Microreview

The struggle for iron – a metal at the host–pathogen interface

Manfred Nairz, Andrea Schroll, Thomas Sonnweber and Günter Weiss*
Department of Internal Medicine I, Clinical Immunology and Infectious Diseases, Medical University of Innsbruck, Austria.

Summary
Iron holds a central position at the host–pathogen interface because mammalian and microbial cells have an essential demand for the metal, which is required for many metabolic processes. In addition, cross-regulatory interactions between iron homeostasis and immune function are evident. While iron affects the secretion of cytokines and the activity of transcription factors orchestrating immune responses, immune cell-derived mediators and acute-phase proteins control both systemic and cellular iron homeostasis. Additionally, immune-mediated strategies aim at restricting the supply of the essential nutrient iron to pathogens, which represents an effective strategy of host defence. On the other hand, microbes have evoked multiple strategies to utilize iron because a sufficient supply of this metal is linked to pathogen proliferation, virulence and persistence. The control over iron homeostasis is a central battlefield in host–pathogen interaction, in which both opponents compete for iron (Weiss, 2002). Accordingly, a key pathway in host defence is the preclusion of invading microbes from iron, for which the term nutritional immunity was coined (Weinberg, 2000).

Iron rusts from disuse – competition for an essential nutrient in infectious diseases
Iron is an essential nutrient for both mammals and microorganisms (Schaible and Kaufmann, 2004; Hentze et al., 2010). The metal is a central component of haem groups, iron–sulfur cluster-containing proteins and enzymes in mitochondrial respiration and DNA synthesis. In addition, iron exerts subtle effects on the proliferation and functionality of immune cells. On the one hand, iron is a cofactor for the generation of antimicrobial oxygen radicals by neutrophils and macrophages and essential for the clonal expansion of T cells. On the other hand, iron overload of mononuclear cells is associated with decreased cell-mediated immune function and impaired control of intracellular microbes, whose proliferation and radical detoxification are iron-dependent (Weiss, 2002). The control over iron homeostasis is thus of central importance in host–pathogen interaction, in which both opponents compete for iron (Weiss, 2002; Schaible and Kaufmann, 2004). Accordingly, a key pathway in host defence is the preclusion of invading microbes from iron, for which the term nutritional immunity was coined (Weinberg, 2000).

In extracellular microenvironments including blood vessels, interstitial spaces or epithelial surfaces, plasma proteins such as haptoglobin, haemopexin, lactoferrin (Lf), transferrin (Tf), lipocalin-1 and lipocalin-2 (Lcn2) bind different forms of iron in order to render this trace element unavailable for microbes. The subsequent uptake of these iron-shuttling proteins into the mononuclear phagocyte system (MPS) and the incorporation of iron into ferritin (Ft) multimers result in its withdrawal from extracellular pathogens and in the development of hypoferraemia (Weiss, 2005). However, many pathogens including bacterial, mycobacterial, protozoan and fungal pathogens have both extracellular and intracellular lifestyles. Immune-mediated mechanisms of iron withholding are different between extracellular and intracellular host compartments and can be specific for a given pathogen or its preferred intracellular habitat.

For host defence against intracellular pathogens, the mechanisms of iron restriction may contrast those initiated...
upon presence of extracellular bacteria. Both the downregulation of iron uptake pathways and the upregulation of iron export or storage mechanisms may participate in iron withholding from intracellular microbes (Fig. 1).

Pathways in systemic host iron homeostasis and their linkage to antimicrobial immune responses

