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# The macrophage: A cellular factory at the interphase between iron and immunity for the control of infections

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## Introduction

Likewise the macrophage is the most important cellular player at the interface between iron and immunity. This is due to the facts that macrophages need iron to produce highly toxic hydroxyl radicals, 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. At the same time iron modulates macrophage effector pathways by regulating cytokine activities, the induction of anti-microbial effector pathways of macrophages and indirectly via regulating lymphocyte proliferation and activities which then affect macrophage differentiation and activation. Since iron is an essential compound for microbial growth and proliferation the control over iron homeostasis is a central battlefield deciding about the fate of an infection. Thus, it is not surprising that phagolysosomal proteins which are associated with resistance towards infections with intracellular pathogens also act as iron transporters. Thus, alterations in immune function affect iron homeostasis and *vice versa* (Porto *et al.* 1994; Roy & Andrews 2001; Meyer *et al.* 2002; Weiss 2002b).

This minireview should provide an overview of our current knowledge of the iron immunity network and how this interplay may be favorably affected in order to obtain a better control over certain infectious agents.

## Pathways for iron acquisition by macrophages

A sufficient supply of iron is essential for all living and proliferating organisms and thus, in the case of an infectious disease also for both- the host and the microbe. This relates to the essential role of iron in oxygen transport by hemoglobin and myoglobin, in electron transport for mitochondrial respiration as being a part of complex I and II enzymes, in the regulation of transcription via its role as central component of ribonucelotid reductase or by modulating the binding affinities of central transcription factors via its catalyzing role for radical processes. Accordingly, both, mammalian cells and microbes, have evoked sophisticated strategies to acquire iron even under conditions of limited availability. The divergent pathways by which micro-organisms can acquire iron have been recently reviewed elsewhere (Hantke 2001; Winkelmann 2002). When keeping focused on the immune system iron not only affects the proliferation and differentiation of immune cells but also modulates their anti-microbial effector pathways. Thus, both, iron deficiency and iron overload exert detrimental effects on immune function.

Especially, lymphocytes are dependent on a sufficient supply of iron needed for their growth (Brock 1981; Latunde-Dada & Young 1992). All lymphocyte subsets are dependent on transferrin/transferrin receptor (TfR) mediated iron uptake

(Seligman *et al*. 1992), however, the dependence on this iron uptake pathway differs between the various lymphocyte subtypes. 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 (de Sousa *et al*. 1991). Moreover, Th-1 cells are very sensitive to environmental changes of iron availability, which is not the case for Th-2 cells (Thorson *et al*. 1991). This is of importance since Th-1 and Th-2 cells and their offspring cytokines have contrasting effects on immune function. Th-1 cells are generated and activated during the acute phase of an inflammatory process or an infection, resulting in the release of interferon (IFN)-$\gamma$ and tumor necrosis factor (TNF)-$\alpha$ and $\beta$ which then activate effector pathways in macrophages, such as the formation of pro-inflammatory cytokines, like TNF-$\alpha$, IL-1 or IL-6, and the production of toxic radicals such as oxygen free radicals and nitric oxide (NO). By contrast, Th-2 cells produce IL-4, IL-5, IL-9 and IL-13, which in part exert anti-inflammatory actions via inhibition of various macrophage functions. In addition, a third subspecies with immuno-suppressive properties, Th3 and T- regulatory $CD25^+$ $CD4^+$ cells ($T_{REG}$) has been described. 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-$\beta$) or IL-10, respectively (Farrar *et al*. 2002).

Immune cells have evoked various pathways to acquire, store and re-circulate iron (Oppenheimer 2001; Weiss 2002a). While the ''classical'' mammalian iron uptake mechanism via transferrin/TfR mediated endocytosis is the preferred pathway in lymphocytes, macrophages have an increased capacity to acquire the metal via transmembrane ion channels, lactoferrin and most importantly via phagocytosed senescent erythrocytes.

