

The Effect of the Host's Iron Status on Tuberculosis

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Several lines of evidence have suggested that iron is critical for *Mycobacterium tuberculosis* growth in macrophages. Macrophage iron loading in patients with African iron overload increases the risk of tuberculosis (TB) and may worsen TB outcome. Likewise, macrophage iron loading may contribute to an increased predisposition toward TB in HIV infection. Human genetic disorders or variations may increase the risk of TB or worsen its outcome through macrophage iron loading, including the haptoglobin 2-2 phenotype, *NRAMP1* polymorphisms (at least in Africans and Asians), and possibly ferroportin 1 mutations, but not *HFE* hemochromatosis. Thus, the host's iron status may be an important yet underevaluated factor in TB prevention and therapy and in TB vaccine design.

In this review of the relationship between tuberculosis and iron, we wish to bridge biological and clinical issues, emphasizing human conditions that might result in macrophage iron loading. The degree of evidence from clinical studies is rated by grades: 1 (randomized controlled), 2 (non-randomized controlled), and 3 (uncontrolled studies, descriptive studies, and expert opinion).

We searched in Medline, without date restriction, for the terms “mycobacterium,” “tuberculosis,” “iron,” and “macrophage” and searched for them in interaction with “immune reaction”; “immunology”; “Th1, Th2, cytokines”; “African iron overload”; “malaria”; “plasmodium”; “hemozoin”; “tobacco”; “smoking”; “alcohol”; “etha-

no”; “alcoholism”; “HIV”; “AIDS”; “genetics”; “hemochromatosis”; “HFE”; “haptoglobin”; “NRAMP1”; “SLC11A1”; “p-aminosalicylic acid”; “isoniazid”; “ethambutol”; “tuberculosis vaccine”; and “BCG.” Because of space limitations, only those articles considered by all authors to be most relevant were retained.

MACROPHAGE IRON HOMEOSTASIS

As shown in figure 1 and as reviewed by others [1], macrophages engulf and digest senescent erythrocytes, degrade heme, and, thus, accumulate iron into the cytosol. Cytosolic iron may be partly incorporated into ferritin, but normally most of it egresses the macrophage to be bound by plasma transferrin for delivery to bone-marrow erythroid precursors. Macrophage iron export is mediated by ferroportin 1 (also termed “SLC40A1”), the receptor for hepcidin, a peptide secreted by the liver in response to inflammation or excess iron [2]. The hepcidin-ferroportin 1 complex is internalized and degraded in lysosomes, locking iron inside macrophages (as well as enterocytes) [2]. Erythrophagocytosis and subsequent iron export via ferroportin 1 constitutes the major iron flux in-

volving macrophages. Iron also enters macrophages via the transferrin-transferrin receptor (TfR) pathway [1], the binding of ferric lactoferrin (hololactoferrin) to lactoferrin receptors [3], and the binding of hemoglobin-haptoglobin (Hp) to the macrophage CD163 receptor during hemolysis [4]; these pathways may assume greater importance depending on the specific macrophage location and functional specialization. Inflammation induces macrophage iron retention, as observed in patients with inflammation, whose blood monocytes show down-regulated ferroportin 1 expression that correlates with increased ferritin levels and mirrors increased urinary hepcidin concentrations (used as surrogate for plasma hepcidin) [5].

ACQUISITION OF IRON BY MYCOBACTERIUM TUBERCULOSIS

Importance of iron acquisition. As confirmed in patient alveolar macrophages, *M. tuberculosis* resides within the macrophage phagosome and prevents phagosome-lysosome fusion by an unclear mechanism [6, 7]. The *M. tuberculosis*-containing phagosome has early endoso-

