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MINIREVIEW

Hepcidin: the Missing Link between Hemochromatosis and Infections

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The aphrodisiacal potential of oysters is the stuff of maritime folklore, having roots in tales of Aphrodite (literally "foam born"), the goddess of love, said to have arisen from the sea on an oyster shell. However, mariners' tales chronicling oysters' lethal potential, despite having abounded since the 5th century B.C., have received less attention. *Vibrio vulnificus*, originally described in 1979 (6; J. J. Farmer III, Letter, Lancet **ii**:903, 1979), is a particularly virulent, lactose-fermenting, motile gram-negative halophilic bacterium that inhabits temperate coastal waters, notably of the Gulf of Mexico. *V. vulnificus* is concentrated in fish and filter feeders such as oysters and clams (up to 50% culture positive) and crabs (up to 11% culture positive) (18, 72), and infection associated with exposure to this organism may range from wound infections to fatal septicemic shock (38, 69). Moreover, as probably the leading cause of seafood-associated fatalities in the United States, this organism is considered a significant public health hazard, since despite early recognition, aggressive antibiotic therapy, and surgical debridement, the morbidity, mortality, and cost associated with *V. vulnificus* infections remain substantial (48, 49).

Risk factors for *V. vulnificus* infection include the *V. vulnificus* subtype (various genetically distinct subgroups of biotype 1 identified by randomly amplified polymorphic DNA PCR appear to be especially virulent), immunocompromised state (human immunodeficiency virus, cancer, bone marrow suppression, achlorhydria, and diabetes), end-stage renal impairment, liver impairment (particularly cirrhosis [infection risk, 200-fold]) (33), and hemochromatosis (primary or secondary such as the hemolytic anemias and thalassemias or porphyria cutanea tarda) (11, 30, 31, 51, 71). Interestingly, these same patients, especially those with iron overload, also have a striking predisposition to other aggressive bacteria, including *Listeria monocytogenes*, *Klebsiella* sp., and *Yersinia* sp. (the last of these, perhaps coincidentally, is also a potentially waterborne pathogen found in fish) (1, 14, 32, 43, 51, 61, 63, 70, 76; J. Collazos, E. Guerra, A. Fernandez, J. Mayo, and E. Martinez, Letter, Clin. Infect. Dis. **21:**223-224, 1995; M. L. Delforge, J. Devriendt, Y. Glupczynski, W. Hansen, and N. Douat, Letter, Clin. Infect. Dis. **21:**692-693, 1995).

This review focuses particularly on *V. vulnificus* but also on other organisms—as an archetypal group of iron-sensitive pathogens—in order to summarize the conventional mechanisms through which bacterial virulence factors and host factors have been suggested to interact to cause disease in patients with iron overload. I subsequently argue that although many of these established factors unequivocally contribute to disease and indicate that iron excess has profound effects both on the host immune response and is also essential for pathogen survival, they fail to provide a unifying explanation for specific host susceptibility to these pathogens. I propose that an effective host response will at least in part be augmented by hepcidin, a recently identified cysteine-rich cationic antimicrobial peptide central to iron metabolism (35). I develop the hypothesis that hepcidin represents an important host response to *V. vulnificus* and similar iron-sensitive pathogens. Failure of adequate hepcidin expression in patients with liver disease and functional impairment due to high iron concentrations in hemochromatosis may impair effective hepcidin bactericidal activity, predisposing the patient to severe infection. Translation of this hypothesis from bench to bedside will be of significance for the counseling and management of those predisposed to *V. vulnificus* and other infections and may more broadly inform our understanding of more-complex, iron-dependent host-pathogen interactions.

BACTERIAL VIRULENCE AND HOST RESPONSE

V. vulnificus boasts a vast armamentarium of putative bacterial factors that may contribute to its pathogenesis (39, 68), including an acidic polysaccharide capsule (which mediates bacterial resistance to phagocytosis and complement-mediated lysis) (2, 67, 73, 74), type IV pili (fimbriae) (which mediate attachment), and degradative enzymes conferring invasiveness (e.g., a metalloprotease, a hemolysin-cytolysin, and a phospholipase). *Yersinia* species analogously express a constellation of thermogenically regulated proteins such as the Inv and YadA proteins, which mediate adhesion and invasion of the epithelium, partially through attachment mediated by the β-1 integrins. They also similarly express Ail proteins and *Yersinia* outer membrane proteins, which, among other functions, protect against complement-mediated bacterial lysis, interfere with phagocytosis and cellular signaling, reduce local tumor necrosis factor alpha (TNF-α) secretion, and impair cellular function (9, 15, 16). Additionally, both these pathogens carry lipopolysaccharides (LPSs), which in conjunction with elements such as constituents of the complex polysaccharide capsule of *V. vulnificus*, may modulate the innate immune response and have the capacity to participate in mediating septic shock. They do so by engaging CD14-toll-like receptor 4–MD-2 complex, hence stimulating NF-B and elaborating TNF-α/NO. However, although this panoply of factors may