Specifically, the divalent metal transporter (DMT-1) is highly expressed on macrophages. DMT-1 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. DMT-1 is also of importance for the transfer of iron from the endosome into the cytoplasm, and mutations of this transporter are associated with the development of iron deficient anemia (for review see (Andrews, 1999)). DMT-1 co-operates 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 DMT-1(McKie *et al*. 2001). Accordingly, human macrophages take up iron-chelates with a higher efficacy than diferric Tf by a temperature dependent but pH independent process (Olakanmi *et al*. 1994, Ludwiczek *et al*. 2003).

Immune cells also express receptors for H-ferritin (Moss *et al*. 1992) which may be involved in the iron turnover and exchange between lymphocytes, hepatocytes and macrophages.

The iron binding protein, lactoferrin (Lf), is a member of the transferrin family and able to bind 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(Brock, 2002). Lf is taken up after binding to specific receptors, Lf receptors (LfR), which are found at the cell surface of all lymphocyte types and macrophages. The Lf/LfR is internalized most likely via an endocytotic process (Crouch *et al*. 1992). Evidence for the existence of Lf re-circulation in macrophages was provided by experiments, demonstrating that Lf is released from macrophages which have been incubated in Lf free media (Brock, 2002).

In addition, macrophages can acquire iron via phagocytosis of hemoglobin–haptoglobin (Hb–Hp) complexes. The receptor being responsible for this endocytotic process has been named, CD163, and is a member of the scavenger receptor cysteine rich (SRCR) domain family. This specific receptor-ligand interaction leads to removal of Hp–Hb complexes – but not free Hp or Hb – from the circulation (Graversen *et al*. 2002).

A major pathways by which monocytes and macrophage acquire iron is erythrophagocytosis. Senescent erythrocytes are taken up via phagocytosis and are then destroyed within monocytes/macrophages. Within the macrophage iron is then released from hemoglobin, a enzymatic step exerted by the enzyme heme-oxygenase-1, and rapidly shifted to re-utilization via incorporation into iron proteins or iron storage within ferritin (Kitagawa *et al*. 1996; Moura *et al*. 1998; Zuckerbraun & Billiar 2003).

Once iron enters cells it is either stored within ferritin or utilized upon incorporation into iron containing enzymes. An estimate of 10–20% per-

cent remain in the labile iron pool which is important for macrophage effector functions and the regulation of cellular iron homeostasis (Oppenheimer, 2001). The latter is maintained mainly at the postranscriptional/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 amino levulinic 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 review see (Rouault & Klausner 1997; Eisenstein & Ross 2003; Hentze *et al*. 2004). 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. 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 (Drapier *et al*. 1993; Weiss *et al*. 1993; Pantopoulos & Hentze 1995; Cairo *et al*. 2002; Schneider & Leibold 2003).

Importantly, macrophages/monocytes release iron which is essential for iron re-circulation form degraded erythrocytes. HFE is a non-classical MHC class I molecule which is ubiquitously expressed on cells (Feder *et al*. 1996) and which has been found to be mutated in 80% of patients suffering from hereditary hemochromatosis (Feder *et al*. 1996; Anderson & Powell 2002; Pietrangelo, 2004). HFE interacts with Tf mediated iron uptake by forming a stoichiometric complex with TfR which lowers the affinity of TfR for iron loaded transferring and thus, HFE affects cellular iron homeostasis (Parkkila *et al*. 1997; Lebron *et al*. 1998; Fleming & Sly 2002; Ludwiczek *et al*. 2004). Interestingly, HFE also serves as a ligand for the gamma-delta T-cell-receptor which may be of importance for enterocyte differentiation. (Ten Elshof *et al*. 1999). In addition, HFE blocks the iron release from macrophages (Drakesmith *et al*. 2002), as transfection of macrophages from hemochromatosis patients carrying the C282Y mutation with wild-type HFE resulted in an increased iron content of these cells (Montosi *et al*. 2000).