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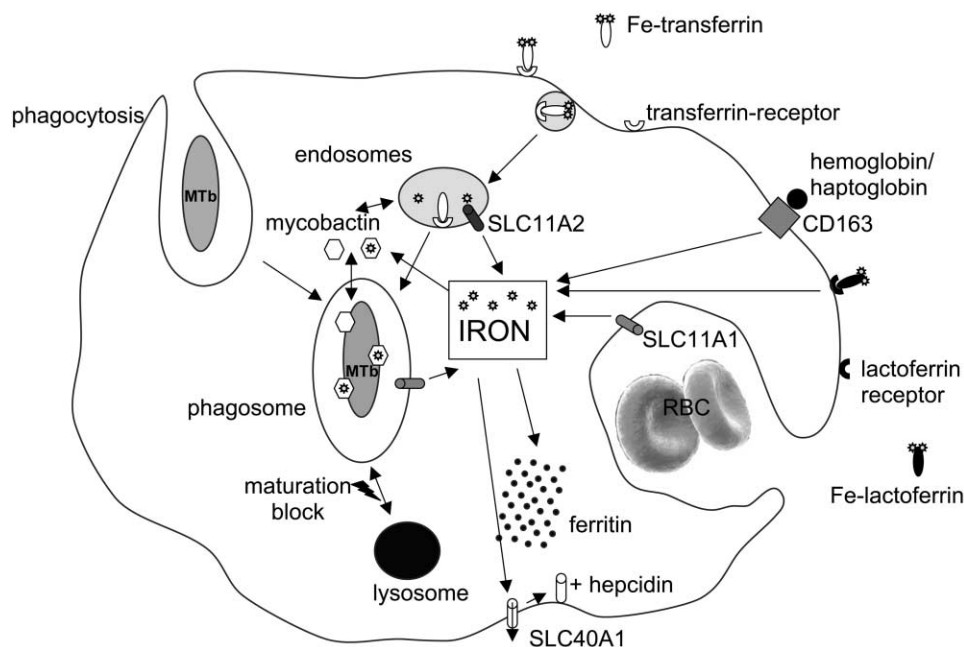


Figure 1. Schematic representation of a *Mycobacterium tuberculosis* (MTb)-infected macrophage and its iron metabolism. MTb resides in an early phagosome, whose maturation to the late phagosome and phagolysosome stages is blocked and whose acidification is limited. Macrophage iron sources are: (1) heme-bound iron from red blood cells (RBCs, main source; the presence of SLC11A1/NRAMP1 within the RBC vacuole is still speculative) and from the hemoglobin-haptoglobin complex, taken up via the hemoglobin scavenger receptor CD163; and (2) iron bound to transferrin and lactoferrin. On release into the cytoplasm, iron can be incorporated into proteins, including the iron-storage protein ferritin, and its presence can be sensed by iron regulatory proteins (not shown). Iron is exported from the cell via SLC40A1 (ferroportin 1), at least when the serum concentration of hepcidin is low to normal; when serum hepcidin is high (as occurs in iron excess or in the presence of inflammation), hepcidin binds to ferroportin 1, the complex is internalized, and iron export is inhibited, resulting in the iron-retention characteristic of inflammation. Binding of iron-bound lactoferrin to its cellular receptor and subsequent iron release are ill defined. Serum iron-bound transferrin binds to the transferrin receptor on the cell surface, and the complex is internalized in the early endosome, where low pH separates iron from the complex, allowing the iron-free complex to recycle to the cell surface and iron to be released into the cytoplasm via SLC11A2 (DMT1). Within its phagosome, MTb can capture iron either from cytoplasmic sources (represented by the central rectangle) or from the transferrin/transferrin receptor complex by interacting with the early endosome. These captures are made possible by a dual mycobacterial siderophore system made of mycobactins. These consist in a water-soluble carboxymycobactin T, which is thought to vehicle iron to a lipophilic mycobactin T associated with the MTb cell wall. Some evidence suggests that phagosomal iron can be pumped out via the SLC11A1 (NRAMP1) efflux pump.

mal characteristics, interacts with early endosomes, and does not acidify below pH 6.3–6.5 [6, 7]. *M. tuberculosis* perceives the phagosome as a low-iron environment [8, 9]: hence the importance of iron-acquisition strategies in the face of the host's iron-withholding mechanisms [9, 10]. Iron limitation induces at least 35 *M. tuberculosis* genes, including *mbtA–J*, which encode enzymes involved in siderophore biosynthesis, but represses *bfrA*, which encodes an iron-storing bacterioferritin [8]. Consistent with the concept of iron restriction during immune control of *M. tuberculosis* replication, *mbtB* mRNA levels increase and *bfrB* mRNA levels

decrease several weeks after infection in mice [11, 12].