Approximately 60–70% of body iron (2.500 mg) is bound within haemoglobin (Hb). Most of the iron needed for erythropoiesis is recovered by macrophages from senescent red blood cells (RBCs) via erythrophagocytosis (Knutson et al., 2005). Upon intravascular haemolysis, free Hb and haem molecules are captured in the circulation by two plasma proteins, i.e. haptoglobin and haemopexin respectively. Macrophages express CD163 for receptor-mediated endocytosis of both Hb–haptoglobin complexes and free Hb molecules (Moestrup and Moller, 2004), whereas CD91 mediates the uptake of haemopexin-bound haem molecules (Hvidberg et al., 2005). Haem-carrier protein 1 (Hcp1), a putative haem transporter, which, however, has also been shown to shuttle folate, may shift free haem molecules through the cell surface or the endosomal membrane to intracellular compartments for subsequent degradation by haem oxygenase-1 (Hmox1), an enzyme that yields ferrous iron, biliverdin and carbon monoxide (Schaer et al., 2007). Other cell types may take advantage of the haem-regulated gene-1 (Hrg1) protein to mediate cellular and endosomal/lysosomal haem transport (Hentze et al., 2010). The endocytosis of haemoglobin via CD163 is associated with an anti-inflammatory macrophage phenotype (Recalcati et al., 2010), which may be attributable to the induction of Hmox1 or to interleukin-10 (IL-10) secretion. In being scavenger receptors, CD163 and CD91 have additional functions in infectious diseases. While CD163 participates in uptake of streptococci, staphylococci and Escherichia coli into macrophages, thus inducing the secretion of pro-inflammatory cytokines, CD91 is involved in antigen presentation by dendritic cells (DCs) and subsequent activation of CD4 T cells (Wessling-Resnick, 2010). Of interest, haem may also act as a danger signal as it is recognized by toll-like receptor-4 (TLR4), resulting in secretion of tumour necrosis factor-α (TNF-α) and the murine IL-8 orthologue keratinocyte chemokine, thus promoting the generation of reactive oxygen species (ROS) and neutrophil recruitment (Figueiredo et al., 2007).

Extracellular bacteria such as Staphylococcus aureus damage erythrocytes by toxic haemolysins in order to exploit these cells as iron sources, while intracellular pathogens including protozoa are able to acquire Hb or haem molecules via cell surface receptors (Schaible and Kaufmann, 2004). Leishmania species express specific Hb and haem receptors because haem acquisition is of pivotal importance for survival of these parasites, which lack the pathway for haem biosynthesis (Sengupta et al., 1999). Trypanosoma species can utilize both haem and ionic iron, although the preferred uptake pathway differs between the acute and the chronic phase of infection, and iron overload of the MPS is associated with reduced survival in systemic trypanosomiasis (Stijlemans et al., 2008). Plasmodium falciparum is able to utilize Hb and haem within RBCs and to avoid haem-mediated oxidative damage by metabolizing it either to haemozoin or to biliverdin (Francis et al., 1997). Mammalian Hmox1 is induced in malaria, which may alter the availability of haem–iron for the parasite. The anti-inflammatory effects of Hmox1, however, are associated with increased parasite numbers in the liver (Epiphanio et al., 2008).

Intriguingly, a number of intracellular pathogens including influenza virus, mycobacteria, salmonellae and certain fungi can induce infection-associated haemophagocytic syndromes. These are characterized by the engulfment of RBCs by macrophages, a process driven by interferon-γ (IFN-γ). Haemophagocytic macrophages provide a survival niche for the growth of intracellular microbes as the engulfed Hb serves as a radical scavenger and as an iron source. Furthermore, the phagocytosis of RBCs impairs the ability of macrophages to kill intracellular microbes (Silva-Herzog and Detweiler, 2008).

Macrophages/monocytes express the haem transport protein feline leukaemia virus subgroup C receptor (Flvcr) for export of haem (Hentze et al., 2010). Flvcr-deficient macrophages exposed to immunoglobulin-coated RBCs present with increased intracellular Ft concentrations. This indicates, first, a role of the haem export protein Flvcr in erythrophagocytosis, and second, that haem derived from senescent RBCs is not fully degraded within the macrophages. A certain amount of this molecule is thus directly released into the circulation, which may limit the access of intracellular pathogens to haem.

