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Sequestration and Scavenging of Iron in Infection

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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.

Iron's capacity to readily donate or accept electrons makes it essential for important cellular redox processes of nearly all organisms. However, this redox reactivity can also be deleterious if uncontrolled. Ferrous iron is potentially toxic through its ability to catalyze the production of reactive oxygen and nitrogen species, including the highly reactive hydroxyl radical (1). These reactive species can damage biological molecules, including DNA (2). Iron within heme is in the ferrous (Fe^{2+}) state and readily participates in redox reactions. Moreover, the heme molecule is lipophilic and can disrupt membrane permeability (3) and alter cytoskeletal protein conformation in certain cell types (4). Redox reactions of bound heme (e.g., myoglobin and hemoglobin) are similar to those of free heme, although they occur more slowly (5). Autooxidation of globin-bound Fe^{2+}-protoporphyrin (heme) produces the ferric (Fe^{3+}) form (hemin) with concomitant production of superoxide (O_2^-), generating methemoglobin and metmyoglobin. Hydrogen peroxide can also oxidize these hemin-containing proteins, generating ferryl (Fe^{4+}) iron, which decays to regenerate ferric iron (6, 7). The potential toxicity of iron is managed in both pathogen and host by highly sophisticated and tightly controlled systems dedicated to balancing cellular and whole organismal iron acquisition, storage, and utilization.

IRON HOMEOSTASIS IN HUMANS

The human body contains approximately 3 to 4 g of total iron. Iron loss arises from epithelial cell sloughing and minor bleeding and totals less than 2 mg per day on average (8). Because regulated iron excretion systems do not exist in humans, total body iron homeostasis is regulated at the level of dietary absorption (9, 10). Dietary nonheme iron is ferric and must be reduced to the ferrous state for membrane transport. This is accomplished by membrane-associated reductases at the duodenal brush border (11, 12). The ferrous iron is then transported into the enterocyte by the membrane transporter, divalent metal transporter 1 (DMT1) (13). Redox cycling is a conserved mechanism that minimizes exposure to reactive ferrous iron by oxidizing it to the relatively inert ferric form upon release from the cell. Conversely, ferric iron reductases return it to the active state prior to its transport across the membrane and incorporation into cellular machinery (14). Cellular iron can either be stored in ferritin or released into the plasma by ferroportin; iron oxidation is coupled to basolateral transport by the ferroxidase hephaestin (15). Ceruloplasmin functions as a ferroxidase in the plasma, where it is most important in situations involving high levels of iron demand, such as stress erythropoiesis (16). Plasma Fe^{3+} is bound to the transport protein transferrin for delivery to sites of storage (as intracellular ferritin) and utilization (primarily as heme but also in iron-sulfur proteins and other iron-containing enzymes) (9, 17). The related protein lactoferrin binds iron with higher affinity than transferrin and is able to retain it under acidic conditions (18, 19). It is found in most exocrine secretions and is a component of the secondary granules of neutrophils (20). Consequently, it is able to bind iron at mucosal surfaces and in plasma.

Iron stored within ferritin is in the ferric state and sequestered from availability to participate in redox reactions. Hemosiderin, a lysosomal degradation product of ferritin, is produced more abundantly under conditions associated with iron overload, hemorrhage, or hemolysis (21–23). Hemosiderin contains heterogenous iron mineralization products that differ from that of ferritin (24). Iron release from hemosiderin is inefficient at neutral pH but does occur under acidic conditions and has been implicated in hydroxyl radical production *in vitro* (25).

The majority of transferrin-bound iron uptake occurs in the bone marrow, where erythroid precursors incorporate the iron into the heme moiety during synthesis of hemoglobin (26). Hemoglobin in circulating erythrocytes accounts for the vast majority of iron-containing heme proteins in the body (27). This pool is salvaged by phagocytosis of senescent erythrocytes by reticuloendothelial (RE) macrophages. Recycled iron in the macrophage can be either stored in ferritin or released into circulation through ferroportin (28). Reoxidation is mediated by ceruloplasmin (28, 29) and followed by binding to transferrin. Ferroportin-mediated iron release from RE cells is one of the primary mechanisms for controlling plasma iron concentrations.

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The hepatocyte is central to iron homeostasis, serving as both a storage site for iron and the principal site of production of the iron regulatory hormone hepcidin (28, 30, 31). Hepcidin is the master regulator of plasma iron concentration. It is induced in response to iron (32) and inflammation and suppressed in response to anemia, hypoxia, and erythropoiesis (33, 34). It regulates the concentration of iron in plasma through its ability to bind with and promote the internalization and subsequent degradation of ferroportin (35). As a consequence, the release of iron into the circulation from sites of storage (RE cells) and absorption (enterocytes) is decreased. Iron released into the circulation is bound to transferrin for transport under normal conditions. Under pathological conditions, such as hemochromatosis, iron release can exceed the binding capacity of transferrin. Iron is then bound to low-molecular-weight molecules (e.g., citrate), resulting in the generation of "non-transferrin-bound iron" (36). Thus, unbound iron is probably not present in serum. In the unlikely event that iron release surpasses the binding capacity of low-molecular-weight molecules, any free iron present will rapidly form insoluble ferric hydroxide. Estimates derived from the solubility constant of ferric hydroxide predict that free Fe^{3+} at a concentration above $\sim 10^{-18}$ M is insoluble at physiologic pH (37, 38).