The cellular mechanism underlying these observations may be traced back to modulation of iron release by ferroportin (Pietrangelo, 2002). 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. Mutations of ferroportin lead to iron overload disorders. After being transported by ferroportin ferrous iron undergoes oxidation which is maintained by the membrane bound ferrioxidase hephaestin, and ferric iron release from cells can than be transferred for incorporations to transferrin (Vulpe *et al*. 1999).

## Regulation of iron homeostasis by cytokines

Under inflammatory conditions, both Th-1 and Th-2 derived cytokines, as well as short lived radicals and acute phase proteins affect iron metabolism both by IRP/IRE dependent and independent pathways leading to typical changes of body iron homeostasis (Figure 1).

The injection of pro-inflammatory cytokines, such as IL-1 or TNF-$\alpha$, into mice resulted in hypoferremia and hyperferritinemia (Alvarez-Hernandez *et al*. 1989) which could be traced back to transcriptional induction of ferritin expression by cells of the reticulo-endothelial system (Konijn *et al*. 1981; Torti & Torti 2002). In addition, IL-1 and IL-6 increase ferritin expression translationally via stimulation of an ''acute phase box'' located within the 5′ untranslated region of ferritin mRNA (Torti & Torti 2002).

However, only limited information is available on how the induction of ferritin synthesis may lead to hypoferremia since pro-inflammatory cytokines (TNF-$\alpha$, IL-1, IFN-$\gamma$) rather down-regulate TfR expression (Byrd & Horwitz 1993; Weiss 2002b; Ludwiczek *et al*. 2003). As outlined above iron acquisition by macrophages is exerted by multiple pathways. Under inflammatory conditions erythrophagocytosis by resident and circulating monocytic cells is increased which can be referred

