacking various iron-containing human proteins (such as transferrin, lactoferrin, haemoglobin and ferritin) as a source of iron[80–82]. The uptake of transferrin by *Neisseria* species is specific to hominid transferrin, indicating that this specialized uptake mechanism facilitates their residence in the human host and several other hominids[83]. Analysis of hominid transferrin sequences and structures has yielded evidence of a mutational ‘arms race’ between bacterial receptors evolving to bind and take up transferrin as a source of iron, whereas the human C-lobe of transferrin, which is targeted by these receptors[84], has evolved mutational variants that escape uptake by these bacteria. As an example of another evasive microbial strategy, microorganisms may modify their siderophores[85] to avoid their sequestration by host-derived siderocalin.

Intracellular microorganisms and the hepcidin–ferroportin system

Hepcidin-induced iron sequestration in macrophages (FIG. 3) may paradoxically enhance iron availability for intracellular microorganisms, including *Leishmania* spp.[86], *Chlamydia* spp. and *Legionella* spp.[87], as well as *S.* Typhimurium[88]. *S.* Typhimurium elicits this response by a mechanism that is dependent on IL-6 and oestrogen-related receptor-γ (ESRRγ)[88]. IL-6 is increased early in most microbial infections and it stimulates hepcidin transcription via the JAK2–STAT3 pathway in a manner dependent on STAT3-binding motifs in the promoter of *HAMP* (the hepcidin gene)[89–91]. A recent study[88] indicated that the IL-6-inducible nuclear receptor ESRRγ enhances hepcidin induction in response to IL-6 in primary hepatocytes. Chemical inhibition of the ESRRγ pathway in a mouse model significantly decreased bacterial burden and modestly improved the survival of infected mice[88]. Although the hepcidin–ferroportin system seems to function maladaptively during infections with intracellular microorganisms, this host response could be mitigated in infected macrophages by local mechanisms that counteract the systemic effects of hepcidin. Indeed, evidence from mouse models of *S.* Typhimurium infection suggests that during infections with intracellular microorganisms, the post-translational downregulation of ferroportin by hepcidin may be opposed by the effects of IFNγ- and bacteria-stimulated nitric oxide production[92], which increases transcription of the gene encoding ferroportin (FIG. 3). NRAMP1 expression is also stimulated by IFNγ[72], and NRAMP1-mediated iron export from phagosomes containing intracellular microorganisms may help to counter the effect of systemic hepcidin. Nevertheless, these mice develop hypoferraemia, indicating that the nitric oxide-dependent mechanism operates at a local level and requires the presence of bacteria within the macrophage, leaving most iron-recycling macrophages under the dominant regulatory influence of hepcidin. It remains to be seen whether ferroportin regulation by nitric oxide also occurs in human macrophages, which produce much less nitric oxide than mouse macrophages after activation.

Intracellular microorganisms and erythrophagocytosis

Erythrocytes are by far the richest source of iron, and therefore residence in erythrophagocytic vacuoles of macrophages might be particularly advantageous for intracellular microorganisms. In chronic infection with *S.* Typhimurium, the bacteria may induce erythrophagocytosis and the conversion of hepatic and splenic macrophages into an M2 phenotype, a process that also requires host signals but has not been characterized in molecular detail[93]. In this way, these macrophages provide the bacteria with a source of iron from erythrophagocytosis and an M2 macrophage environment with attenuated antimicrobial mechanisms favouring the survival of the bacteria.

Therapeutic opportunities and challenges

Although targeting iron acquisition in pathogenic microorganisms is an appealing strategy for the treatment of infection, the design of iron chelators as antimicrobial agents is very challenging. An effective iron chelator must be nontoxic, must have higher affinity for ferric iron than bacterial siderophores and must not be recognized by microbial siderophore transporters[94]. Despite several decades of research in this area that have yielded three successful drugs for the treatment of iron overload disorders, and evidence of *in vitro* microbistatic activity for several compounds[95], no chelator has yet been adopted as an antimicrobial therapeutic.