BACTERIAL IRON AND HEME ACQUISITION SYSTEMS

The optimal iron concentration for growth of most bacteria is much higher than the concentration that is freely accessible in the mammalian host (39). For example, *in vitro* studies indicate that a siderophore mutant strain of *Escherichia coli* requires 0.05 μM iron for growth, and the growth rate of this strain increases as iron concentrations are increased up to 2 μM (40). This disparity provides strong selective pressure favoring the evolution of systems able to overcome the severe iron sequestration encountered in the mammalian host, and successful bacterial pathogens can exploit almost every major host iron-binding protein (Table 1). In fact, many of the components of these systems are required for pathogenesis in animal models of infection (Table 2). Notably, many pathogens possess multiple iron acquisition systems. When several different iron sources are available in any given host niche, the elimination of a single system may not be sufficient to attenuate virulence. Alternatively, a particular iron system may be required for virulence in one animal model but not another, depending on the iron sources available in each model.

Microbial iron acquisition systems have been extensively characterized in Gram-negative bacteria. In general, an outer membrane protein receptor binds a specific iron- or heme-containing compound or protein and transports the iron or heme from it into the periplasmic space in an energy-dependent manner. The proton motive force at the cytosolic membrane supplies energy through the ExbB/ExbD complex which, in turn, induces conformational changes in TonB that allow the transduction of energy to outer membrane proteins (41). Many virulence studies have taken advantage of the fact that TonB is universally required for iron and heme uptake in Gram-negative bacteria and they have targeted it directly to circumvent issues with overlapping iron acquisition systems (discussed above). TonB-dependent receptors are structurally conserved and share an amino acid consensus sequence termed the "TonB box," which interacts with TonB (42, 43). Once the heme or iron has been transported across the outer membrane, a substrate-specific periplasmic binding protein ferries the substrate to an ATP-binding cassette (ABC) transport system that moves it into the cytosol (39) (Fig. 1A).

TABLE 1 Examples of bacterial heme and iron uptake systems

System type	Example	Representative organism(s) (reference)
Ferrous iron uptake	Feo	*V. cholerae* (51), *E. coli* (52), *Shigella flexneri* (141), *S. enterica* serovar Typhimurium (55)
	Mts	*Streptococcus pyogenes* (142)
Ferric iron receptor	FecA	*E. coli* (143), *S. flexneri* (144)
Siderophore system	Ybt	*Y. pestis* (46)
	Fhu	*Haemophilus influenzae* (145), *Streptococcus agalactiae* (146)
	Snfa	*S. aureus* (147)
	Mbt	*M. tuberculosis* (148)
	Vib	*V. cholerae* (149)
	Ent	*E. coli* (150), *Shigella dysenteriae* (151), *S. enterica* serovar Typhimurium (152)
Transferrin receptor	Tbp	*N. meningitidis* (48), *H. influenzae* (153)
	Tpn	*S. aureus* (154)
Lactoferrin receptor	Lbp	*Neisseria gonorrhoeae* (155)
	Unnamed	*H. influenzae* (156)
Heme receptor	HutA	*Bartonella quintana* (157), *V. cholerae* (158)
	HxuC	*H. influenzae* (159)
	Shr	*S. pyogenes* (65)
	ChuA	*E. coli* (160)
	ShuA	*S. dysenteriae* (161)
Hemoglobin receptor	IsdB	*S. aureus* (44)
	HmbR	*N. meningitidis* (162)
Haptoglobin receptor	HarA	*S. aureus* (163)
	HhuA	*H. influenzae* (164)
	HpuAB	*N. meningitidis* (165)
Hemophore system	IsdX1X2	*B. anthracis* (74)
	Rv0203	*M. tuberculosis* (166)
	HasA	*Serratia marcescens* (167)
Hemoglobin protease	Hbp	*E. coli* EB1 (08-K43) (168)
Ferritin receptor	IlsA	*B. cereus* (61)

Considerably less is known about iron and heme uptake systems in Gram-positive bacteria. The absence of an outer membrane results in some differences in the overall design of iron and heme acquisition systems; neither outer membrane receptors nor the TonB/ExbB/ExbD system is necessary. In the most well-studied examples, substrate specificity is determined by cell wall-anchored proteins that transfer the substrate to an ABC transporter, which then delivers it to the cytoplasm (Fig. 1B) (39, 44, 45). Specific microbial nonheme iron and heme acquisition systems, as well as iron storage systems, are discussed in detail below.

Nonheme iron acquisition systems. One bacterial strategy for the acquisition of ferric iron is the production and secretion of high-affinity siderophores that, in addition to being able to bind

TABLE 2 Examples of iron-related bacterial genes required to establish or maintain infection

Organism	Gene(s)	Function	Disease model	Reference
S. aureus	*fur*	Iron-responsive regulator	Murine model of pneumonia	169
	isdB	Hemoglobin receptor	Murine model of abscess formation	44
V. cholerae	*irgA*	Enterobactin receptor	Newborn mouse model of cholera	170
Brucella abortus	*bhuA*	Heme receptor	Murine macrophage and chronic spleen infection	171
Y. pestis KIM5	*yfeAB*	Components of iron and manganese ABC transport system	Murine models of plague	172
	irp2	Yersiniabactin biosynthetic enzyme	Murine models of plague	173
	psn	Yersiniabactin receptor	Murine models of plague	173
Bordetella pertussis	*tonB*	Energy transducer	Murine respiratory infection model	174
K. pneumoniae	*tonB*	Energy transducer	Murine model (intraperitoneal and intragastric inoculation)	175
S. enterica serovar Typhimurium	*fur*	Iron-responsive regulator	Systemic murine infection model	176
H. influenzae type b	*hbpA*	Heme binding lipoprotein	Weanling rat model of bacteremia	177
E. coli 018:K1:H7	*iroN*	Salmochelin receptor	Rat model of neonatal meningitis	178