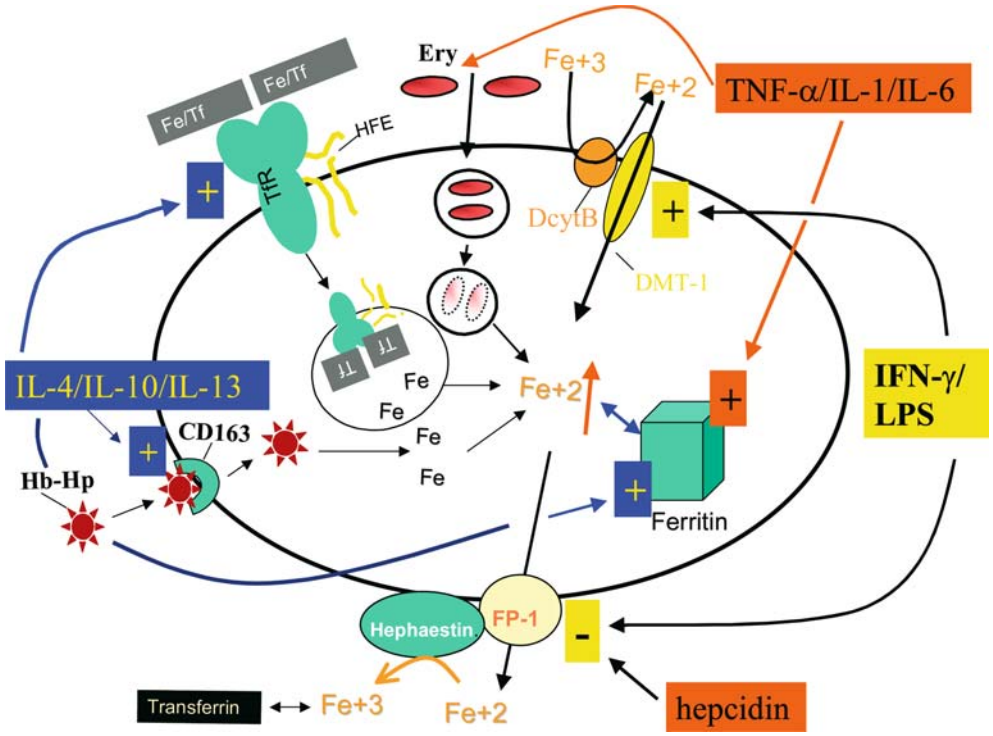


*Figure 1*. Pathways for the regulation of iron homeostasis in macrophages by pro-and anti-inflammatory cytokines. The pro-inflammatory cytokines TNF-α, IL-1 and IL-6 induce ferritin synthesis and thus iron storage. TNF-α in addition increases the erythrophagocytosis, which is further enhanced by damaging of erythrocyte membranes by cytokine induced radicals. IFN-γ and LPS increase intracellular iron levels stimulating the up-take of ferrous iron via induction of DMT-1 expression and by inhibiting the expression of the iron export protein ferroportin (FP-1). In addition, FP-1 mediated iron export is also blocked by circulating acute phase protein hepcidin. The anti-inflammatory cytokines IL-4, IL-10 and IL-13 counteract IFN-γ/LPS mediated effects on iron proteins, thereby increasing ferritin translation and TfR expression. Moreover, IL-10 promotes the uptake of hemoglobin/haptoglobin complexes into macrophages by stimulating CD163 expression.

to the action of TNF-α which induces the expression of C3bi (CD11b/CD18) receptors on macrophages and thus erythrophagocytosis. The latter process is also enhanced via damaging of erythrocyte membranes by cytokine induced radical products (Moldawer *et al*. 1989).

In parallel, pro-inflammatory stimuli, IFN-γ, lipopolysacharide (LPS) or TNF-α, induce DMT-1 expression and subsequently ferrous iron uptake in activated macrophage (Yang *et al*. 2002; Ludwiczek *et al*. 2003). The intriguing relationship between immunity and iron homeostasis went into a new dimension upon identification of the acute phase protein hepcidin (Ganz 2003; Loreal & Brissot 2003) which is a liver derived, anti-microbial, pro-peptide of 84 amino acid length subsequently cleaved to its active form of 25 aminoacids. The expression of hepcidin is increased by iron loading while iron deficiency or anemia decrease it (Pigeon *et al*. 2001; Nicolas *et al*. 2002). Hepcidin is also induced by LPS and IL-6 while TNF-α blocks its expression in hepatocytes (Ganz 2003; Nemeth *et al*. 2004a). Injection of IL-6 into volunteers resulted in increased hepcidin expression and induction of hypoferremia within 24 h. In addition, IL-6 knock out mice which were treated with turpentine in order to induce an inflammatory state did not develop hypoferremia (Nemeth *et al*. 2004a). The mechanism by which hepcidin may induce hypoferremia include an inhibition of duodenal iron absorption and blockage of macrophage iron release by interacting with ferroportin (Fleming & Sly 2001; Andrews 2004; Nemeth *et al*. 2004b).

Another acute phase protein, α1-anti-trypsin interferes with cellular iron homeostasis by competitively blocking the binding of transferrin to TfR thus reducing TfR mediated iron uptake especially by erythroid cells, while macrophages are not affected. (Graziadei *et al*. 1994).

The central Th-1 mediated cytokine IFN-$\gamma$ has distinct effects on iron homeostasis. IFN-$\gamma$ induces ferritin transcription but also affects ferritin translation which can be referred to activation of IRP binding affinity by the cytokine. This is due to stimulation of nitric oxide NO formation by IFN-$\gamma$ in murine macrophages (Drapier *et al.* 1993; Weiss *et al.* 1993) and subsequent activation of IRP-1. In addition, pro-inflammatory cytokines induce the formation of oxygen radicals by macrophages. One of these products, hydrogen peroxide, is an activator of IRP-1 by a process involving kinase/phosphatase signal transduction pathways thus affecting TfR and ferritin expression (Pantopoulos & Hentze 1995; Hentze *et al.* 2004).