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significantly contribute to bacterial pathogenesis and to host morbidity and mortality, animal models coupled with genetically modified bacteria suggest that the majority of these factors such as the type IV pili, hemolysin, and metalloprotease are not essential for bacterial virulence (21, 29, 64, 88). Similarly, although the capsular components of *V. vulnificus* are capable of producing cytokines that may result in septicemia resembling endotoxic shock, it appears that its LPS is relatively innocuous and its contribution to bacterial virulence is limited (46, 58). By contrast, *V. vulnificus*'s type IV leader peptidase-*N*-methyltransferase, otherwise termed it prepilin peptidase (which executes pleiotropic functions, including forming adherence pili and general type II extracellular protein secretion), and the diverse iron-scavenging modalities do appear at least in some animal models to be essential for virulence (55).

IRON AND PATHOGENS

Following seminal observations that *V. vulnificus* exhibited reduced growth in serum unless iron-bound transferrin or hematin was added, that the intraperitoneal injection of iron to mice resulted in a dramatic lowering of the 50% lethal dose from 10^6 to 1.1 cells and in a reduction in the time of death following infection, and that virulence correlated directly with host iron availability (89), attention has focused on the mechanisms of essential iron acquisition and how this relates to virulence. The growth and metabolism of bacteria are exquisitely sensitive to iron—which is ultimately utilized in the active sites of enzymes, constrained sites of proteins, or directly in redox reactions to mediate otherwise kinetically improbable electron transfer reactions. Despite being one of the earth's most abundant elements, the levels of iron in the environment and in mammalian hosts (with elaborate iron-withholding defense systems described below) are often too low (some estimates suggest this may be as low as 10^{-9} M or even lower) to sustain this iron requirement that is destined for use in DNA and RNA metabolism, in the complex redox reactions of intermediate metabolism, and in electron transport. This situation is exacerbated in aerobic environments at neutral pH, as ferrous iron spontaneously oxidizes to its ferric Fe(III) state, predominantly existing as its insoluble inorganic hydroxide or oxyhydroxide (44).

As an adaptive strategy, many bacteria as well as other organisms have therefore developed siderophores (over 500 have been described, with bacterial varieties being predominantly of the hydroxymate and catechol-phenolate class), which are high-affinity iron-binding molecules that retrieve essential elemental iron from the host transferrin or lactoferrin and carry it in the Fe(III) state (87). Thus, either iron is delivered to the cell surface directly as elemental Fe(II) in conjunction with porins in the outer bacterial membrane and is facilitated by proteins such as FeoA and FeoB (these proteins have ATP/GTP-binding motifs suggestive of a role in facilitated uptake at the cytoplasmic membrane) or, alternatively, iron is transported in the form of complexes consisting of transferrin, lactoferrin, or heme and hemophores or siderophores, which are transported through the outer membrane by specific surface receptors that are energetically sponsored (through proton motive forces) by regulators such as the TonExbB-ExbD system. Iron complexes are subsequently imported through the cytoplasmic membrane in conjunction with periplasmic binding protein-dependent transport complexes (PBTs) (e.g., *Escherichia coli* FecB, FepB, and FhuD). PBTs either direct siderophore-bound iron to ferric reductases on cell surface membranes which release iron in its Fe(II) state or simply internalize siderophore complexes for intracellular processing (Fig. 1) (22). Importantly, in some instances, the Ton system can also transcriptionally drive iron uptake, as in the *E. coli* siderophore-mediated TonB-dependent receptor FecA. However, under different circumstances, excess bacterial iron is detrimental; transitional metal complexes can mediate unintended electron transfer either directly or through reactions such as the Fenton and Haber-Weiss reactions, producing superoxide or hydroxide anions. These electron transfers are highly toxic and damaging to macromolecules, including proteins and DNA (75). Scavenging systems are therefore exquisitely controlled through transcriptional repressors such as the *fur* system (22). This extensive and complex machinery, especially in light of the evolutionary imperative for genetic parsimony, evidences the importance of iron to bacterial viability.

