# Review of Literature on Targeting Iron Sequestration a Novel Strategy to Inhibit Bacterial Pathogens

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**Abstract:** The proliferative capability of many invasive pathogens is limited by the bioavailability of iron. Pathogens have thus developed strategies to obtain iron from their host organisms. In turn, host defense strategies have evolved to sequester iron from invasive pathogens. This review explores the mechanisms employed by bacterial pathogens to gain access to host iron sources, the role of iron in bacterial virulence, and iron-related genes required for the establishment or maintenance of infection. Host defenses to limit iron availability for bacterial growth during the acute-phase response and the consequences of iron overload conditions on susceptibility to bacterial infection are also examined. The evidence summarized herein demonstrates the importance of iron bioavailability in influencing the risk of infection and the ability of the host to clear the pathogen.
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## PATHOGENS

### Introduction

Iron is essential to nearly all life forms on Earth, required for the proper function of enzymes involved in, for example, respiration, photosynthesis, the tricarboxylic acid cycle, nitrogen fixation, electron transport, and amino acid synthesis. The utility of iron in biological processes hinges on its chemical properties as a transition metal, engaging in single electron transfers to interconvert between the ferrous (Fe2+) and ferric (Fe3+) states. While this clearly makes iron advantageous, the same property provides the explanation for why excess, or "free," iron is inherently toxic. Ferrous iron– catalyzed Fenton chemistry results in the generation of the highly toxic hydroxyl radical (OH•) that can compromise cellular integrity through damage to lipids, proteins, and nucleic acids. Aside from Borrelia burgdorferi and Treponema pallidum, iron is essential to all microbial pathogens, yet perhaps the most difficult issue facing pathogens is accessing enough iron to support growth. The concentration of iron under physiological conditions (10−8 to 10−9 M) is orders of magnitude below the ∼10−6 M required for bacterial growth, owing to the formation of insoluble ferric oxyhydroxide precipitates, and host sequestration mechanisms further decrease the available concentration to the range of ∼10−18 M. As such, iron plays a fundamental role in host-pathogen interactions, and coevolution has shaped both bacterial and host iron acquisition/sequestration mechanisms.

Iron is an essential nutrient for the growth, survival and virulence of almost all bacteria. To access iron, many bacteria produce siderophores, molecules with a high affinity for iron. Research has highlighted substantial diversity in the chemical structure of siderophores produced by bacteria, as well as remarkable variety in the molecular mechanisms involved in strategies for acquiring iron through these molecules. The metal-chelating properties of siderophores, characterized by their high affinity for iron and ability to chelate numerous other metals (albeit with lower affinity compared with iron), have also generated interest in diverse fields. Siderophores find applications in the environment, such as in bioremediation and agriculture, in which emerging and innovative strategies are being developed to address pollution and enhance nutrient availability for plants. Moreover, in medicine, siderophores could be used as a tool for novel antimicrobial therapies and medical imaging, as well as in haemochromatosis, thalassemia or cancer treatments. This Review offers insights into the diversity of siderophores, highlighting their potential applications in environmental and medical contexts.

### Review of Literature

This review highlights some of the many interactions between iron metabolism and the immune response such as the effects of iron on distinct immune cell types as well as the pathophysiology of the AI. We also summarize microbial mechanism for iron uptake in prevalent pathogens in as much as they are

relevant to the understanding of counteracting strategies of immune-mediated iron withdrawal from these agents. The multifaceted role of iron homeostasis in the pathogenesis of infections with subcellular agents such as viruses and prions, in contrast, is not covered by this but by other reviews (Drakesmith and Prentice, 2008; Schmidt, 2019; Weinberg, 1996; Wessling-Resnick, 2018).

In living cells, iron is predominately bound within functional groups such as heme and iron-sulfur clusters (Muckenthaler et al., 2017). Yet, minute amounts of iron are also present in a free and thus metabolically active form, which is known as the labile iron pool (LIP) (Epsztejn et al., 1997). Due to its ability to act as cofactor for electron transfer during the shift between its divalent (ferrous) and trivalent (ferric) forms, iron is involved in many biochemical processes. For example, proteins involved in redox reactions, mitochondrial respiration and nucleic acid synthesis utilize iron as central cation in their prosthetic moieties. These functions are evolutionary conserved in all forms of life, from unicellular organisms to human beings. Therefore, bacteria, fungi, protozoa and helminths all require iron for basic metabolic pathways just as mammals do. Comprehensibly, whenever microorganisms and human subjects interact, such as during commensalism or infections, the shared requirement for iron shapes these interactions.