Although, IFN-$\gamma$ treatment of monocytes leads to down-regulation of TfR expression (Byrd & Horwitz 1993; Weiss 2002b; Ludwiczek *et al.* 2003), the cytokine induces DMT-1 expression thus stimulating the uptake of ferrous iron and its subsequent incorporation into ferritin (Ludwiczek *et al.* 2003). At the same time IFN-$\gamma$/LPS induce iron retention in macrophages by down-regulating the transcriptional expression of ferroportin, thus blocking iron release from these cells (Yang *et al.* 2002; Ludwiczek *et al.* 2003)

Some of these IFN-$\gamma$ mediated pathways are counter-acted by anti-inflammatory, Th-2 cells derived, cytokines which include IL-4, IL-10 or IL-13. These cytokines suppress IFN-$\gamma$ inducible NO formation and IRP activation which concomitantly enhances ferritin translation and counteract the IFN-$\gamma$ induced inhibition of TfR expression (Weiss *et al.* 1997a). In addition, IL-10 as IL-6 affect macrophage iron acquisition by stimulating the expression of hemoglobin scavenger receptor, CD163, and thus promoting the uptake of hemoglobin/haptoglobin complexes into monocytic cells (Graversen *et al.* 2002).

The role of Th-2 derived cytokines for the development of hyperferritinemia under chronic inflammatory processes has been confirmed by a clinical study involving patients suffering from Crohn's disease. Therapeutic application of human recombinant IL-10 as part of a placebo controlled, double blinded study resulted in the development of a normocytic anemia which was preceded by a significant increase in serum ferritin levels (Tilg *et al.* 2002). Both, anemia and hyperferritinemia spontaneously resolved within 2–4 weeks after stopping IL-10 therapy. Thus, Th-2 derived cytokines may increase iron uptake via induction of TfR and CD163 and promote iron incorporation into ferritin within activated macrophages.

The combined action of these cytokines and their by-products leads to a diversion of iron traffic resulting in decreased iron absorption, increased iron storage within cells of the reticuloendothelial system while circulating iron levels are reduced and the availability of iron for erythroid progenitor cells is restricted. Thus, under conditions of chronic immune activation hypoferremia and hyperferritinemia occur which are the diagnostic hallmarks for the anemia of chronic disease (Matzner *et al.* 1979; Weiss 2002c; Means 2003; Andrews 2004). Anemia of chronic disease (ACD) is the most frequent anemia in hospitalized patients occurring frequently in subjects suffering from chronic inflammatory disorders, such as auto-immune diseases, chronic infections, cancer or post transplantationem. ACD can be diagnosed according to the underlying diversion of iron traffic described above (Weinstein *et al.* 2002; Weiss 2002c; Goodnough *et al.* 2003; Means 2003).

Although, the development of anemia is associated with detrimental effects especially in relation to cardiac function, quality of life, growth and mental development the underlying hypoferremia and the diversion of iron from the circulation may also harbor some potentially positive effects, especially when the diseases underlying ACD are cancer or infections.

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 (Weinberg 1999).

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

Third, reducing the concentrations of metabolically active iron strengthens the immune response directed against invading pathogens and tumor cells by stimulating cell mediated immune effector pathways of macrophages and by affecting

the differentiation of lymphocyte subsets as detailed above.

Moreover, iron overload in association with immune activation may generate increased amounts of toxic radicals which can cause tissue damage.

## Modulation of macrophage immune effector pathways by iron

The direct effects of iron on cell mediated immune function became first evident by the observation that iron loading of macrophages results in an inhibition of IFN-$\gamma$ mediated pathways in macrophages such as formation of the pro-inflammatory cytokine TNF-$\alpha$, reduced expression of MHC class II antigens and ICAM-1, decreased formation of neopterin, and impaired tryptophan degradation via IFN-$\gamma$ mediated induction of indole-amine-2,3-dioxygenase (Weiss *et al*. 1992; Recalcati *et al*. 1998; Oexle *et al*. 2003). As a consequence of this iron loaded macrophages have an impaired potential to kill various bacteria, viruses, parasites and fungi by IFN-$\gamma$ mediated pathways (Kontoghiorghes & Weinberg 1995; Weiss 2002b). 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 (Bogdan 2001). 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 (MacMicking *et al*. 1997) 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 (Weiss *et al*. 1994; Melillo *et al*. 1997; Dlaska & Weiss 1999). According to the regulatory feed back loop, NO produced by activated macrophages activates the IRE-binding function of IRP-1, leading to inhibition of ferritin translation (Drapier *et al*. 1993; Weiss *et al*. 1993), thus linking maintenance of iron homeostasis to NO formation for host defense.