Cellular iron uptake pathways and infections

Transferrin is the major iron shuttle in the circulation. Accordingly, monocytes and macrophages express transferrin receptor-1 (TfR1) to utilize Tf-bound iron. However, many intracellular bacteria gain access to Tf-bound iron. For instance, Mycobacterium avium colocalizes with TfR1 in early endosomes thus being able to acquire TfR1-delivered iron for its own demands (Halaas et al., 2010). Mycobacteria can arrest endosome/phagosome maturation, thus maintaining access to Tf-bound iron while avoiding the activation of bactericidal macrophage effector mechanisms. Of note, the ability of mycobacteria to interfere with phagosome maturation is dependent on their ability to acquire iron (Kelley and Schorey, 2003).
Francisella tularensis, which enters macrophages through early endosomes subsequently escaping to the cytosol, is able to access Tf-bound iron and to actively upregulate TIR1 expression in infected host cells. In contrast, inhibition of TIR1 expression is associated with reduced bac-

Fig. 1. Interplay between macrophage iron homeostasis and immune function. Macrophages possess a range of mechanisms implicated in uptake and utilization of different iron sources including (left-hand side, clockwise) erythrophagocytosis, CD91, CD163, Dmt1, TIR1, LcnR and LFR. Iron export pathways are limited to Flvcr as haem–iron exporter and Fpn1 (right-hand side), which exports ionic iron and is regulated by hepcidin as negative feedback signal (orange arrow). In addition, macrophages express FTRs, which may shuttle Ft from the cells to the circulation and presumably vice versa (lower left). Engulfed bacteria residing in the cytosol or within phagolysosomes are recognized by TLRs and NLRs, resulting in adaptive changes in the expression and activities of macrophage iron transporters. These are also regulated by the Th1 cytokine IFN-γ, which reduces TIR1-mediated iron uptake (red arrow), while increasing Slc11a1, Fpn1 and Lcn2 levels (green arrows) as mechanisms of microbial iron withdrawal (purple arrows). Employing counter-acting iron acquisition strategies, bacteria access macrophage iron uptake pathways as well as the cytosolic iron storage protein Ft (blue arrows). Iron and IFN-γ cross-regulate each other. Iron inhibits the expression of TNF-α, Lcn2, MHC-II and iNOS (red arrows), while IFN-γ stimulates these pathways (green arrows).

Abbreviations: CD-91, haem scavenger receptor; CD-163, haemoglobin scavenger receptor; Dmt1, divalent metal transporter-1; Tf, transferrin; TIR1, TIR receptor-1; Lcn2, lipocalin-2; LcnR, Lcn2 receptor; Lf, lactoferrin; LFR, Lf receptor; Flvcr, feline leukaemia virus subgroup C receptor; Fpn1, ferroportin-1; Ft, ferritin; FTR, FT receptor; TLR, Toll-like receptor; NLR, NOD-like receptor; IFN-γ, interferon-γ; Slc11a1, solute carrier 11 family member a1; TNF-α, tumour necrosis factor-α; MHC-II, major histocompatibility complex class II; iNOS, inducible NO synthase; phox, phagocyte oxidase; NF, nuclear factor; HIF, hypoxia-inducible factor; STAT, signal transducer and activator of transcription; ?, putative mechanisms.
Several pro-inflammatory cytokines, including IFN-γ, downregulate TFR1 expression in macrophages, thus reducing the intramacrophage access of iron for intracellular pathogens such as mycobacteria, Legionella pneumophila and Salmonella Typhimurium (Byrd et al., 1989; Zhong et al., 2001; Olakanmi et al., 2002).