Tools used. Salicylate-derived *M. tuberculosis* siderophores, termed “mycobactins,” are composed of 2 entities: hydrophilic mycobactin T (carboxymycobactin) and the more lipophilic *M. tuberculosis* cell wall-associated mycobactin T, which appears to transfer iron captured by hydrophilic mycobactin across the mycobacterial cell wall [13]. An *M. tuberculosis* strain with a deleted *mbtB* gene has interrupted mycobactin synthesis, impaired intraphagosomal iron acquisition, and retarded growth within THP-1 cells [14, 15]. The product of another *M. tu-*

berculosis gene, *ideR*, represses *mbt* expression in an iron-dependent manner but activates *bfrA* [16, 17]. Two other genes, *irtA* and *irtB* (for iron-regulated transporters A and B), encode enzymes that possibly translocate Fe-carboxymycobactin into the mycobacterial cytoplasm independently of mycobactin [18].

Host iron sources. *M. tuberculosis* scavenges iron from the host-cell transferrin-iron acquisition pathway [6] (figure 1). Mycobactins are able to extract iron from exogenous holotransferrin [6, 19]. Phagosomes containing live *M. tuberculosis* interact with early endosomes to which holotransferrin traffics (figure 1) [6]. *M.*

tuberculosis within the macrophage phagosome is able to concentrate more iron from transferrin than the nonpathogenic *M. smegmatis* [15]. Mycobactins are also able to acquire iron from cytoplasmic pools supplied by holotransferrin [19] and from hololactoferrin, which is more available at the pulmonary infectious site than is holotransferrin [20, 21]. In a recent study [22], gallium-loaded lipophilic mycobactin J from *M. paratuberculosis* (a surrogate for ferric-mycobactin J and structurally close to mycobactin T) was added exogenously to macrophages and was found to (1) equilibrate throughout infected and even surrounding cells and distribute between macrophage aqueous and membrane domains; (2) scavenge both holotransferrin-released and cytoplasmic iron; and (3) be recruited as Fe-mycobactin to lipid droplets delivered through lipid trafficking to *Escherichia coli*-containing phagosomes. Although these results are interesting, the exogenous addition of mycobactin does not mimic the in vivo situation, nor does an *E. coli*-infected phagosome mimic an *M. tuberculosis*-infected phagosome. In summary, *M. tuberculosis* appears to evade host defense by preventing phagosome acidification and lysosome fusion, to acquire iron from host endosomal holotransferrin, and—if confirmed—to utilize host lipid trafficking for the delivery of additional iron by mycobactin.

Effect of modifying iron status on *M. tuberculosis* growth. Adding iron experimentally has always—with a single exception [23]—resulted in enhanced *M. tuberculosis* growth, both in vitro (axenic medium or macrophage infection) [24–28] and in mice [29–32] (table 1). Furthermore, *M. tuberculosis* growth is reduced by interrupting iron availability, whether by (1) treatment with an exogenous iron chelator, such as deferoxamine [26, 30], silybin [27], or a phytosiderophore [33]; (2) exposure to the endogenous iron chelators apo-transferrin [29] and apo-lactoferrin [26]; (3) the addition of

an anti-TfR antibody to *M. tuberculosis*-infected macrophages [27]; or (4) incubation with gallium salts or gallium transferrin that occupy iron pathways with a metal that cannot be utilized by *M. tuberculosis* [34]. Genetic studies have confirmed the importance of iron for *M. tuberculosis*. First, as mentioned previously, a mutant defective in mycobactin synthesis has decreased growth in macrophages [14]. Similarly, an *irtAB* mutant compromised in the utilization of iron from Fer-carboxymycobactin has also been shown to have defective growth [18]. Finally, β_2 -microglobulin knockout mice, given that they are iron overloaded, are more susceptible to tuberculosis (TB) than are control mice [26]. However, these mice

are of questionable relevance for studying the relationship between TB and iron, because they also have several T cell deficiencies [35].