free iron ions, are able to extract iron from mammalian iron-binding proteins and deliver it to specific outer membrane receptors or lipoproteins. Hundreds of siderophores have been described, and they are generally classified by the nature of the ligand employed to bind iron (e.g., hydroxamates and catechols) (39). A typical example of siderophore-mediated iron acquisition is the yersiniabactin system of *Yersinia pestis*, which is able to extract iron from mammalian transferrin and lactoferrin proteins and deliver it to a specific outer membrane protein. Unless inoculated directly into the bloodstream, mutant strains of *Y. pestis* lacking yersiniabactin are avirulent in mouse models of bubonic plague, indicating that siderophore-mediated iron uptake is essential to pathogenesis in this model (46).

Citrate can be used by some pathogens as an iron-chelating molecule, and receptors specific for the uptake of ferric citrate are typically grouped with siderophore receptors (47). Whether the bacteria produce the citrate or utilize host citrate is uncertain, as it has been noted that certain pathogens may not produce sufficient citrate for iron uptake (39). In cases of iron overload, some evidence suggests that ferric citrate may be the primary form of non-transferrin-bound iron present in humans (36). Although this does not rule out the possibility that bacterial pathogens produce citrate as a siderophore, it does suggest that ferric citrate may be a biologically relevant iron source in either case.

An alternative strategy to siderophore-mediated iron uptake is the utilization of outer membrane receptors that directly recognize mammalian iron-binding proteins, such as transferrin and lactoferrin. For example, all clinical isolates of pathogenic *Neisse-*

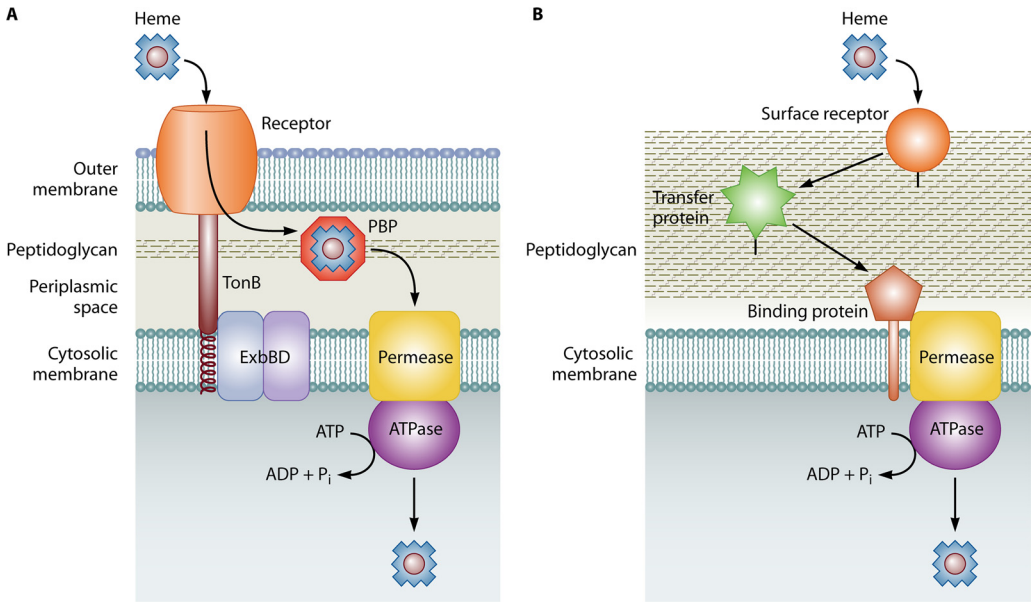

FIG 1 Schematic of bacterial heme acquisition systems. (A) In Gram-negative bacteria, heme binds an outer membrane (OM) receptor that transports it to the periplasmic space by using energy transduced from the cytosolic membrane via the TonB/ExbBD complex. A periplasmic binding protein (PBP) transfers the heme to a membrane-spanning permease, and transport across the cell membrane is mediated by an ATPase. (B) In Gram-positive bacteria, the absence of an OM eliminates the need for TonB/ExbBD and the PBP. In general, a cell wall-anchored surface receptor binds heme and relays it to an intermediate cell wall-anchored receptor (labeled a transfer protein in this diagram), which then transfers the heme to the binding protein and permease of an ABC transporter at the cell membrane. Energy for transport across the membrane is provided by an ATPase. Peptidoglycan is shown for orientation.

ria spp. encode a set of transferrin-binding proteins (TbpA and TbpB) dedicated to the acquisition of iron from human transferrin. TbpA is a TonB-dependent receptor that binds the C-terminal lobe of transferrin, where it extracts iron through a distortion of the iron-binding site and transports it across the outer membrane. TbpA alone is sufficient for transferrin utilization, but the coreceptor TbpB enhances the efficiency of uptake by binding transferrin and increasing its concentration for subsequent utilization by TbpA (48). A distinct set of neisserial proteins (LbpA and LbpB) is dedicated to the utilization of human lactoferrin. Molecular modeling, based on limited sequence homology, suggests that the Lbps are functionally similar to the Tbps. In contrast to TbpB, LbpB does not increase the efficiency of lactoferrin uptake. In addition to functioning as a coreceptor, it may play a protective role by binding and thereby neutralizing the antimicrobial cleavage product of lactoferrin, lactoferricin (49). These examples illustrate the fact that pathogens have several strategies to circumvent mammalian iron sequestration mechanisms.