Via its de-activating effect towards IFN-$\gamma$ function iron then affects the Th-1/Th-2 balance with Th-1 effector functions being weakened while Th-2 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 (Mencacci *et al*. 1997; Weiss *et al*. 1997b). 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 phagocytoze foreign particles (Patruta *et al*. 1998).

Thus, both iron overload and iron deficiency have unfavorable immunological effects *in vivo*. Accordingly, mice kept on an iron rich diet had a reduced production of IFN-$\gamma$ as compared to mice fed with a normal diet, while animals receiving an iron deficient diet presented with a decreased T-cell proliferation (Omara & Blakley 1994). 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.

Several studies investigated the effects of iron homeostasis on the course or incidence of infections (Weinberg 1999). Interestingly, iron deficient children had a reduced incidence of certain infections as compared to children with a balanced iron status (Weinberg 1999; Oppenheimer 2001; Nyakeriga *et al*. 2004). Such observations were traced back to limitation of iron availability to invading pathogens which inhibits their growth by reducing the amount of this essential nutrient (Weinberg 1999) while at the same time iron deficiency strengthens Th-1 mediated immune function (Weiss 2002b).

Accordingly, children suffering from the most severe form of Plasmodium falciparum infection, cerebral malaria, and who were treated with the iron chelator desferrioxamine in addition to a standard anti-malarial 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 (Gordeuk *et al*. 1992). Children receiving desferrioxamine had higher levels of Th-1 cytokines and NO while serum concentration of Th-2 cytokines (IL-4) tended to be lower (Weiss *et al*. 1997b) which indicated that withdrawal of iron increases Th-1 mediated immune function also *in vivo* (Fritsche *et al*. 2001).

The association between iron, immunity and disease surveillance has been described in other infections with enormous impact on worldwide health.

In hepatitis C virus (HCV) infection iron accumulation in the liver may increase tissue

damage by catalyzing the formation of toxic radicals (Rosen *et al*. 1995) but the metal also impairs the Th-1 immune response directed against the virus (Weiss *et al*. 1999) while iron itself promotes HCV translation by stimulating the expression of the translation initiation factor eIF-3 (Theurl *et al*. 2004). In line with this, increased iron concentrations in the liver are associated with impaired responses of patients to IFN-$\alpha$ treatment and with a faster progression of disease (Shedlofsky 1998).

In Africa, an endemic form of secondary iron overload traced back to the consumption of traditional iron-containing beer is associated with an increased incidence and mortality from tuberculosis (Gordeuk 2002). These data are supported by *in vitro* findings showing that changes in intramacrophage iron availability stimulate the proliferation of mycobacteria and may weaken anti-mycobacterial defense mechanisms of macrophages (Olakanmi *et al*. 2002; Schnappinger *et al*. 2003).

The role of iron for disease progression has been evaluated in HIV infection. Dietary supplementation of iron, a specific haptoglobin polymorphism, or increased iron stores in the bone marrow, are associated with a poor clinical course (Gordeuk *et al*. 2001).

Other infectious diseases ranging from bacterial, viral, fungal to parasitic diseases where iron overload is associated with an unfavorable course of the infection and/or an impaired immune response have been well summarized in a recent review (Weinberg 1999).