HOST DEFENSE AGAINST *M. TUBERCULOSIS*

As reviewed by others [36–38], defense against TB involves immune recognition by macrophages and dendritic cells, which leads to tumor necrosis factor (TNF)- α production and cooperative interaction with T cells, mainly CD4⁺ T lymphocytes and NK cells. The resulting Th1 type pro-inflammatory reaction includes interleukin (IL)-18, IL-12, interferon (IFN)- γ , and IL-1 β production and is responsible

Table 1. Influence of alterations in iron status on the growth of *Mycobacterium tuberculosis* (MTb) both in vitro and in vivo.

Growth, conditions	MTb growth [references]	
	Enhanced	Inhibited
In vitro growth		
Growth in broth or serum		
Iron salts	[24–27]	
Serum transferrin		[29]
Desferrioxamine		[26]
Silybin		[27]
Phytosiderophore		[33]
Growth in the macrophage		
Murine macrophage		
Iron salts	[28]	
Lactoferrin		[26]
Anti-TfR antibody		[26]
Siderophore-deficient MTb mutant		[14]
Human macrophage		
Iron salts	[27, 28]	[23]
Gallium-transferrin		[34]
<i>irtAB</i> mutation of MTb		[18]
In vivo growth (murine infection)		
Iron enrichment of drinking water	[26]	
Parenteral iron	[29–32]	
Desferrioxamine		[26, 30]
$\beta_2\mu$ KO mice	[26]	
$\beta_2\mu$ KO mice treated with (vs. untreated $\beta_2\mu$ KO mice)		
Intranasal lactoferrin		[26]
Desferrioxamine		[26]
<i>irtAB</i> mutant of MTb		[18]

NOTE. $\beta_2\mu$, β_2 -microglobulin; KO, knockout; TfR, transferrin-transferrin receptor.

for killing or controlling *M. tuberculosis*. Effector mechanisms involve reactive oxygen and nitrogen intermediates and apoptosis. Antiinflammatory cytokines such as IL-4, IL-10, and transforming growth factor- β , the latter 2 of which are produced by regulatory T (T_{reg}) cells, suppress Th1 type responses and interfere with effector T cell activation.

Of *M. tuberculosis*-infected humans, 5%–10% develop clinical TB, whereas the majority develop latent infection characterized by quiescent *M. tuberculosis* with a strong Th1 and a possibly suppressed Th2 cytokine response [38]. Clinical TB is associated with a mixed Th1/Th2 response and Th2 dominance [38], and therapeutic recovery from TB usually involves restored Th1 dominance over Th2 [39].

EFFECT OF CYTOKINES ON MACROPHAGE IRON STATUS

IFN- γ , which is pivotal for protection against TB, also influences cellular iron status. Indeed, IFN- γ activation of human monocytes down-regulates TfR numbers on the cell surface [40] and the rate of macrophage iron acquisition from holotransferrin [19], and it decreases iron availability to intracellular microorganisms that utilize transferrin iron, such as *Legionella pneumophila* [40] and *M. tuberculosis*, as discussed above. *M. tuberculosis* remains able, at least in vitro, to access other iron sources, despite the IFN- γ -induced limitation in macrophage iron acquisition [19]. Macrophage *M. tuberculosis* infection does not subvert this IFN- γ -induced decrease in TfR expression [19, 41].