An alternative approach, based on animal models of infection with *V. vulnificus* or *Y. enterocolitica*, is the development of so-called minihepcidins, which are small peptide mimetics of hepcidin. These agents were effective against fulminant *V. vulnificus* infection in hepcidin-deficient mice[52]. In this model, mini-hepcidins rapidly decreased iron concentrations in the plasma and extracellular fluid, and thereby decreased the concentrations of non-transferrin-bound iron, which is a likely source of iron for siderophilic bacteria. Although the logistics of providing these agents to patients with fulminant infections is formidable, mini-hepcidins could prove useful in these lethal and often treatment-resistant infections.

Conclusions and perspectives

The scarcity of bioavailable iron has made iron a target of a variety of host defence mechanisms that limit the extent to which microbial invaders can access this essential trace metal. In turn, pathogenic microorganisms have evolved mechanisms that counter iron sequestration and allow microbial proliferation within the host. Many aspects of the struggle for iron between microorganisms and their hosts remain unexplained and are important subjects for further research; these include the impact of inflammatory hypoferraemia on common pathogens that are not considered to be siderophilic; understanding iron availability and its regulation in the subcellular environment of intracellular microorganisms; and comprehensive analysis of the effects of iron deficiency on host defence and inflammation. Finally, the exquisite dependence of most microorganisms on iron provides a rationale for the design of new antimicrobials that target iron uptake mechanisms in pathogenic microorganisms.

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Glossary

β-thalassaemia	An autosomal-recessive genetic disorder in which there is a mutation in the gene encoding β-globin chains that make up haemoglobin. Thalassaemia can also be caused by mutation of the gene encoding the α-globin chain. Mutations lead to reduced globin chain synthesis, causing anaemia of variable severity. Carriers of the thalassaemia mutations are partially protected from malaria infection. This selective advantage is thought to contribute to the persistence of this potentially harmful mutation in the human genetic pool
Siderophilic bacteria	Bacteria that become more pathogenic when iron concentrations in the blood or tissues are increased. *Vibrio vulnificus* and *Yersinia entercolitica* are the most prominent examples
Siderophore	An organic molecule that is synthesized by microorganisms to chelate environmental iron and deliver it for microbial uptake
Minihepcidins	Synthetic peptides that act as potent hepcidin agonists. Their design is based on the amino-terminal 7–9 amino acid sequence of hepcidin, which is modified to have greater stability, duration of action and potency by substitution of natural for unnatural amino acids and by covalent addition of a fatty or bile acid moiety
M2 phenotype	Macrophages develop diverse phenotypic characteristics in response to signals from their tissue environment. M1 (inflammatory) macrophages develop following stimulation with Toll-like receptor ligands and interferon-γ, whereas M2 (alternatively activated) macrophages develop in the presence of interleukin-4 (IL-4) and/or IL-13. Relevant to iron metabolism, macrophages of the M2 phenotype express ferroportin and recycle iron from damaged tissues

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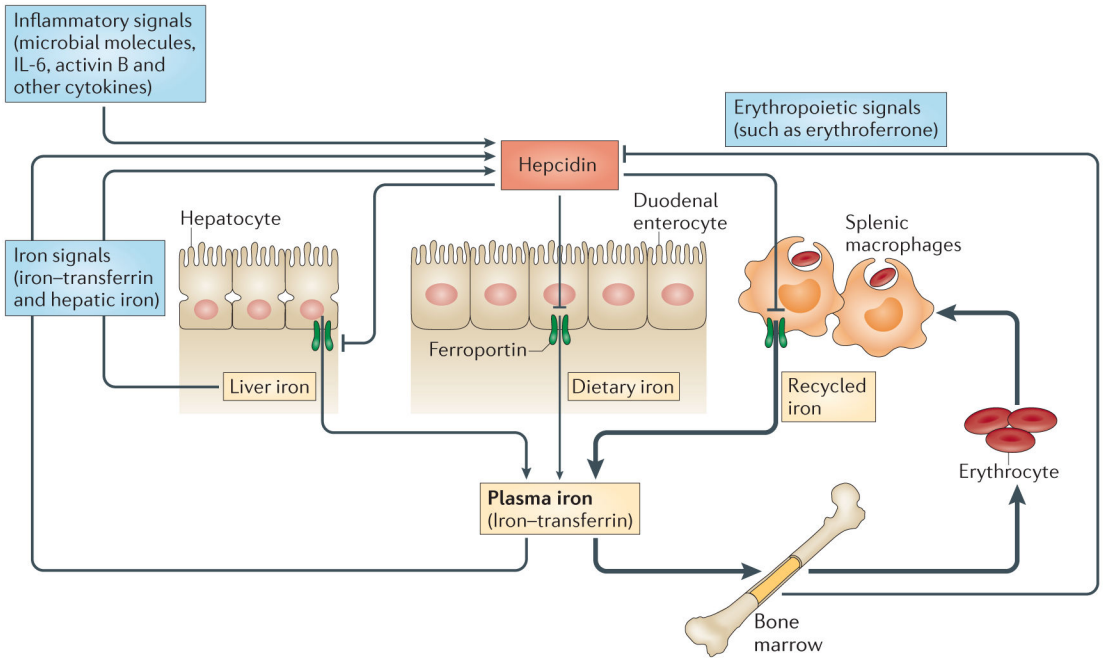