Certain bacteria, including the pathogens *V. vulnificus* and *Yersinia* species, are particularly dependent on these scavenging techniques for survival (81, 82). Table 1 lists the wide variety of organisms whose virulence is enhanced by iron (81). Correspondingly, the inability to express the catechol siderophore vulnibactin in *V. vulnificus* reduced bacterial virulence by reducing iron acquisition (41). Additional defects in the *V. vulnificus* protease metalloprotease further limits the pathogen's ability to cleave transferrin and lactoferrin and to release iron to siderophores. More recently the *vuuA* gene, which encodes the vulnibactin receptor, has been cloned, and an internal deletion causing the loss of expression of the 72-kDa protein resulted in the loss of the ability to use transferrin or vulnibactin as a source of iron and a consequent loss in virulence (80). Assessment of isogenic *fur* mutants has also allowed the identification of downstream proteins such as the 77-kDa putative *V. vulnificus* heme receptor, *hupA* (40), which is also downstream of HupR, a positive regulator of *hupA* transcription under low-iron conditions in the presence of heme. Finally *V. vulnificus* also utilizes ferric reductases to further facilitate iron acquisition from siderophores into the cell (45). These extensive observations reaffirm the role of iron in *V. vulnificus*'s virulence.

Yersinia species are similarly highly dependent on iron. These gram-negative bacilli grow in iron-enriched host fluids as well as in macrophages, and interestingly the highly pathogenic forms carry a pathogenicity island, the high-pathogenicity island, which encodes genes for the synthesis of the siderophore yersiniabactin (12). This high-pathogenicity island has a wide distribution among different enterobacteria such as *E. coli*, *Klebsiella* sp., *Citrobacter* sp., and *Salmonella enterica*. Similarly to *V. vulnificus*, downstream of a *fur* iron repressor consensus protein-binding sequence, *Yersinia* species express an iron-repressible outer membrane protein, FyuA, operating as a receptor with the dual functions of acting as a receptor for the *Y. pestis* bacteriocin pesticin and as a receptor for yersiniabactin. Furthermore, desferrioxamine can be used by *Yersinia* spp. as a surrogate siderophore (5, 61), binding the bacterium's FoxA siderophore receptor (82). Rather like the *Vibrio* species, they also contain *hemR*, which encodes the receptor for heme.