The interaction between Tf-bound iron and TFR1 is modified by Hfe, an atypical major histocompatibility complex (MHC) class I molecule (Hentze et al., 2010). At the cellular level, Hfe influences transmembrane iron fluxes partly through its interaction with TFR1. In monocytes and macrophages in contrast, the absence of functional Hfe causes increased iron export and reduces intracellular iron levels (Drakesmith et al., 2002). At the systemic level, however, hepatocellular Hfe is involved in sensing of circulating levels of Tf-bound iron by a complex mechanism sequentially involving both TFR1 and TFR2 (Schmidt et al., 2008). Lack of Hfe results in reduced production of the master regulator of iron homeostasis, hepcidin, and subsequently, in increased duodenal iron absorption and parenchymal iron overload (Vujic Spasic et al., 2008). Of relevance, monocytes of patients with type I hereditary haemochromatosis (HH) carrying the C282Y mutation of HFE restrict iron from Mycobacterium tuberculosis more efficiently than monocytes isolated from healthy individuals, resulting in reduced numbers of intracellular mycobacteria (Olakanmi et al., 2002). Furthermore, the absence of functional Hfe is associated with increased production of the siderophore-binding peptide Lcn2 by murine macrophages, thus reducing the access of Salmonella Typhimurium to iron (Nairz et al., 2009). In contrast, Hfe-deficient mice display increased susceptibility to M. avium infection, which appears to be attributable to increased serum iron levels (Gomes-Pereira et al., 2008). Opposing susceptibilities of Hfe−/− mice to M. avium and Salmonella Typhimurium may result from different subcellular localization of these pathogens or from differences in the efficacy of their iron-acquisition systems. Interestingly, β2-microglobulin−/− mice which lack both Hfe and MHC class I expression on the cell surface display high susceptibility to mycobacterial infection which can be corrected by treatment with Lf-mediated iron chelation (Schaible et al., 2002).

In addition, macrophages can acquire molecular iron via the divalent metal transporter-1 (Dmt1), which is also involved in the transfer of iron acquired via erythropagocytosis from the phagolysosome to the cytoplasm (Soe-Lin et al., 2009). M. tuberculosis and Salmonella Typhimurium can acquire iron, and these pathogens as well as Chlamydia pneumonia and F. tularensis can upregulate Dmt1 mRNA levels (Zhong et al., 2001; Paradkar et al., 2008; Pan et al., 2010). While the cytokine-mediated induction of Dmt1 by macrophages
the iron binding moiety of enterobactin (2,3-DHBA), and interacts with Lcn2. The iron–2,5-DHB–Lcn2 complex is required for iron shuttling within mammalian cells and delivers iron to mitochondria, the organelles of haem biosynthesis (Devireddy et al., 2010). In addition, Lcn2 binds iron in association with catechols, which can be derived from both mammalian and bacterial metabolic pathways (Bao et al., 2010). Interestingly, Lcn2 is trafficked within the cell by a mechanism distinct from Tf. Although both are initially taken up into early endosomes, Tf-bound iron is subsequently released by a reduction of endosomal pH, reduced by six-transmembrane epithelial antigen of the prostate-3 (Steap3) and shifted to the cytosol via Dmt1. The H-subunit (H-Ft) is catalytically active and responsible for the storage of iron. Moreover, H-Ft is able to rescue TNF-α-stimulated cells from apoptosis by inhibiting the generation of ROS (Pham et al., 2004). Relevantly, mammalian cell surface receptors for Ft subunits (FtRs) have recently been described. Whereas H-Ft appears to be recognized by the T-cell immunoglobulin and mucin domain-2 (Tim-2) protein in the mouse and by Tfr1 in humans, scavenger receptor member 5 (SCARF) is the cell surface receptor for L-Ft (Hentze et al., 2010). Of interest, Ft can be exported from macrophages and monocytes by a lysosomal secretory pathway (Cohen et al., 2010). Some microbes, such as Neisseria meningitidis, are able to utilize cytosolic Ft as an iron source. In addition, the growth of Listeria monocytogenes in liquid cultures is promoted by Ft, and Entamoeba histolytica can acquire Ft via endocytosis, suggesting that taxonomically unrelated pathogens are able to utilize multimeric Ft as an iron source (Larson et al., 2004; Schaible and Kaufmann, 2004). Macrophages infected with Salmonella Typhimurium reduce the incorporation of iron into Ft in order to withhold iron from Salmonella, which can utilize holo-Ft for proliferation (Nairz et al., 2007). The storage of iron within Ft is a bi-directional pathway because iron can be mobilized via sequential proteasome-mediated Ft degradation and Fpn1-facilitated iron export (De Domenico et al., 2006; Hentze et al., 2010). Moreover, extracellular Ft has immuno-modulatory functions since it stimulates the production of cytokines including IL-1β (Ruddell et al., 2009; Wessling-Resnick, 2010).