Figure 1. Iron homeostasis and its modulation by erythropoiesis and inflammation

Hepcidin blocks major iron flows into the plasma (predominantly from splenic macrophages recycling erythrocytes but also from duodenal absorption and from stores in hepatocytes) by causing degradation of its receptor, the iron exporter ferroportin. Hepcidin production by the liver is upregulated by increasing levels of plasma iron and liver iron stores. Infection and inflammation also stimulate transcription of the gene encoding hepcidin. By contrast, after erythropoietic stimulation, erythroid precursors secrete mediators (such as erythroferrone) that suppress hepcidin production in the liver. Thicker arrows indicate dominant flow of iron. IL-6, interleukin-6.

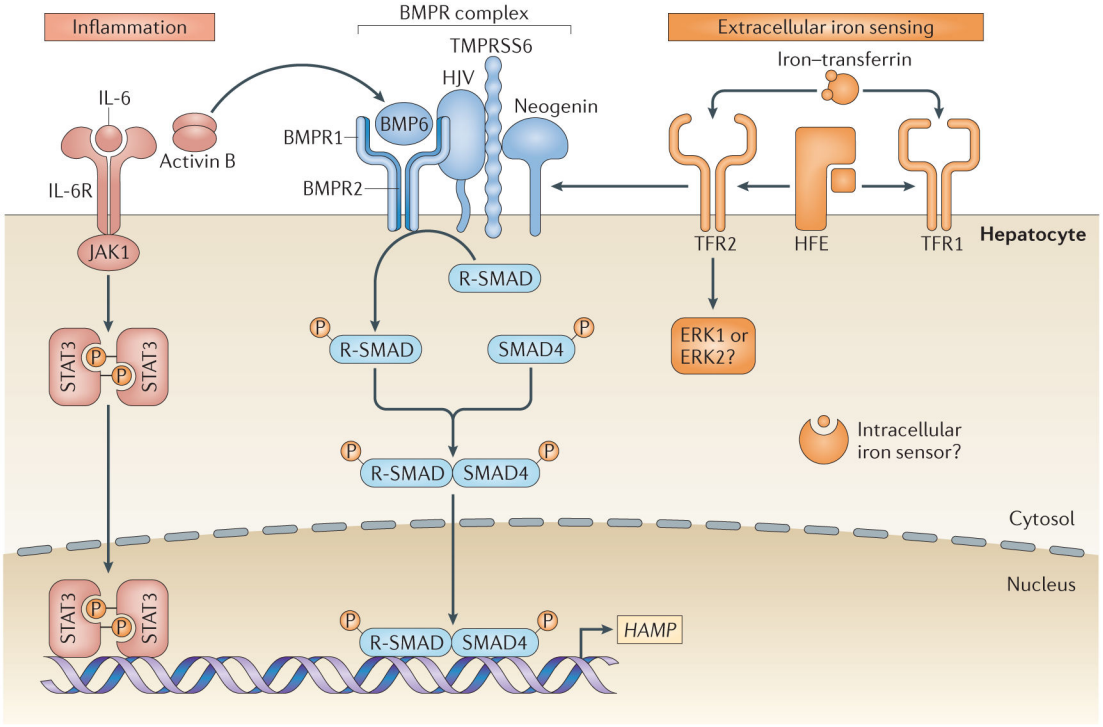

Figure 2. Regulation of *HAMP* transcription

The SMAD and signal transducer and activator of transcription 3 (STAT3) pathways are the main known regulators of transcription of the gene encoding hepcidin (*HAMP*) in response to iron-related and inflammatory signals. The SMAD pathway is activated by the interaction of bone morphogenetic protein 6 (BMP6) and other BMPs with a heterodimeric BMP receptor (BMPR) containing type 1 and type 2 subunits. Haemojuvelin (HJV) is an iron pathway-specific ligand of the BMPR and BMPs that increases BMPR signalling. Transmembrane protease serine 6 (TMPRSS6) is a negative regulator of iron-related BMPR signalling and acts by cleaving HJV. Neogenin may facilitate the interaction of HJV with TMPRSS6 and also perhaps with other components of the BMPR complex. The concentration of extracellular iron–transferrin is sensed by transferrin receptor 1 (TFR1) and