Macrophages express a phagolysosomal protein which links resistance to infections with iron homeostasis. The natural resistance associated macrophage protein-1 (NRAMP-1) is an integral 56 kD membrane protein composed of 12 highly hydrophobic transmembrane domains and its expression is associated with resistance to infections with intracellular microbes, such as *Leishmania*, *Salmonella* or *Mycobacteria* spp. (Blackwell *et al*. 2000; Forbes & Gros 2001). NRAMP-1 is highly glycosylated and is thus found in membrane fractions of neutrophils or macrophages in a molecular weight between 90–100 kD. Studies in inbred mouse strains have shown that the susceptibility to suffer from an infection with intracellular pathogens is associated with a single base mutation in NRAMP-1 leading to an amino acid exchange at position 169 (Gly169Asp). This mutation results in loss of NRAMP-1 function. In humans mutations of NRAMP-1 or polymorphisms within its promoter are associated with an increased susceptibility to infections with intracellular pathogens such as Mycobacterium tuberculosis (Bellamy 1999).

The function of NRAMP-1 in host resistance is far from being clear. Recent evidence suggested that NRAMP-1 causes phagolysosomal acidification, thus leading to strengthening of immune defense against intracellular pathogen. This is supported by the finding that NRAMP-1 is expressed on late phagosoms in close proximity to intracellular pathogens such as *Leishmania*, *Salmonella* or *Mycobacteria* and by the observation that functionality of NRAMP-1 is associated with fusion of phagosomes with lysosomes e.g. in Mycobacterium avium infection (for review see (Blackwell *et al*. 2000; Forbes & Gros 2001)).

Moreover, evidence is accumulating that NRAMP-1 acts as a metal-proton co-transporter (Kuhn *et al*. 1999; Zwilling *et al*. 1999; Baker *et al*. 2000; Mulero *et al*. 2002).

It has been shown that NRAMP-1 is able to transport, $Mn^{2+}$, $Zn^{2+}$ and $Fe^{2+}$ most likely by a proton gradient-dependent mechanism, however, there is discrepancy as to whether such an transport is from the cytoplasm to the phagosome or *vice versa* and if such a driving force is pH dependent. Interestingly, NRAMP-1 expression appears to be regulated by iron perturbations, with increased NRAMP-1 mRNA and protein levels in macrophages loaded with iron (Baker *et al*. 2000), which would suggest that NRAMP-1 and iron metabolism may regulate each other by a feed back loop.

Mutations in NRAMP-1 have been associated with a reduction of IFN-$\gamma$ triggered immune effector pathways such as release of nitrate from macrophages, a mechanism being indicative for endogenous NO formation (Barton *et al*. 1995).

The attractive hypothesis that NRAMP-1 expression may confer resistance towards 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 the finding of different immune gene expression patterns along with changes in intracellular iron distribution in cells knocked out for NRAMP-1 (Alter-Koltunoff *et al*. 2003; Fritsche *et al*. 2003). Most importantly, a

mutation of NRAMP-1 results in impaired formation of NO which can be traced back to a reduced transcriptional induction of iNOS in NRAMP-1 susceptible cells (Fritsche *et al*. 2003).

This further supports the notion that the control over intracellular and extracellular iron availability is one of the central battlefields deciding about the fate of an infection.

In a line with this, macrophages challenged with bacterial pathogens produce and secrete the protein lipocalin which sequesters iron-loaded bacterial siderophores thus limiting their growth (Flo *et al*. 2004; Fluckinger *et al*. 2004).

Macrophage and T-cells produce cytokines to retain iron within the reticulo-endothelial system and to withhold it from invading pathogen while this procedure at the same time increases the immune effector potential of macrophages to better withstand infectious microbes. On the other hand, microbes have evoked sophisticated strategies to acquire iron, which they need for their growth, and in many microbes the expression of the iron uptake system is associated with pathogenicity (Schrettl *et al*. 2004). Getting more knowledge of host-pathogen interaction and the pivotal role of iron in this network may hold a key for the development of new treatment strategies to successfully fight infections in an era of increasing anti-microbial resistance towards antibiotics.

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