The Feo system of Gram-negative bacteria supports direct uptake of ferrous iron. FeoB is a transport GTPase protein located in the cytosolic membrane and is usually associated with FeoA, a small cytoplasmic protein of unknown function (50). An additional open reading frame, designated *feoC*, may be present in the operon, and its importance is unknown (51). Feo systems appear to be preferentially expressed and used under anaerobic and microaerobic conditions when ferrous iron is expected to be the dominant species (52). Attenuated colonization of mouse gastric mucosa and intestine by *feo* mutants of *E. coli* (53), *Helicobacter* sp. (54), and *Salmonella* sp. (55) has been reported. In contrast, a *feo* mutant of *Vibrio cholerae* is fully virulent in mouse models, suggesting that redundant or alternative systems may be present (51). Uptake systems dedicated to the acquisition of ferrous iron suggest that extracellular reduction of ferric iron and subsequent uptake might be an alternative strategy to uptake of ferric iron. Accordingly, evidence has been presented for the secretion of extracellular ferric reductases by several pathogens (56–58).

Some bacterial pathogens utilize human ferritin as an iron source. *Neisseria meningitidis* does so indirectly by decreasing transferrin uptake, which induces a cellular iron starvation response. Ferritin is thought to be degraded by the host cell to meet its own iron needs; *Neisseria* is able to access the released iron as well (59). In contrast, in *Burkholderia cenocepacia*, an opportunistic pathogen of cystic fibrosis patients, ferritin degradation and subsequent iron release appear to be directly mediated by a secreted or surface-bound serine protease (60). Ferritin levels are known to be higher in the lungs of people with cystic fibrosis relative to healthy individuals, suggesting a direct link to the pathogenesis of this organism. *Bacillus cereus* is also able to use ferritin through a surface-localized NEAT (near iron transporter) domain protein (61). Although not clearly defined, extracellular pathogens, such as *Streptococcus pneumoniae*, may access ferritin following its release from cells damaged or lysed by virulence determinants or may utilize serum or secreted ferritin (62). Additional studies are needed to fully define the mechanisms of ferritin utilization, but these reports provide yet another example of the fact that bacterial pathogens employ multiple mechanisms to promote their survival in the iron-limiting environment of the mammalian host.

Heme acquisition systems. In accordance with the abundance of heme in mammals, many pathogens use dedicated heme acquisition systems to obtain iron and/or heme. In addition to preserving the structural architecture characteristic of TonB-dependent receptors, Gram-negative heme receptors share amino acid homology, including conservation of FRAP/NPNL domains. Two conserved histidine residues, one of which is in the FRAP/NPNL motif, are required for heme utilization in *Yersinia* (63). The Gram-negative heme receptors are classified by substrate specificity into either "heme scavenger" receptors, which are able to obtain heme from a variety of heme-containing proteins, such as the HemR receptor of *Yersinia enterocolitica*, or more specific hemoglobin receptors, as exemplified by HmbR of *N. meningitidis* (63, 64). Dedicated mechanisms for the utilization of myoglobin as a heme source have not been reported to date. However, myoglobin can serve as an iron source for some organisms *in vitro*. Potential mechanisms include recognition and binding of myoglobin by broad-specificity hemoprotein receptors (63, 65), utilization of spontaneously released heme following oxidation of myoglobin (66), and utilization of haptoglobin-bound myoglobin (67). The degree of myoglobin availability and utilization *in vivo* is uncertain.

In pathogenic *E. coli*, heme acquisition is facilitated by the production of autotransporter proteins that have hemoglobin protease activity. In general, autotransporter proteins have diverse functions but share a unique secretion mechanism (type V secretion system) whereby a signal sequence traffics the protein to the periplasm. Once there, the C terminus of the protein generates a β-barrel in the outer membrane that allows the passenger domain to exit the cell (68). Hemoglobin proteases from *E. coli* belong to the SPATE (serine protease autotransporter proteins of *Enterobacteriaceae*) family of proteins and are only found in pathogenic strains (68, 69). *E. coli* hemoglobin proteases are thought to bind the released heme and deliver it to an unknown surface receptor (68).

Heme acquisition systems in Gram-positive bacteria differ somewhat from those of Gram-negative bacteria. The iron-regulated surface determinant system (Isd) of *Staphylococcus aureus*, encoded by 10 genes, extracts heme from hemoglobin with the cell wall-anchored proteins IsdH and IsdB and then sequentially passes the heme to various proteins in the Isd system until it is transported into the cytosol (70). Once there, it is either incorporated into bacterial heme proteins or degraded by the heme oxygenases, IsdG and IsdI (71). The cell wall-anchored components of this system share one or more NEAT domains, which facilitate heme binding and transfer (72).

Analogous to the extracellular scavenging of iron by siderophores, hemophores are high-affinity binding molecules that either extract heme from hemoproteins or bind free, extracellular heme and deliver it to appropriate surface receptors. In the Gram-positive pathogen *Bacillus anthracis*, two secreted proteins, IsdX1 and IsdX2, are involved in extracellular heme capture (73). IsdX1 binds heme and delivers it to either IsdX2 or the cell wall-associated IsdC protein. Akin to the *S. aureus* heme acquisition system, the interaction of these proteins with heme and each other is thought to be mediated by the NEAT domains of each respective protein (74). Gram-negative hemophores have also been described (42).