In contrast to classical macrophage activation by bacterial products or IFN- γ , alternative macrophage activation by Th2 cytokines has 2 potential implications for macrophage iron status. First, during experimental TB, alternatively activated mouse macrophages express the gene encoding arginase-1 [41]. Arginase-1 competition with inducible nitric oxide synthase (iNOS) for L-arginine results in decreased NO production and, hence,

possibly less iron efflux [42] and more iron being available to *M. tuberculosis*. Whether the differential expression of arginase-1 versus iNOS applies to humans is unclear. Second, alternative activation of uninfected macrophages results in increased expression of both TfR and CD163, resulting in increased TfR-related and CD163-mediated iron acquisition [41, 43]. Furthermore, in a comparative gene-expression study of *M. tuberculosis*-infected murine macrophages activated by either IL-4 or IFN- γ , IL-4 led to increases in both host TfR expression and *M. tuberculosis* iron availability as manifested by decreased mycobactin and increased bacterioferritin synthesis, whereas IFN- γ was associated with opposite findings [41]. Whether these interesting experimental results apply to human *M. tuberculosis* infection is speculative. The fact that serum ferritin levels increased in a dose-dependent manner during recombinant human IL-10 treatment of patients with Crohn disease (evidence grade 1) [44] is consistent with the concept that immunosuppressive cytokines increase macrophage iron availability.

EFFECT ON TB OF CONDITIONS THAT MAY AUGMENT RETICULOENDOTHELIAL IRON STORES

In some conditions, reticuloendothelial iron loading originates from the alimentary tract, the respiratory route, or hemolysis, whereas, in others, it results from chronic inflammation. The administration of iron-dextran to rats results in bronchoalveolar macrophage iron loading [45]. Hence, it is conceivable that human iron-loading conditions might enhance *M. tuberculosis* growth in alveolar macrophages. Iron loading affects the immune system in a way that resembles alternative macrophage activation. When erythropoietin-treated patients undergoing hemodialysis were randomized to receive or not receive intravenous iron, those receiving iron developed lower TNF- α but higher

IL-4 serum concentrations (evidence grade 1) [46]. In volunteers receiving 120 mg of oral iron daily for 7 days, IL-4 serum levels increased modestly but significantly [47].

Some clinical conditions characterized by generalized or local reticuloendothelial iron loading are possibly associated with TB. In African iron overload and in HIV/AIDS, the available evidence suggests a pathophysiological role of iron loading that increases the risk of TB or worsens its outcome. In other conditions that increase the risk of TB and/or worsen its outcome (e.g., tobacco smoking and alcoholism), there is some degree of iron loading, but its pathogenetic role is hypothetical; in human malaria, another condition with increased macrophage iron, the association with TB, is conjectural. Although inconclusive, data on tobacco smoking, alcoholism, and malaria are presented as basis of future research.

African iron overload. Iron overload of both macrophages and hepatocytes is common in sub-Saharan African populations drinking a traditional beverage of high iron content that is brewed in non-galvanized steel containers. In addition to high dietary iron, a non-*HFE* genetic defect may be implicated in iron loading [48]. African iron overload was first described in autopsies performed in the 1920s on black Africans whose cause of death was recorded, and hepatic, splenic, and lymph-node iron was graded on a scale of 1–5. When these data were reanalyzed in the 1990s, splenic (macrophage) iron was the variable most significantly associated with death from TB—the odds of death from TB were 16.9-fold higher in persons with the highest, compared with the 2 lowest, grades of splenic iron (evidence grade 2) [49]. In a prospective study of the relationship between pulmonary TB and increased dietary iron (lifetime consumption of traditional beer) among 98 Zimbabwean patients with pulmonary TB and 98 control subjects, increased dietary iron was associated with a 3.5-fold increase in the estimated odds of developing pulmonary TB and with

a trend toward higher mortality among the patients with pulmonary TB (evidence grade 2) [50].