**Iron efflux as antimicrobial defence strategy against intracellular pathogens**

Although several avenues exist for iron uptake into cells, the best-characterized export route is Fpn1-mediated (Fig. 1). The presence of Fpn1 within the cell surface membrane is regulated at multiple levels including transcriptional, post-transcriptional and translational mechanisms (Hentze et al., 2010). Following phagocytosis of the intracellular bacterium Salmonella Typhimurium, Fpn1 expression is stimulated, resulting in increased iron export from infected macrophages and in reduced iron availability for this microbe (Nairz et al., 2007). Although the individual contributions of different Fpn1 transcripts to this process remain to be determined, the induction of Fpn1 expression is linked to the presence of viable bacteria. Accordingly, overexpression of Fpn1 efficiently limits intracellular Salmonella replication (Chiosta et al., 2006). In contrast, TLR4 ligation with lipopolysaccharide (LPS) causes downregulation of Fpn1 mRNA expression in macrophages (Yang et al., 2002; Ludwiczek et al., 2003). In addition, reduced Fpn1 cell surface levels result from targeting of the protein by hepcidin. Binding of hepcidin leads to Fpn1 internalization and proteasomal degradation, thus blocking cellular iron egress (Nemeth et al., 2004b). Nonetheless, the exact biophysical transport characteristics by which Fpn1 shuttles ferrous iron through the membrane, which is then oxidized by membrane-anchored hephaestin or circulating ceruloplasmin, remain to be elucidated (Hentze et al., 2010). Of
relevance, hepcidin–Fpn1 interaction induces signalling via signal transducer and activator of transcription-3 (STAT3), with subsequent transcriptional reprogramming of hepcidin-exposed macrophages (De Domenico et al., 2010). Exposure of LPS-stimulated macrophages to hepcidin results in a reduced mRNA expression of TNF-α and IL-6, which may be a consequence of increased iron retention in macrophages because iron blocks these pro-inflammatory immune effector pathways (Oexle et al., 2003; Weiss, 2005; De Domenico et al., 2010). However, in mice the expression of hepcidin was reduced following infection with *Schistosoma japonicum*, resulting in increased iron absorption and promotion of pathogen growth (McDonald et al., 2010).

Of interest, Fpn1 is also present within *Mycobacterium*-containing phagosomes. Thereby, Fpn1 may contribute to mycobacterial iron restriction (Van Zandt et al., 2008). Accordingly, cytokines such as IFN-γ exert part of their anti-bacterial activity by reducing the intramacrophage availability of iron to intracellular microbes (Olakanmi et al., 2002). In addition, macrophage-derived cytokines such as IL-6 and IL-1 stimulate hepatocellular synthesis of the hepcidin (Nemeth et al., 2004a). High circulating hepcidin levels then negatively regulate plasma iron concentration, which may lead to the development of anaemia of chronic disease (ACD) (Weiss and Goodnough, 2005). Presumably, this response to inflammatory and infectious stimuli limits the availability of iron for infectious agents and malignant cells, both of which need the metal for their growth and proliferation. Hypoferraemia and ACD are thus thought to occur as pathophysiological adaptations to the presence of microbes and tumour cells respectively. Iron supplementation to treat anaemia under these conditions is controversial as it may promote the progression of the underlying infectious disease, but there is a lack of data from prospective clinical trials to support this hypothesis (Weiss and Goodnough, 2005). However, clinical studies are required to determine the optimal treatment regimen in ACD and the risk–benefit ratios of iron supplementation, endogenous iron mobilization by modifying cytokine and hepcidin-mediated iron restriction in monocytes/macrophages, treatment with erythropoietic agents and combinations thereof in ACD as a function of the underlying disease, sex as well as baseline and target Hb levels.