Once heme has been transported into the cytosol, it can be directly incorporated into bacterial proteins or degraded by bacterial heme oxygenases (HOs) to release the iron. One class of heme oxygenase, homologous to human heme oxygenase 1 (HO-

1), generates iron, carbon monoxide, and biliverdin upon cleavage of heme (75). A second class is exemplified by the IsdG family. Through a mechanism that hasn't been fully defined, this family of enzymes generates iron, the novel oxo-bilirubin chromophore staphylobilin, and formaldehyde, rather than carbon monoxide, upon the cleavage of heme (76–78). ChuS of *E. coli* O157:H7 may represent a third, structurally distinct class of HOs (79).

In other Gram-negative pathogens, ChuS homologues protect against heme toxicity at high heme concentrations and are required for efficient heme utilization at low heme concentrations, but they appear to function as heme trafficking proteins rather than heme oxygenases (80, 81). In particular, PhuS of *Pseudomonas aeruginosa* has been shown to bind heme and deliver it to the HO, HemO (82). In certain Gram-positive pathogens, protection against heme toxicity is conferred by the ABC transporter proteins HrtAB. These proteins are proposed to function in heme efflux, effectively detoxifying excess heme by exporting it from the cell (83, 84). In parallel with the HOs, the heme trafficking and putative efflux proteins promote the use of heme as an iron source and protect pathogens against heme toxicity.

Bacterial iron storage systems. Irrespective of the mechanism used to acquire iron, once it has been obtained it can be stored intracellularly. Bacterial storage systems with similarities to mammalian ferritin have been described, including ferritin and bacterioferritin, both of which consist of a hollow sphere comprised of 24 subunits (85, 86). The ferroxidase center of these proteins is highly similar to the ferroxidase center of the mammalian ferritin heavy (H) chain (87). *E. coli* ferritin is reportedly able to accommodate ~2,000 iron atoms, while bacterioferritin has the capacity for ~1,800 iron atoms (85, 86). Bacterioferritin is unique in that a heme b molecule is bound between every two subunits, and evidence suggests that it promotes electron transfer for the reduction and subsequent release of iron from the core (88). These proteins provide a system for the accumulation and storage of iron reserves that can be tapped when iron becomes scarce.

Dps (DNA-binding protein from starved cells) may represent an alternative iron storage system; homologues have been identified in many species, although not all of them are able to bind DNA. The *E. coli* Dps consists of 12 identical subunits that generate a hollow core for iron storage and possess a markedly different ferroxidase center than that found in ferritin and bacterioferritin. Dps reduces one hydrogen peroxide molecule for every pair of ferrous iron ions oxidized, which bypasses generation of the hydroxyl radical through a single electron transfer and neutralizes the hydrogen peroxide. These activities contribute to its role in oxidative stress resistance, regardless of its ability to physically shield DNA (89). It has, in fact, been hypothesized that the primary function of Dps is stress resistance rather than iron storage. In support of this hypothesis, the Dps core only contains ~500 iron atoms (89). Additional studies are needed to fully explore the capacity of the Dps protein to serve as an iron source under iron-limiting conditions.

ALTERATION OF HOST IRON-RELATED PROTEINS DURING THE ACUTE-PHASE RESPONSE

The extensive iron acquisition strategies employed by bacterial pathogens may render the host's basal iron homeostasis suboptimal for a successful defense. A marked change in iron metabolism is a central component of a larger systemic host response to infection (as well as other stressors, such as tissue injury, inflammation, and cancer), collectively termed the acute-phase response (APR). The APR is initiated by the innate immune system in an attempt to neutralize the source of infection or injury while minimizing collateral tissue damage. The characteristic APR includes fever, certain hormonal changes, leukocytosis, and alterations in the hepatocellular production of multiple plasma proteins. Plasma proteins that increase in concentration during the APR are termed "positive" acute-phase proteins (APPs), while those that decrease are termed "negative" APPs (90). The APPs include protease inhibitors, secreted pathogen recognition receptors, clotting and coagulation factors, and complement proteins, as well as multiple proteins relevant to iron metabolism (91).

Alterations in hepatocellular APP synthesis are initiated by certain inflammatory cytokines produced by activated macrophages and monocytes. Interleukin-6 (IL-6) and IL-1 are the principal regulators of the APPs, but additional cytokines have been implicated, including tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) (91). For some cytokines, including IL-6, homodimerization of the receptor-associated molecule gp130 is required to transduce the signal upon binding to their cognate receptors (92, 93). Intracellular signaling molecules include STAT3 (for IL-6) and NF-κB (92). Glucocorticoids have also been shown to modify expression levels of certain APPs (91).

The APR markedly alters host iron metabolism in ways that serve to minimize iron bioavailability to pathogens (Fig. 2). These include (i) decreased iron release into the circulation (for which the APP mediator is hepcidin), (ii) increased intracellular iron storage (for which the APP mediator is ferritin), (iii) decreased accessibility of extracellular nonheme iron (for which the APP mediators are ceruloplasmin and lactoferrin), and (iv) decreased accessibility of extracellular heme iron (for which the APP mediators are hemopexin and haptoglobin). Each of these processes is discussed in detail below.