In innate immunity, iron fine-tunes the function of myeloid cells because it controls the activity of transcription factors and of enzymes and thus the production of antimicrobial effectors such as nitric oxide ($NO^{\bullet}$) and hydroxyl ($^{\bullet}OH$) radicals. In the adaptive immune system, iron is an essential growth-factor for the clonal expansion of lymphocyte subsets (Ganz and Nemeth, 2015; Soares and Weiss, 2015; Weiss, 2002).

Iron overload (IO) can result from environmental (e.g. dietary or therapeutic) or genetic (hereditary) causes or a combination of both. In thalassemia syndromes for instance, both inadequately high iron absorption and the need for repetitive blood transfusions contribute to IO (Rivella, 2018; Zimmermann et al., 2008). The IO affects both parenchymal organs, such as liver and myocardium, and the MPS. As major consequences, chronic liver or heart failure attributable to iron-catalyzed radical formation and subsequent cellular damage develop. In addition, iron loading is a risk factor for infections which substantially contribute to an increased morbidity and mortality of patients with IO disorders (Vento et al., 2006).

Infections in transfusion-dependent thalassemia are caused by distinct gram-negative and gram-positive bacteria. Klebsiella pneumoniae, Pseudomonas aeruginosa, *Escherichia coli*, Salmonella enterica and Staphylococcus aureus are some of the agents most often recovered from microbiological samples of these patients (Wang et al., 2003). Various therapeutic approaches such as apo-TF, HAMP analogues and FPN1 inhibitors promise to ameliorate IO, erythropoiesis and/or transfusion dependence in hemoglobinopathies (Casu et al., 2016, 2019; Li et al., 2010). But whether these have positive or negative effects on the rate and severity of infections remains largely unknown.

The need for a thorough consideration of the risk of infections upon therapeutic or dietary manipulation of iron status, however, is one of the lessons learned from iron supplementation studies in developing countries (Armitage and Moretti, 2019; Paganini and Zimmermann, 2017; Prentice et al., 2013). In Sub-Saharan Africa for example, iron supplementation to infants and young children increased morbidity and mortality from infections, possibly because of iron-induced growth of *Plasmodium*, the causative agent of malaria, or expansion of pathogenic *E. coli* (Jaeggi et al., 2014; Sazawal et al., 2006; Tang et al., 2017). However, when bed nets were available to reduce vector-borne transmission of *Plasmodium*, no increased malaria risk was observed in subjects supplemented with iron (Zlotkin et al., 2013). These discrepant outcomes show both, the importance of a considerate selection of inclusion criteria and supporting measures and the need for additional prospective randomized controlled trials (RCT).

Specific point mutations in the *HFE* gene, which encodes a major histocompatibility complex (MHC) class I-like molecule, are the cause of classical hereditary hemochromatosis (HH), the most prevalent IO disorder in subjects of Caucasian ancestry (Brissot et al., 2018). Numerous alterations in T cell subsets and functions have been reported for HH patients. For example, IO due to HH results in low numbers of $CD8^+$ T cells and a divergent T cell receptor (TCR) repertoire in this population, possibly because IO continuously sustains the activation and subsequent exhaustion of $CD8^+$ T cells which is associated with their reduced cytotoxicity (Arosa et al., 1997; Cardoso et al., 2001; Costa et al., 2015; Cruz et al., 2006). Some of these observations may be attributable to the fact that the presence of wildtype HFE but not its mutated version on antigen presenting cells (APC) inhibits the activation of $CD8^+$ T cells (Reuben et al., 2014). Vice versa, low numbers of $CD8^+$ T cells and high numbers of $CD4^+$ T cells are associated with a higher degree of IO in HH patients (Thorstensen et al., 2017). These and other observational data demonstrate that reciprocal interactions exist between iron homeostasis and immunity which are relevant for the

function of immune cells and for the clinical course of infectious diseases.

Not only are red blood cells (RBC) the most abundant cell type of the human body, they also contain the largest quantity of iron. Each RBC has approximately $2.5 \times 10^8$ HB molecules, each of which harbors four heme moieties with a central cation, ferrous iron. Thus, there are about $1 \times 10^9$ atoms of iron in a human RBC (Chalmers et al., 2017; Zborowski et al., 2003). For comparison, *E. coli* has an iron content of $10^5$–$10^6$ molecules per cell (Andrews et al., 2003). It is noteworthy and unexpected that the most common pathogens that infect human RBC, the two protozoan species *Plasmodium* and Babesia, may not directly use host HB or heme for iron supply. Therefore, it appears counterintuitive that human subjects with quantitative (thalassemic syndromes) or qualitative (hemoglobinopathies) HB defects are partially resistant to these infections (Atkinson et al., 2014; Ndila et al., 2018; Taylor et al., 2012). This is especially true for (heterozygous) sickle cell trait or (homozygous) sickle cell disease (Cursino-Santos et al., 2019).