TFR2 assisted by HFE, and HFE and TFR2 convey a stimulatory signal to the BMPR complex through interactions that have not yet been characterized. The BMPR phosphorylates regulatory SMADs (R-SMADs), which complex with SMAD4 to enter the nucleus and stimulate the transcription of *HAMP*. Inflammation increases *HAMP* transcription through increased levels of interleukin-6 (IL-6), activin B and other cytokines. IL-6 binds to the IL-6 receptor (IL-6R) and signals through Janus kinase 1 (JAK1)-induced phosphorylation of STAT3 and the binding of phosphorylated STAT3 to cognate motifs in the *HAMP* promoter. Activin B probably signals through the BMPR pathway. ERK, extracellular signal-regulated kinase.

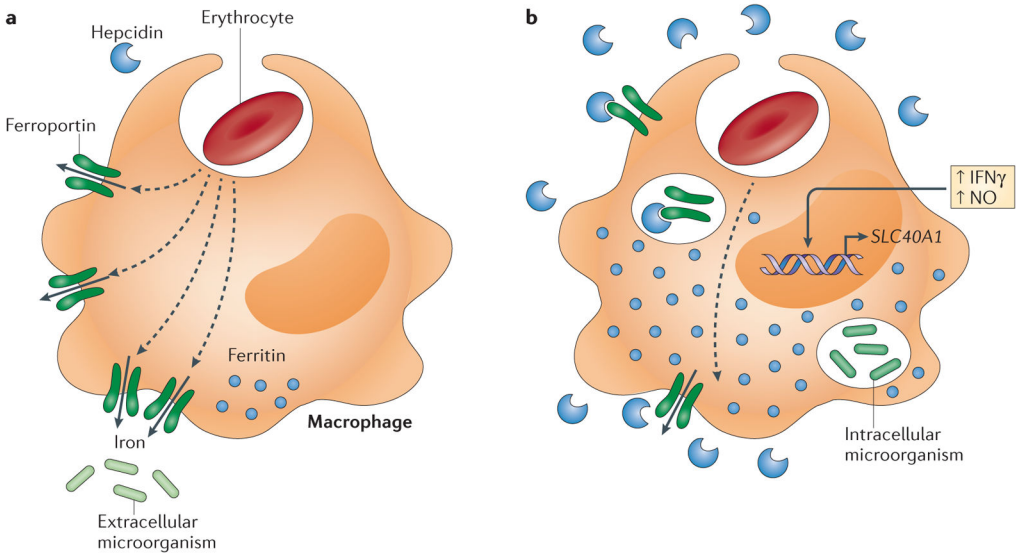

Figure 3. Hepcidin-induced sequestration of iron in macrophages

The flow of iron in macrophages is depicted by dashed arrows. **a** | When hepcidin concentrations are low, ferroportin exports iron from macrophages, resulting in relative macrophage iron depletion. Low levels of intracellular iron stores (ferritin) are inhibitory to intracellular microorganisms, but higher extracellular iron levels may promote the growth of extracellular microorganisms. **b** | Increased hepcidin concentrations cause ferroportin degradation and iron sequestration by ferritin in macrophages, which restricts iron availability for extracellular microorganisms but may promote the growth of intracellular microorganisms. The favourable effect on intracellular microorganisms is opposed by local production of interferon-γ (IFNγ) and nitric oxide (NO), which stimulate transcription of the gene encoding ferroportin (*SLC40A1*) in an autocrine or paracrine manner.