Decreased iron release into the circulation. Originally described as an antimicrobial peptide, hepcidin is arguably the major APP that contributes to the hypoferremia associated with the APR (94). The hepcidin-mediated degradation of ferroportin decreases the efflux of iron by RE macrophages and hepatocytes, resulting in a net decrease in the plasma iron concentration. Dietary iron absorption is likewise decreased through the inhibition of iron efflux in duodenal enterocytes (95). IL-6 is the major inflammatory cytokine that upregulates hepcidin expression in the APR (96); however, IL-1 and IL-22 have also been implicated (97). In cultured cells, induction of hepcidin is synergistic when cells are treated with BMP6 (a signaling molecule for the BMP/SMAD pathway that is responsible, in part, for the hepatic upregulation of hepcidin in response to iron), when combined with either IL-6 or IL-22. This is of particular interest because iron-loaded ferritin has been shown to increase hepatic *Bmp6* mRNA levels in mice (98). These observations suggest a potential feed-forward mechanism for maintaining high levels of hepcidin despite decreased circulating iron. Activin B, itself upregulated by lipopolysaccharide (LPS) challenge in mice, also induces hepcidin via the BMP/SMAD pathway in response to inflammation and also synergizes with the IL-6/STAT3 pathway in human hepatoma cells (99). The observation that mice with a liver-specific knockout of *Smad4* do not upregulate hepcidin in response to IL-6 injection provides additional evidence suggesting that an intact BMP signaling pathway is required for the upregulation of hepcidin by inflammation (100).

It has been proposed that, in addition to altering iron efflux,

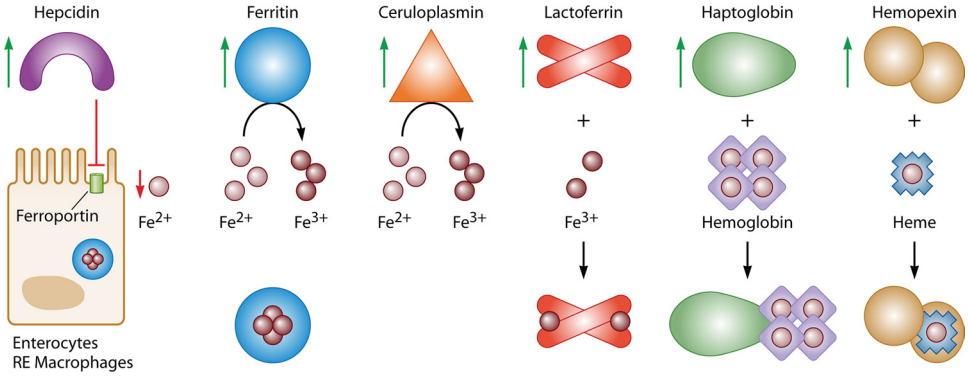

FIG 2 Iron-related APPs coordinate hypoferremia in response to infection and inflammation. Infection and inflammation result in the production of proinflammatory cytokines, such as IL-6, by immune effector cells. In turn, proinflammatory cytokines bind to cognate receptors on hepatocytes, triggering a signaling cascade that results in increased synthesis (indicated by green arrows) of several iron-related APPs. Decreased release of iron into the circulation is facilitated by hepcidin upregulation, which results in a net decrease in plasma iron by binding to and promoting degradation of ferroportin on hepatocytes, RE macrophages, and duodenal enterocytes. Increased intracellular iron storage results from the induction of ferritin. Decreased bioavailability of nonheme iron is mediated by ceruloplasmin's ferroxidase activity, combined with binding of Fe^{3+} by lactoferrin. Decreased availability of extracellular heme iron is mediated by the induction of hemopexin, which results in the binding of free heme, while haptoglobin binds free hemoglobin and promotes its clearance. This coordinated response is thought to deprive invading microorganisms of iron while simultaneously protecting tissue from unnecessary oxidative stress resulting from the interaction of iron with immune mediators.

the hepcidin-mediated internalization of ferroportin modulates the macrophage production of inflammatory cytokines via the activation of JAK2 and subsequent phosphorylation of STAT3. In support of this hypothesis, hepcidin treatment is protective against lethal challenge with LPS in murine models (101). However, recent evidence suggests that internalization of ferroportin by hepcidin is mediated by ubiquitination and is independent of JAK2/STAT3 activation (102). Direct antimicrobial activity, including disruption of bacterial membrane integrity and inhibition of growth, has also been reported for hepcidin present at a concentration of 200 μg/ml (103). Whether such a concentration is locally achievable *in vivo* is uncertain.

While decreased iron release into the circulation may be beneficial in limiting iron availability to extracellular pathogens, it is possible that the concomitant increase of intracellular iron may facilitate its availability to certain intracellular pathogens. For organisms that proliferate intracellularly, whether a low hepcidin state or a high hepcidin state protects against or facilitates iron availability may depend on the cell type in which it proliferates. It has been suggested, for example, that the low hepcidin state in HFE-associated hereditary hemochromatosis (HH) may provide a selective advantage by decreasing macrophage iron and protecting the host against *Salmonella enterica* serovar Typhimurium, a bacterium that proliferates in this cell type (discussed below) (104).

Increased intracellular iron storage. Expression of genes encoding the L-type and H-type ferritin proteins is increased in the APR (105). Moreover, hepcidin-mediated cellular iron retention serves to increase translation of the ferritin proteins (through an iron response element in the ferritin mRNAs) (106). The ferritin H-chain possesses ferroxidase activity, facilitating a rapid decrease in redox-active iron from both the extracellular space and the intracellular labile iron pool. The increased retention and storage of iron as ferritin in macrophages (107) contributes to the characteristic fall in serum iron concentrations and increase in serum ferritin concentrations observed in the APR. In general, increased intracellular iron storage is expected to increase resistance to infection. The decreased intracellular labile iron pool is expected to limit the growth of intracellular pathogens, and the decreased serum iron concentration is expected to limit the growth of extracellular pathogens. However, as noted above, certain pathogens are able to circumvent these decreases in available iron by utilizing ferritin as an iron source. Therefore, the increased ferritin in macrophages may ultimately be beneficial to a pathogen, such as *B. cenocepacia*, that can survive in a macrophage and utilize ferritin (60, 108).