Sickle cell disease is based on a missense mutation in the β-globin chain that results in the substitution of the amino acid in position 6 of the peptide. This mutation is one of the most common genetic defects in the world and its high frequency has been attributed to the fact that heterozygous carriers are more resistant to malaria. On the one hand, the presence of sickle cell HB (HBS) results in an altered biochemical microenvironment within RBC that impairs the growth of *Plasmodium* and counteracts the adhesion of infected RBC to endothelial cells. On the other hand, HBS activates the transcription factor NRF2 (for NF-E2-related factor-2). NRF2 coordinates the antioxidative stress response as it induces the expression of several cytoprotective mechanisms including heme oxygenase-1 (HO1) and FT (Nguyen et al., 2009). As a consequence, the balance between damaging prooxidative molecules such as free heme or iron and antioxidative factors such as carbon monoxide (CO) and bilirubin is shifted towards cytoprotection (Cholera et al., 2008; Ferreira et al., 2011; Gong et al., 2013; Jeney et al., 2014).

In a similar fashion, hemizygous males and healthy heterozygous females carrying a mutation in the *G6PD* (for glucose-6-phasphate dehydrogenase) gene are protected from severe malaria (Guindo et al., 2007). One possible explanation is that the genetic defect in the supply of RBC with NADPH (nicotinamide adenine dinucleotide phosphate) also brings a disadvantage for intraerythrocytic *Plasmodium*, thus impairing its growth. Alternatively, the uptake of infected G6PD deficient RBC by myeloid cells appears to be enhanced which downsizes the pathogen's habitat and undermines one of its key strategies of immune evasion (Cappadoro et al., 1998). Similar observations have been made for healthy carriers of sickle cell trait, α- and β-thalassemia trait (Ayi et al., 2004).

Although *Plasmodium* does produce hemolysins, functionally more important ones are expressed by gram-positive bacteria and, in contrast to their protozoan counterparts, enable them to harvest HB as iron source (Moonah et al., 2014). Such a strategy is highly evolved in *Streptococcus pyogenes*, a pathogen that also binds RBC membrane fragments to the S protein on its surface to avoid recognition and phagocytosis by macrophages (Wierzbicki et al., 2019). Following lysis of RBC during bacteremia or of myocytes in soft tissue infections, *Streptococcus pyogenes* is able to bind HB, HB-haptoglobin complexes and myoglobin by Shr (streptococcal hemoprotein receptor). This may be relevant in severe infections such as necrotizing fasciitis (Bates et al., 2003). Similarly, *S. aureus* produces several hemolysins and other exotoxins including the panton-valentine leukocidin, which enables it to cause necrotizing pneumonia and severe soft tissue infections (Kebaier et al., 2012; Lehman et al., 2009). The expression of Isd (for iron-regulated surface determinant system) complements these virulence mechanisms because it enables *S. aureus* to extract heme from HB and myoglobin and use it as iron source (Pishchany et al., 2013).

But heme's function in infectious diseases is not restricted to its role as iron source for certain pathogens. Rather, heme and the heme-catabolizing enzyme HO1 have a range of immune-modulatory functions in systemic infections (Cunnington et al., 2012; Korolnek and Hamza, 2015; Mitterstiller et al., 2016; Saha et al., 2019). For example, excessive hemolysis during sepsis, the most severe course of systemic infection, results in a dysbalance between free heme and its scavenger in plasma, hemopexin (HPX) (Larsen et al., 2010). The presence of free heme in the circulation is deleterious for the affected individual because it promotes the catalysis of radicals and thereby causes oxidative tissue damage which contributes to immune paralysis partly via inhibiting the migration and phagocytosis of myeloid cells (Martins et al., 2016). Therefore, the induction of HO1 breaks down excess heme and protects from organ dysfunction such as acute renal failure, and reshapes innate immunity thus reducing mortality from sepsis (Ramos et al., 2019; Wegiel et al., 2014). However, the induction of HO1 may reduce pathogen elimination in malaria, leishmaniasis or systemic *Salmonella* infection suggesting that the tolerance to pathogen- or immune-driven tissue damage may come

at the cost of impaired control of other microbes (Cunnington et al., 2011; Mitterstiller et al., 2016; Saha et al., 2019).