Monocytes and macrophages secrete hepcidin in the presence of inflammatory stimuli, and the autocrine formation of hepcidin limits iron egress to extracellular pathogens by diminishing Fpn1 functionality (Peyssonnaux et al., 2006; Theurl et al., 2008). However, this strategy will be counter-productive in infections with intracellular pathogens (Fig. 1). In *Mycobacteria*-infected macrophages, hepcidin is present within the phagolysosomal compartment where it may exert direct anti-mycobacterial effects (Sow et al., 2007). Hepcidin expression can be also induced via TLR2-mediated signalling, which results in limitation of iron availability to *Borrelia burgdorferi* (Koening et al., 2009). Whether intracellular patterns recognition receptors (PRRs) such as NOD-like receptors (NLRs) influence hepcidin production is currently unknown (Fig. 2). Of note, T cells also produce hepcidin in response to T-cell receptor (TCR) ligation. The subsequent downregulation of membrane Fpn1 is suggested to increase intracellular iron levels and to promote T-cell proliferation (Pinto et al., 2010).

The solute carrier family 11 member a1 (Slc11a1, formerly termed Nramp1) is a transmembrane protein expressed in the late phagolysosomes of macrophages, in secondary granules of neutrophils and in organelles of DCs (Valdez et al., 2008). The expression of Slc11a1 is associated with resistance to infections with the intracellular pathogens mycobacteria, salmonellae and leishmaniae. A key function of Slc11a1 appears to be the modulation of iron availability in the pathogen-containing vacuole (Blackwell et al., 2001; Forbes and Gros, 2001) (Fig. 2). In *Salmonella* Typhimurium infection, Slc11a1 functionality in macrophages and DCs is associated with increased formation of pro-inflammatory effector molecules such as TNF-α, IL-6, IL-12 and inducible NO synthase (iNOS) and a reduced expression of the anti-inflammatory cytokine IL-10 (Fritsche et al., 2008). In line with these observations, Slc11a1-mediated resistance against *Leishmania* and *Mycobacteria* is attributable to a predominant Th1-type immune response (Forbes and Gros, 2001). Furthermore, Slc11a1 promotes the maturation of *Salmonella*-containing phagosomes and stimulates the acidification of phagolysosomes (Jabado et al., 2000). Of interest, Slc11a1 functionality also enhances macrophage iron release (Fritsche et al., 2007). This may be the consequence of more efficient haem–iron recycling from phagocytosed RBCs and subsequent increase of Fpn1 expression and iron-mediated translational induction of Fpn1 expression (Hentze et al., 2010). The predominant Th1 immune effector function in Slc11a1-expressing cells may result from cellular iron depletion as iron negatively affects pro-inflammatory immune effector pathways in macrophages (Weiss, 2002).

**Networks between iron homeostasis and immune effector pathways**

There are numerous cross-regulatory interactions between iron homeostasis and immune responses (Weiss, 2005). Cytokines such as IFN-γ and TNF-α affect systemic iron homeostasis and cellular iron fluxes, while iron is an important regulator of immune effector functions, immune cell proliferation and cytokine production (Ludwiczek et al., 2003; Weiss, 2005). Iron availability and
Th1-type immune responses are mutually linked (Fig. 1). Iron antagonizes the IFN-γ-induced expression of MHC class II, iNOS and TNF-α (Weiss et al., 1994; Oexle et al., 2003; Nairz et al., 2007). This may be attributed to a direct influence of iron on the binding affinities of transcription factors such as NF-IL6, interferon regulatory factor (IRF)-1 or hypoxia-inducible factor (HIF)-1α to target gene promoters (Wessling-Resnick, 2010). These effects contribute to impaired killing of intramacrophage Salmonella by iron-laden macrophages. On the other hand, iron overload inhibits IFN-γ activity and shifts the Th1/Th2 differentiation towards a Th2 type reaction characterized by increased production of IL-4 and IL-10 (Mencacci et al., 1997). Studies in mice extend these findings to mucormycosis, in which the application of an iron chelator results in an improved survival paralleled by Th1/Th2 shift towards the Th1 phenotype and increased expression of protective, pro-inflammatory cytokines (Ibrahim et al., 2007). Of interest, IFN-γ regulates the expression of several genes of iron metabolism. IFN-γ induces hepcidin expression in macrophages infected with M. tuberculosis by activation of the transcription factors NF-kB and STAT1 (Sow et al., 2009). In monocytes, IFN-γ stimulates Dmt1 expression and triggers the uptake of non-Tf bound iron, while decreasing the mRNA expression of both TfR1 and Fpn1 (Ludwiczek et al., 2003). Intriguingly, IFN-γ is also important for the control of Cryptosporidium parvum proliferation, and the supplementation of infected intestinal cells with ferrous iron counteracts the growth-limiting effects of IFN-γ (Pollok et al., 2001).