Decreased bioavailability of extracellular nonheme iron. Ceruloplasmin is a positive APP, with blood concentrations increasing up to 6-fold in response to inflammation (109). By virtue of its ferroxidase activity, ceruloplasmin promotes the loading of iron onto transferrin and lactoferrin. The oxidation of extracellular ferrous iron also prevents it from participating in Fenton chemistry. Ceruloplasmin scavenges superoxide, a neutrophil immune effector (110). This property decreases the availability of reactants for the Haber-Weiss cycle, which can generate Fe^{2+} required to interact with H_2O_2 during Fenton chemistry from Fe^{3+} and superoxide (111).

Transferrin is a negative APP (112), i.e., transferrin synthesis and circulating transferrin levels decrease during inflammatory states. The biological significance of this downregulation is not fully understood. Because circulating iron and transferrin levels both decrease during the APR, the measured transferrin saturation is generally normal, or only moderately decreased. Nonetheless, the total concentration of iron-loaded transferrin in the circulation is decreased. At the same time, iron availability to the host erythron for hemoglobin synthesis is decreased. The consequent iron-restrictive erythropoiesis contributes to the anemia of inflammation (or anemia of chronic disease) (113). Circulating inflammatory cytokines also contribute to the hypoproliferative state by disrupting erythrocyte maturation (114).

Lactoferrin is a positive APP produced in sufficient amounts to compensate for the concomitant decrease in transferrin (115). Increased hepatic synthesis of lactoferrin occurs in response to IL-6, IFN-γ, and TNF-α in mice. Serum concentrations of lacto-

ferrin also increase due to proinflammatory cytokine-mediated neutrophil degranulation at sites of inflammation (20, 116). Apolactoferrin binds and sequesters iron, thereby limiting the amount of iron available to support pathogen growth and react with oxygen-dependent immune effectors, such as hydrogen peroxide and superoxide. Moreover, positively charged peptides derived from lactoferrin have direct antimicrobial activity through their interaction with negatively charged bacterial membrane components (117, 118).

Overall, decreased bioavailability of extracellular nonheme iron is expected to target the iron acquisition of extracellular pathogens and limit their growth. Increased ceruloplasmin decreases the concentration of any available extracellular Fe^{2+} and promotes the loading of Fe^{3+} onto transferrin and lactoferrin. The decreased production of transferrin likely limits iron availability to those pathogens capable of extracting iron from it. Increased production of lactoferrin is expected to bind and sequester iron. This may result in increased iron availability for those pathogens able to use lactoferrin as an iron source. However, the potential benefit of additional lactoferrin-bound iron may be fully offset by the antimicrobial activity of lactoferrin-derived peptides (117, 118). Facultative intracellular pathogens, such as *Neisseria* spp., able to utilize these molecules are likely to be affected in the same manner as extracellular pathogens, whereas these changes would presumably not have a major impact on the accessibility of iron to obligate intracellular pathogens (48, 49).

Decreased bioavailabilty of heme iron. The APR leads to an increase in liver haptoglobin synthesis, a process mediated by IL-6 and glucocorticoids. Neutrophils also contribute to increased concentrations of haptoglobin through local degranulation at sites of inflammation and injury (119). Haptoglobin binds free hemoglobin released during hemolysis and facilitates its uptake through the CD163 hemoglobin scavenger receptor present on monocytes and macrophages. Of particular relevance during an inflammatory response, haptoglobin binding protects hemoglobin from peroxide-mediated damage that would otherwise prevent its uptake by CD163 (120). Thus, increased haptoglobin production has an antimicrobial function through its iron-sequestering activities and an antioxidant function by preventing hemoglobin-mediated generation of oxidative species.

When the binding capacity of haptoglobin has been surpassed, free hemoglobin is rapidly oxidized to methemoglobin. Hemin (Fe^{3+}-protoporphyrin) dissociates more readily from globin than heme (Fe^{2+}-protoporphyrin) and is released as a free molecule. Although albumin binds heme in the bloodstream, hemopexin, another positive APP, does so with much higher affinity and is considered the primary heme-scavenging molecule (121). The heme-hemopexin molecule binds the scavenger receptor LRP1 (low-density lipoprotein receptor-related protein 1); upon internalization, the two molecules dissociate, and intracellular heme can be used directly, catabolized by a heme oxygenase, or exported (122).

Extracellular and facultative intracellular pathogens are most likely to experience restricted heme access as a consequence of the upregulation of haptoglobin and hemopexin. This expectation is qualified by the fact that many pathogens have receptors that recognize the heme-hemopexin complex and the haptoglobin-hemoglobin complex. For intracellular pathogens, these changes may result in an increase in the availability of heme and/or iron, depending on the host cell type. The haptoglobin-hemoglobin complex is taken up by macrophages and monocytes; LRP1 is present on many cell types, including macrophages, hepatocytes, and neurons (122).