HIV/AIDS. The HIV epidemic dramatically fuels the coexisting TB epidemic. Macrophage iron loading in the setting of HIV may be due to blood transfusion or to HIV- and opportunistic infection-induced inflammation [51], which would be expected to lead to hepcidin production and macrophage iron trapping. HIV may also contribute to macrophage iron loading by its Nef protein, which interacts with HFE, the protein mutated in classical hereditary hemochromatosis, and markedly decreases its expression on the surface of macrophages. The net effect of this interaction is to increase macrophage iron and ferritin content [52]. Macrophage iron loading during HIV infection may increase oxidative stress and lower intracellular glutathione levels, impairing the anti-TB defense. Indeed, (nitroso-)glutathione inhibits *M. tuberculosis* growth in vitro, whereas the glutathione precursor N-acetyl cysteine improves control of intracellular *M. tuberculosis* cultured in blood from HIV-infected patients [53]. Clinical studies of the adverse influence of high iron stores on the outcome of HIV infection have been reviewed elsewhere [54]. One retrospective study of bone-marrow macrophage iron grades in HIV-seropositive patients found significantly more frequent TB in patients with high, compared with low or normal, macrophage iron grades (evidence grade 3) [55]. The impact of HIV-related macrophage iron loading on TB requires more investigation.

Tobacco smoking. Tobacco smoking is a major risk factor for pulmonary but not extrapulmonary TB infection and death, which implicates a local airborne insult [56]. A parasympathetic antiinflammatory pathway has been identified, whereby activation of macrophage $\alpha 7$ nicotinic acetylcholine receptors in bronchoalveolar macrophages reduces the synthesis and release of TNF- α , IL-1, and IL-6 [57]. Because nicotine is an agonist of these receptors, tobacco smoking should inhibit

alveolar macrophage TNF- α release, impairing the formation of appropriate *M. tuberculosis*-containing granulomas. Tobacco smoking may also favor TB by iron loading alveolar macrophages, given that smoking 1 pack of cigarettes is equivalent to inhaling 1.1 μg of iron [58]. Iron levels in alveolar macrophages are 2-fold higher in asymptomatic smokers and 4.6–7-fold higher in symptomatic smokers than in nonsmokers. Proof that tobacco smoking increases the risk of TB by loading the alveolar macrophages with iron is lacking at present.

Alcoholism. In a cross-sectional study in humans [59], alcoholism was the strongest predictor of TB in tobacco smokers, with an adjusted odds ratio of 7.4 (evidence grade 2). Alcoholism may result in alimentary iron loading because ethanol metabolism down-regulates hepcidin expression [60]. Among 8839 individuals who did not consume alcohol or who had consumed <1, 1–2, or >2 daily alcoholic drinks during the preceding year, serum transferrin saturation and ferritin levels were significantly higher in consumers of >2 daily alcoholic drinks, compared with nonconsumers, after adjustment for potential confounders [61]. After 2 weeks of alcohol consumption, mice had decreased lymphocyte proliferation and IFN- γ production, smaller granulomas, and higher *M. tuberculosis* lung burdens than control mice [62]. At the bronchoalveolar level, chronic alcoholism suppresses Th1 and induces T_{reg} cells responses [63], potentially enhancing the risk of TB, but it is unknown whether human alcoholism may favor TB by inducing alveolar macrophage iron loading. In one study, chronic alcohol exposure of rats did not increase iron levels in alveolar macrophages, in agreement with alcohol-induced down-regulation of hepcidin, but the sample size was limited [64].

Malaria. In most reports, a Th1-biased response clears an uncomplicated acute human *Plasmodium falciparum* infection [65]. Malaria suppresses the immune response to both parasite and non-

parasite antigens, probably because of macrophage accumulation of hemozoin, a heme (ferri-protoporphyrin IX) polymer derived from hemoglobin ingested by intraerythrocytic plasmodia [66]. Hemozoin-fed human monocyte-derived macrophages produce IL-10 in a dose-dependent manner [67] and show impaired differentiation to mature dendritic cells [67], and T cells activated by these dendritic cells lack effector functions [68]. *M. tuberculosis*-infected mice coinfecting later with *P. yoelii* have higher organ *M. tuberculosis* burdens than mice infected only with *M. tuberculosis* and have higher mortality than mice infected only with *M. tuberculosis* or *P. yoelii*. Hemozoin-related lung and liver pigmentation is stronger in coinfecting mice than in those infected only with plasmodium species, which suggests that there is more malarial hemolysis-induced iron loading that favors *M. tuberculosis* growth in coinfecting mice [69]. At present, it is unclear whether malaria affects the development or outcome of human TB.