Table 1

Examples of iron-containing proteins in vertebrate animals

Function	Examples of proteins
Oxygen transport and storage	Haemoglobin and myoglobin
Oxygen sensing and hypoxic regulation	Hypoxia-inducible factor prolyl hydroxylases
Energy production	Cytochrome *c*, cytochrome *c* oxidase and NADH dehydrogenase
Intermediary metabolism and detoxification	Ribonucleotide reductase, amino acid oxidases, fatty acid desaturases, cytochrome P450 and catalase
Synthesis of hormones and neurotransmitters	Tryptophan hydroxylase, tyrosine hydroxylase and thyroperoxidase
Host defence and inflammation	Myeloperoxidase, NADPH oxidase, indoleamine 2,3-dioxygenase, nitric oxide synthases and lipoxygenases
Iron storage and transport	Transferrin and ferritin

Table 2

Iron distribution in adult humans

Compartment	Major iron-containing protein	Iron content
Erythrocytes	Haemoglobin	1,500–2,500 mg
Storage (hepatocytes and macrophages)	Ferritin	200–1,200 mg
Plasma	Transferrin	3–4 mg
Other tissues	Myoglobin (muscle) and many others	200 mg

Table 3

Regulation of hepcidin: components and pathways

Component	Function	Effect on *HAMP* transcription	Loss-of-function phenotype
BMP receptors (such as ALK1)	Signal via SMAD pathway	Stimulatory	Iron overload, severe in ALK3-deficient mice
BMP6	Ligand of BMP receptors; liver *BMP6* mRNA reflects cellular iron load in the liver	Stimulatory	Severe iron overload in mice
Haemojuvelin	Co-receptor for BMPs	Stimulatory	Severe iron overload in humans and mice
TMPRSS6	Protease that cleaves haemojuvelin	Suppressive	Iron-refractory iron deficiency anaemia in humans and mice
Transferrin receptor 1	Mediates cellular iron uptake and is an iron sensor for iron–transferrin	Stimulatory via HFE	Embryonic lethal in mice
Transferrin receptor 2	Iron sensor for iron–transferrin	Stimulatory	Iron overload in humans and mice
HFE	Adaptor for iron sensing	Stimulatory	Iron overload in humans and mice
Erythroferrone	Erythroid hormone	Suppressive	Delayed recovery from anaemia in mice
IL-6 and IL-6 receptor	Signal via STAT3	Stimulatory	Lack of inflammatory hypoferraemia

ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; *HAMP*, hepcidin gene; IL-6, interleukin-6; IL-6R, IL-6 receptor; STAT3, signal transducer and activator of transcription 3; TMPRSS6, transmembrane protease serine 6.

Table 4

Molecules that mediate iron sequestration from microorganisms during infection

Protein	Distribution	Function	Loss-of-function phenotype*
Extracellular			
Transferrin	Plasma and extracellular fluid	Iron transport protein; iron inaccessible to many but not all microorganisms	Severe anaemia (M, H)
Lactoferrin	Neutrophils, milk and moist epithelia	Binds extracellular iron, even at low pH	Susceptibility to periodontal infection (M)
Siderocalin	Neutrophils, plasma and moist epithelia	Sequesters extracellular iron from catechol-type bacterial siderophores	Susceptibility to systemic infections with Gram-negative bacteria (M)
Haptoglobin	Plasma	Clearance of haemoglobin from the plasma and extracellular fluid	Haemoglobin deposition in the kidney after haemolysis (M)
Haemopexin	Plasma	Clearance of haem from the plasma and extracellular fluid	Increased organ damage after haemolysis (M)
Cellular			
NRAMP1	Macrophages	Iron transport, probably from phagosomes to the cytoplasm, causing iron depletion in phagosomes	Susceptibility to macrophage-based *Mycobacterium bovis* BCG, *Leishmania donovani* and *S.* Typhimurium infections (M), and increased risk of tuberculosis (H)
Ferroportin	Macrophages	Iron export, causing iron depletion of macrophages	Accumulation of iron in macrophages (partial loss of function; M, H)

BCG, bacille Calmette–Guérin; NRAMP1, natural resistance-associated macrophage protein 1; *S.* Typhimurium, *Salmonella enterica* serovar *enterica* Typhimurium.

* In this column, the letters ‘M’ and ‘H’ in brackets indicate whether the phenotype has been observed in mice and humans, respectively.