IRON STATUS AND SUSCEPTIBILITY TO INFECTION

Additional evidence supporting a pivotal role for iron in infection and immunity comes from studies that have examined susceptibility to infection as a function of host iron status. These studies have demonstrated the effects of iron overload, both clinically and in animal models, on infection and inflammatory responses. Mutations in several genes participating in hepcidin regulation can give rise to HH. *HFE*-associated HH is the most common; *HFE* encodes the major histocompatibility complex class I-like hemochromatosis protein (123). Mutations in the repulsive guidance molecule family member hemojuvelin result in juvenile hemochromatosis (124). Mutations in the genes encoding hepcidin or transferrin receptor 2 or gain-of-function mutations in ferroportin also cause HH. In these settings, serum and tissue iron concentrations are high, while iron concentrations in RE macrophages are comparatively low. Individuals with hemochromatosis are more susceptible to infection by certain pathogens. For example, increased risks for infections with *E. coli* (125), *V. cholerae* (126), *Y. enterocolitica* (127), and *Listeria monocytogenes* (128) are associated with iron overload. HH patients are also vulnerable to infection by *Vibrio vulnificus*. The specific role for iron in this susceptibility has been suggested by studies in which the addition of ferric ammonium citrate or hematin to whole blood from control subjects was sufficient to promote the same degree of *V. vulnificus* growth as observed in whole blood taken from hemochromatosis patients (129). Studies in macrophages from murine *Hfe* knockout models indicated that the Toll-like receptor 4 signaling in response to LPS challenge is impaired. Evidence suggests that this signaling impairment involves the TRAM/TRIF adaptor molecules, rather than MYD88, and is associated with decreased production of IL-6 and TNF-α. Moreover, these changes are associated with decreased intestinal inflammation in response to *Salmonella*-mediated enterocolitis (130). However, as discussed above, the decreased macrophage iron consequent to loss of *HFE* may be advantageous in the context of certain infections. For example, $Hfe^{+/-}$ and $Hfe^{-/-}$ mice injected intraperitoneally with *S. enterica* serovar Typhimurium (a pathogen that proliferates in macrophages) show decreased hepatic and splenic bacterial loads and increased survival compared to $Hfe^{+/+}$ mice, and isolated *Hfe*-deficient macrophages show enhanced antimicrobial activity following infection with *S. enterica* serovar Typhimurium compared to wild-type macrophages (104). Likewise, decreased iron acquisition is associated with decreased growth of *Mycobacterium tuberculosis* in monocyte-derived macrophages from HH patients with *HFE* mutations compared to control macrophages (131).

Iron overload results from ineffective erythropoiesis in thalassemia intermedia (132) and from both blood transfusion and ineffective erythropoiesis in thalassemia major. Several factors contribute to increased susceptibility to infection in this population. In particular, the iron chelator deferoxamine (DFO) is a siderophore that can enhance the growth of several pathogens commonly associated with thalassemia, including *Y. enterocolitica* and *Klebsiella pneumoniae* (133, 134). Nonetheless, iron overload is an independent factor predisposing thalassemic patients to infection, as evidenced by the occurrence of these infections in the absence of

chelation therapy and when alternative chelators that are unable to enhance the growth of these organisms are used (134, 135).

Dietary iron loading, as measured indirectly in rural Africans based on consumption levels of traditional beer, which is high in ferrous iron, was associated with a 3.5-fold increase in the odds of developing active tuberculosis, and among those treated for pulmonary tuberculosis, there was a 1.3-fold increase in the hazard ratio of death compared to individuals without increased dietary iron intake (136). Of note, heavy alcohol consumption is associated with the incidence of tuberculosis, as well as the outcome; dose-response relationships have been reported (137). For example, a retrospective study examining the effects of alcohol consumption, based on family member reports, on cause of mortality in Russia found a dose-response relationship between alcohol and tuberculosis. Those in the highest alcohol consumption category, defined by reported consumption of three or more 0.5-liter bottles of vodka per week, had a relative risk of tuberculosis greater than 3.0 compared to controls, who reportedly consumed less than one 0.5-liter bottle of vodka per week (138). Thus, it is possible that the increased odds of developing tuberculosis reported in rural Africans are influenced by alcohol consumption, but the authors of that study pointed out that African traditional beer is low in alcohol content and that histological changes consistent with alcoholism are notably absent in liver biopsy specimens from patients with African iron overload. In a different study, the effects of dietary iron intake as a function of traditional beer consumption and single nucleotide polymorphisms (SNPs) in the gene encoding ferroportin were examined in tuberculosis patients relative to controls. Overall, four SNPs were associated with an increased risk of tuberculosis. Gene-environment interactions were also reported for four SNPs; two of them were associated with a significant increase in the risk of tuberculosis when iron intake was high. Interestingly, the SNP encoding the Q248H ferroportin mutation was not associated with risk for tuberculosis in this study (139). In contrast, the prevalence rates of both pulmonary tuberculosis and *Pneumocystis jirovecii* pneumonia were increased in HIV-positive Rwandese women with the Q248H ferroportin mutation relative to those without the mutation (140). On the whole, these studies emphasize the importance of host iron status with respect to susceptibility and clearance of infection.

FUTURE DIRECTIONS

Several areas of future research are warranted. Targeting conserved bacterial iron uptake systems and mechanisms may result in novel, broad-spectrum therapeutics, a strategy that is especially relevant given the expanding problem of antimicrobial resistance. Expanded exploration of iron-related genes required for virulence may help to identify new targets for vaccine candidates. The role of iron in the outcome of infection implies that both anemia and iron supplementation should be studied carefully to ensure successful management in areas where certain infectious diseases are endemic. Continued investigation of iron-related APPs may improve the treatment of disease. Elucidation of the mechanisms regulating iron metabolism in both the host and pathogen may ultimately result in novel strategies to promote a successful host defense.

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