Tumour necrosis factor-α exerts regulatory effects on systemic iron balance by controlling the expression of intestinal and MPS iron transporters. In contrast, there is no effect of TNF-α on hepcidin expression in vitro (Hentze et al., 2010). Apparently, TNF-α influences the transcriptional expression of Dmt1 and Fpn1, resulting in diminished iron absorption and iron storage within duodenal enterocytes. Moreover, TNF-α stimulates macrophage iron accumulation by promoting Dmt1 and blocking Fpn1 expression (Ludwiczek et al., 2003). In contrast, iron
appears to influence TNF-α production. On the one hand, reduced intracellular iron levels impair TNF-α secretion by interfering with TLR4 signalling and likewise NF-κB activation (Wang et al., 2009) (Fig. 1). On the other hand, macrophage iron overload reduces TNF-α transcriptional expression, although the transcription factors involved in this response remain to be elucidated (Oexle et al., 2003; Wessling-Resnick, 2010).

Of relevance, iron profoundly affects the efficacy of macrophage immune functions. Distinct amounts of iron are required for the generation of ROS by the Fenton reaction and via the catalytic action of phagocyte oxidase (phox). Correspondingly, treatment of Salmonella-infected mice with the iron chelator desferrioxamine (DFO) impairs pathogen clearance due to reduced ROS generation (Collins et al., 2002). In contrast, iron impairs pro-inflammatory effector pathways in macrophages (Fig. 1). Thus, a certain amount of iron is important for the formation of oxygen radicals while iron overload has immune-debilitating effects. It is well established that macrophages can undergo polarization towards either a M1 or a M2 phenotype. Whereas M1 macrophages are classical activated via PRRs in the presence of the Th1 cytokine IFN-γ, M2 type macrophages are considered as alternatively activated through IL-4. Macrophages polarized towards a M1 phenotype have decreased CD163, Hmox1 and Fpn1 levels and increased iron retention within Ft, resulting in a smaller LIP. In contrast, M2 macrophages have reduced iron storage and IRP activation secondary to increased TIR1, Hmox1 and Fpn1 levels (Ludwiczek et al., 2003; Recalcati et al., 2010; Wessling-Resnick, 2010). Future studies will reveal to which extent iron contributes to different effector functions of M1 and M2 macrophages in infectious diseases.

All lymphocyte subsets, including B cells, T cells and natural killer (NK) cells, require sufficient amounts of iron for growth and proliferation. Consequently, iron has effects on the expansion of Th cell populations following TCR engagement. Studies using anti-TIR1 antibodies suggest that Th1 cells are more susceptible to iron limitation as compared with Th2 cells (Weiss, 2002). Correspondingly, patients with HH or secondary iron overload display quantitative and qualitative alterations in T-cell subsets (Porto and De Sousa, 2007).