In total, all TF that exists in the extracellular space and circulates in the bloodstream contains as little as 3–4 mg of iron (Fig. 1). Therefore, the body's pool of TBI is turned around 8–10 times per day to supply TFR1-expressing cells such as developing erythroblasts (EB) with iron. During the APR to infections, the concentration of TF in the plasma, normally 2–4 g/l, and its saturation with iron, normally 15–45%, are substantially reduced. This immune strategy aims at limiting the microbial iron availability because TBI is a potential iron source for many extracellular pathogens. These may harvest iron from TF by several mechanisms. Some pathogens such as Neisseria species express their own TFR whose function is coupled to TonB to provide the energy for uptake of TBI (Noinaj et al., 2012; Pogoutse and Moraes, 2017). In Mycobacterium tuberculosis, the task of TBI acquisition is carried out by GAPDH (for glyceraldehyde-3-phosphate dehydrogenase) present on the cell surface whereas enterobacteriaceae may use porins to bind TBI (Boradia et al., 2014; Sandrini et al., 2013). Many other pathogens secrete siderophores whose binding affinity for iron exceeds TF's by several orders of magnitude, which may allow them to get ahold of TBI, especially in an infected tissue characterized by low pH (Brock et al., 1991; Ford et al., 1988; Kalidasan et al., 2018; Sipe and Murphy, 1987). In conclusion, human pathogens have evolved several mechanisms to utilize TBI, and mechanisms to exploit LF as iron source are very similar (Malhotra et al., 2017).

NTBI is typically cleared from the circulation by classical monocytes but can occur in the plasma of patients with primary IO, i.e. HH, or secondary IO such as transfusion-dependent sickle cell disease or thalassemia (Batey et al., 1980; Esposito et al., 2003; Haschka et al., 2019; Wang et al., 1986). Also, NTBI is found in hematologic malignancies such as myelodysplastic syndromes (MDS) or acute leukemias, especially during myelo-ablative chemotherapy and hematopoietic stem cell transplantation (Belotti et al., 2014; Harrison et al., 1994; Petzer et al., 2019; Sahlstedt et al., 2001; von Bonsdorff et al., 2002). A transient increase of NTBI in the circulation can also be found in other conditions, e.g. after iron supplementation, following blood transfusion or during hemodialysis sessions (Brittenham et al., 2014; Hod et al., 2011; Prakash et al., 2005; Schumann et al., 2012). In MDS, the presence of NTBI is associated with increased mortality (de Swart et al., 2017; Wermke et al., 2018). Infections are a major cause of non disease related mortality in patients with IO (Alessandrino et al., 2009; Altes et al., 2004; Wermke et al., 2012). The spectrum of pathogens that have a high dependence on iron and benefit from NTBI for growth and proliferation is limited and includes Yersinia *enterocolitica*, S. epidermidis and Aspergillus *fumigatus* (Matinaho et al., 2001; Petzer et al., 2019). In contrast to *S. epidermidis*, *S. aureus* is not dependent on NTBI yet grows substantially better in its presence following intravenous iron administration (Barton Pai et al., 2006; Lindsay et al., 1995). Therefore, hematologic diseases illustrate the direct connections between iron availability and infections.

Ferritin (FT) is present both in the extracellular space including plasma and in the cytoplasm of all cell types. Plasma FT is secreted by macrophages by well characterized mechanisms (Cohen et al., 2010; Truman-Rosentsvit et al., 2017). It is rich in light (FTL) subunits and relatively iron-poor but forms a potential iron source for extracellular pathogens such as *Streptococcus pyogenes* (Ryc et al., 1985). Cytoplasmic FT in contrast, is composed of cell-type specific proportions of FTL and heavy (FTH) subunits (Rucker et al., 1996). It is present in every organ and tissue in the human body and, due to its size, the normal liver stores 300–1000 mg of FT-associated iron in adults (Pietrangelo, 2016). Macrophages are cells with an extraordinarily high capacity to store iron into FT and appear to express FT that is relatively rich in FTH subunits (Nairz et al., 2010; Wang et al., 2013). This is functionally relevant because FTH is the subunit that exclusively carries the ferroxidase activity that is necessary to oxidize ferrous to ferric iron for subsequent incorporation and storage within FT. In doing so, FT reduces the LIP in the cytoplasm. Therefore, cytoplasmic FT forms a safe storage compartment for iron which renders iron inaccessible for infectious agents that reside either in the extracellular space or, after phagocytosis, in the phagosome.

Of note iron may not only act as an immediate growth factor for pathogens but also alter the homeostatic environment within cells. Septicemia results in a metabolic reprogramming of monocytes and macrophages resulting in stimulation of anaerobic glycolysis while tricarboxylic cycle (TCA) activity is reduced, known as the Warburg effect (Cheng et al., 2016). This metabolic switch is induced upon activation of the mammalian target of rapamycin (mTOR). On the other hand, iron stimulates TCA activity in part via increasing the expression and activity of the TCA enzyme aconitase resulting in promotion of aerobic glycolysis and mitochondrial respiration (Anderson et al., 2012; Oexle et al., 1999; Volani et al., 2018). Interestingly, iron loading of macrophages promotes growth and survival of *S.* Typhimurium not only by serving as a direct nutrient

but also by inducing activation of the TCA thereby antagonizing the Warburg effect which results in a pathogen friendly metabolic environment within the cell (Telser et al., 2019).

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