IRON-RELATED GENES AND HUMAN TUBERCULOSIS

Hereditary iron overload. In classical hereditary hemochromatosis caused by homozygosity for the C282Y mutation of *HFE*, alimentary iron overabsorption results in iron loading of liver, heart, and pancreas parenchymal cells, whereas macrophages are relatively iron deficient. In *M. tuberculosis*-infected macrophages from patients with hemochromatosis, intraphagosomal *M. tuberculosis* acquires less iron from holotransferrin and is 50% growth-retarded, compared with that in normal macrophages [19, 21]. Studies to confirm whether patients with hemochromatosis have a lower risk of TB have not been reported. *M. tuberculosis* has been hypothesized to exert a selective pressure responsible for a high prevalence of *HFE* mutations in certain populations (evidence grade 3) [70]. By contrast, ferroportin 1 mutations often result in macrophage iron loading [71]. Whether they may increase

the risk of TB is unknown. *Salmonella enterica* growth is reduced by macrophage ferroportin 1 expression and increased by its hepcidin-induced down-regulation [72], which is compatible with the suggestion that a ferroportin 1 mutation also might increase TB susceptibility.

Hp. Binding of hemoglobin-Hp to the monocyte/macrophage CD163 results in internalization of heme and, eventually, to heme degradation and IL-10 secretion [73]. There are 3 Hp phenotypes: Hp 1-1, Hp 1-2, and Hp 2-2. Multimeric Hp 2-2, which has a 10-fold higher CD136 affinity than Hp 1-1, may result in greater macrophage iron acquisition [4] and increased serum markers of iron loading, as has been observed in some but not all studies (reviewed in [74]), as well as more macrophage IL-10 production. In the previously mentioned study of Zimbabwean patients with pulmonary TB and control subjects [50], the Hp phenotype did not modulate TB susceptibility, but it did affect treatment outcome; the odds of death were 6.1-fold higher in patients with TB carrying Hp 2-2, compared with those carrying Hp 1-1 [75]. It is uncertain whether this worse TB outcome in individuals carrying Hp 2-2 relates to differences in iron status.

NRAMP1. NRAMP1, the gene product of NRAMP1 (*SLC11A1*), is principally expressed in the membranes of late endosomes, phagosomes, and lysosomes of macrophages. In mice, NRAMP1 activates an early microbicidal mechanism that provides resistance to several intracellular microorganisms, including *M. avium* and *M. bovis* (bacille Calmette-Guérin [BCG]) but not *M. tuberculosis*. A G169D substitution renders NRAMP1 nonfunctional, causing murine susceptibility to these infections. NRAMP1 transports divalent cations (putatively iron) across the phagosomal membrane, but opposing views exist on the flux direction: either Fe²⁺ influx into the bacterium-containing phagosome causes bacterial killing by forming reactive oxygen species [76], or, because macrophages without functional NADPH oxidase have

their resistance unaffected [77], Fe²⁺ efflux restricts bacterial growth by iron deprivation [77, 78]. The latter view is gaining more experimental support (figure 1) [79]. The G169D mutation in human NRAMP1 has never been found, but there are several NRAMP1 polymorphisms in the noncoding human gene regions.

Fourteen studies examining the relationship between human NRAMP1 polymorphisms and TB susceptibility were analyzed in a meta-analysis that involved 2460 case patients and 3227 control subjects and covered the 4 most investigated NRAMP1 polymorphisms [80]. The odds ratio of TB with the 3' untranslated region (UTR) variant was 1.33, that with the D543N variant was 1.67, that with the INT4 variant was 1.14, and that with the 5'(GT)_n variants was 1.32, compared with the corresponding common alleles. Polymorphisms at these 4 loci significantly increased the risk of pulmonary TB in Africans (except the 3' UTR) and in Asians (except INT4) but not in white persons, whose numbers were low (evidence grade 2). More data are needed about the direction of the NRAMP1-associated iron flux and the effect of NRAMP1 polymorphisms on TB susceptibility.