**Magnetic attraction to pathogens – iron in microbial metabolism**

Pathogens have specific iron requirements due to the metal’s role for metabolic functions and proliferation. Moreover, the detoxification of antimicrobial radicals requires iron because superoxide dismutase is an iron-dependent enzyme. Consequently, microbes have multiple routes to acquire different forms of iron including receptors for Hb, haem, Lf, Tf, Ft, ferric iron, ferrous iron, haemophores and siderophores. The expression of iron uptake systems is linked to virulence in a broad range of pathogens including bacteria, protozoa and fungi (Schaible and Kaufmann, 2004; Schrettl et al., 2004; Crouch et al., 2008; Seifert et al., 2008). Gram-negative bacteria require sophisticated iron uptake machineries to cross the outer and inner membranes, respectively, usually involving an outer membrane receptor, a periplasmatic shuttling protein and an inner membrane transporter (Fig. 2). Of relevance, pathogens also compete with commensal microbes for iron. Certain siderophores originating from specific bacteria can be utilized by other microbes which cannot produce them. Of note, excessive amounts of iron can be toxic for microbes, which consequently have acquired the ability to detoxify and store iron (Halsey et al., 2004; Velayudhan et al., 2007).

Remarkably, pathogens have evolved strategies to subvert host pathways of iron restriction. For instance, Leishmania amazonensis is capable of upregulating its ferrous iron importer LIT-1 within phagolysosomes and its expression is further increased during iron starvation as a consequence of Slc11a1-activity by host macrophages (Huynh and Andrews, 2008). Salmonella expresses a set of virulence genes encoded within Salmonella pathogenicity island-2 as a specific response to Slc11a1-mediated iron starvation (Zaharik et al., 2002). Similarly, M. tuberculosis enhances siderophore production when confronted with Slc11a1 activity. In addition, M. tuberculosis residing within IFN-γ-activated macrophages induces expression of genes for mycobactin synthesis whereas bacterioferritin production and thus iron storage is suppressed, implying that the pathogen tries to adapt to the iron-restricted environment created by macrophage activation (Rachman et al., 2006). Notably, toxins associated with virulence of L. monocytogenes and Salmonella Typhimurium are induced upon iron starvation (Schaible and Kaufmann, 2004). Therefore, iron restriction may provide one of the signals to start toxin production.

Based on the central role of iron in infections, the potential of iron targeting strategies to combat infections has been investigated in experimental and clinical studies. In malaria, for instance, DFO is able to promote the recovery from coma in children with cerebral malaria (Gordeuk et al., 1992). This is partly attributable to the immuno-modulatory role of iron chelation via increased NO and decreased IL-4 production in DFO-treated patients, while, on the other hand, DFO also limits plasmodial iron access. DFO also exerts beneficial effects in experimental infections with Histoplasma capsulatum or T. cruzi, whereas it can be detrimental in a certain other infections via its inhibitory role for phox activity (Collins et al., 2002) or because some pathogens, including yersiniae or mucorales, can utilize DFO as siderophore. Orally bioavailable iron chelators

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such as desferasirox and deferiprone limit the growth of *Chlamydia psittaci*, *C. trachomatis* and *L. pneumophila* and may be suitable as add-on therapies in mucormycosis (Ibrahim et al., 2007; Paradkar et al., 2008). Novel approaches to target infectious agents may be based on the interference with iron uptake systems of microbes.

**Conclusion**

With the exception of *B. burgdorferi*, most human-pathogenic bacteria, many viruses, including HIV and hepatitis C virus, parasites and fungi have an essential requirement of iron. The withholding of iron from invading microbes is thus an important strategy of host defence, acting in line with the principal of nutritional immunity, whereby iron availability is limited for invading pathogens. The host's inducible pathways leading to microbial iron restriction are incompletely understood and differ mainly between intra- and extracellular pathogens. Because iron is a significant modifier of host immune responses and immune cell proliferation and differentiation, the tight control of iron homeostasis is a central battlefield designating the fate of an infection. Pharmacological modification of either host iron metabolism and/or microbial iron acquisition may hold the key for the development of new strategies to treat or prevent infections. Iron chelators with improved pharmacokinetic properties may become important antimicrobial drugs. Furthermore, microbial iron transporters and iron acquisition pathways are attractive targets for the development of new medications to fight infections in an era of an increasing burden of antimicrobial drug resistance.

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