ANTITUBERCULAR THERAPY AND IRON

p-aminosalicylic acid inhibits the conversion of salicylate to (carboxy)-mycobactin and therefore targets *M. tuberculosis* iron acquisition [13]. The prodrug isoniazid needs conversion to an active form that inhibits mycolic acid biosynthesis. This conversion requires the heme-containing catalase peroxidase, which is encoded by the *M. tuberculosis katG* gene and protects *M. tuberculosis* against oxidative damage [81]. In a study of the effect of experimental iron loading on the therapeutic efficacy of isoniazid in murine high-inoculum TB infection [32], the bactericidal activity of isoniazid was significantly reduced in iron-loaded compared with normal-iron mice, because of either the growth-promoting or immunological ef-

fects of iron or the massive oxidative stress overwhelming the *katG*-associated protection against reactive oxygen species.

ANTITUBERCULAR VACCINATION AND IRON

BCG vaccination is of low efficacy in persons in developing tropical and subtropical areas, who also face helminth infections, environmental mycobacteria, malaria, HIV, and malnutrition—conditions that may suppress Th1 and enhance Th2/T_{reg} cell responses [38]. It seems possible that an increased macrophage iron burden may be one of the mechanisms exacerbating immune dysregulation and BCG vaccine failure. We suggest that iron status should be taken into account in further studies of anti-*M. tuberculosis* vaccines. For instance, one could (1) compare results of vaccinating animals fed an iron-poor versus iron-enriched diet and (2) test the administration of apolactoferrin together with new TB vaccines, given that apolactoferrin coadministration enhances delayed-type hypersensitivity toward BCG in mice [82] and increases the efficacy of BCG in protecting mice against virulent *M. tuberculosis* [83]. It is uncertain whether this beneficial lactoferrin effect relates to iron binding, immunological effects, or antibacterial activity of its breakdown peptide, lactoferricin.

RESEARCH PROSPECTS

In view of the critical importance of iron in TB pathogenesis, research might be directed at examining the effect of manipulating several stages of the iron pathways of the host and the invading organism: (1) the quantity of iron entering the host via the alimentary, parenteral, and respiratory routes; (2) the amount of hepcidin circulating in the host; (3) the function of ferroportin 1, the hepcidin receptor, at the host macrophage membrane; (4) the direction of iron flux by NRAMP1 at the host phagosomal membrane; (5) host phagosomal maturation and acidification; (6) mycobactin synthesis by *M. tuberculosis*; and (7) the ferric mycobactin trans-

port pathway of *M. tuberculosis*. Furthermore, research on *M. tuberculosis* and iron may result in spin-offs into unrelated fields. For example, iron-free mycobactin reversibly blocks the in vitro growth of vascular smooth-muscle cells because its iron-chelating properties block redox-modulated signaling pathways and several enzymes. One clinical situation where blocking the proliferation of these cells might be desirable is the prevention of coronary restenosis after coronary balloon angioplasty. Intramural application of mycobactin shortly after coronary balloon injury, tested in a porcine model, halved the injury score and reduced neointima thickness [84]. Results in humans are awaited.

CONCLUSION

Trousseau reported in 1872 [85] that patients recovering from active TB had a relapse if they received iron-rich supplements, and he warned against their use. The relationship between iron and TB is now strengthened thanks to the elucidation of the *M. tuberculosis* genome, advances in immunology, and clinical study results. Drugs that target mycobacterial iron-acquisition pathways or host pathways that affect iron availability for *M. tuberculosis* are worth developing and testing for anti-TB activity. The role of iron should be taken into account in future vaccine studies.

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