Other well-recognized iron retrieval strategies include bind-

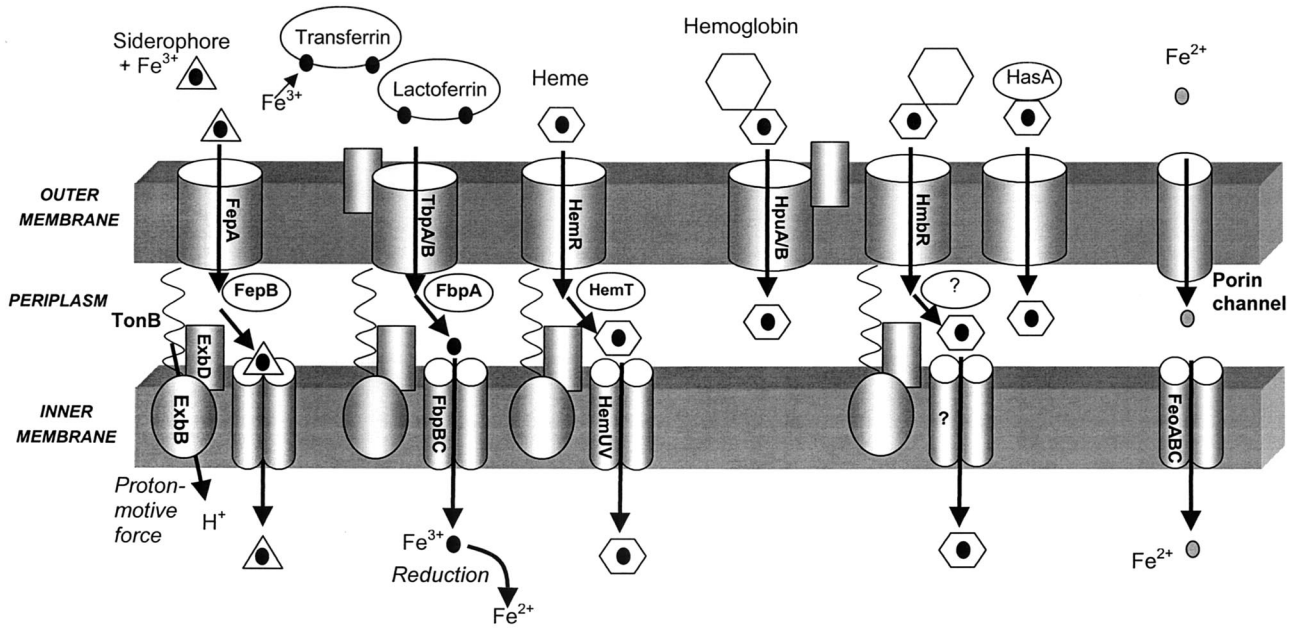

FIG. 1. Iron uptake mechanisms in gram-negative bacteria. Outer membrane receptor proteins (many exist and only a few are shown: FepA, TbpA/B, HemR, HpuA/B, HmbR, and HasR) bind iron-containing ligands (siderophores, lactoferrin, transferrin, heme, hemoglobin, and the *S. marcescens* hemophore HasA) and transport them to the periplasm. PBTs (e.g., FepB, FbpA, and HemT) then facilitate the TonB-ExbB/D-dependent transport to the ATP-driven cytoplasmic-membrane transporters (e.g., FepCDG, FbpBC, and HemUV), a process that depends on the proton motive force. Under anaerobic conditions, outer membrane porins transport soluble Fe^{2+} directly to the periplasm and then enter the cytoplasm via ATP-dependent FeoABC channels. (Adapted from reference 22 with permission of the publisher.)

ing of ferrated siderophilins (e.g., transferrin or lactoferrin) with extraction of iron from host complexes, as observed in *Haemophilus influenzae* and *Neisseria meningitidis. N. meningitidis*, for example, binds specific human ferrated transferrins through the essential TbpA (a TonB-dependent integral membrane protein) and TbpB (a nonintegral membrane protein with apolipoprotein features and a role in modifying the activity of TbpA) and transfers the iron into the periplasmic space, where it is retrieved by a ferric binding protein, Fbp. These ferric binding proteins play a role similar to that of PBTs (17, 34). Similar receptors exist for lactoferrin in other organisms. Direct heme or hemoglobin assimilation may be mediated by complexes such as the *N. meningitidis* Ton-dependent HpuB/HpuA and the HmbR systems and the HasA system from *Serratia marcescens*, which detaches heme from hemoglobin before transport (13). Similar elements may be of particular benefit during erythrocyte lysis, as observed in *Staphylococcus aureus*, *Bartonella* spp., and *L. monocytogenes*. Finally, acquisition of host intracellular iron may occur, as observed in *My cobacterium tuberculosis*, *S. enterica* serovar Typhimurium, *Francisella tularensis*, and *Legionella pneumophila*. Indeed, one remarkable obligate intracellular bacterial pathogen residing in human macrophage/monocyte lineages, *Ehrlichia chaffeensis*, is sufficiently dependent on iron that by activation of the host iron-responsive protein 1 mRNA-binding protein and subsequent stabilization of transferrin receptor mRNA, it induces intracellular inclusions of iron in the infected host cell, which is then ripe for bacterial utilization (4).

IRON AND THE HOST IMMUNE SYSTEM

In response, the host has its own mechanisms of withholding iron from microbes. These include increasing the production of iron binding proteins, reducing dietary iron assimilation, increasing hepatic production of hemoglobin and hemin scavengers (haptoglobin and hemopexin, respectively), and the release of apolactoferrin from neutrophils to sequester iron at sites of bacterial invasion. Conditions under which the level of iron in serum is increased compromise these host defenses and thereby predispose the host to invasion from these iron-requiring microbes (44).

In addition to its direct importance in bacterial growth, excess iron plays a crucial role in impairment of the host immune system (79, 85). Although not as extensively investigated as the immune impairment in iron deficiency states and subject to sometimes contradictory results in different models, it is clear that excessive iron may have profound effects on T-cell function, with increased $CD8^+$ counts at the expense of reduced $CD4^+$ cell counts and a reduced mitogenic response to standard antigens and impaired hypersensitivity responses. Specifically it appears that Th1 responses marshalling cellular immune responses may be impaired (with reduced interleukin-1 [IL-1] and TNF-α production). Furthermore, a well-maintained Th2 axis will through cytokines such as IL-4, further exacerbate this cellular impairment. This is consistent with retained IL-4 production and intact humoral antibody-mediated immunity. More pertinently to the bacterial pathogens discussed in this article, iron overload appears to potently impair the macrophage and neutrophil arms of the innate

TABLE 1. Genera of infectious-disease organisms which require iron for growth and whose virulence is enhanced by iron levels[a]

Genus
Gram-negative bacteria
Acinetobacter
Aeromonas
Campylobacter
Capnocytophaga
Chlamydia
Ehrlichia
Enterobacter
Escherichia
Klebsiella
Legionella
Moraxella
Neisseria
Pasteurella
Proteus
Pseudomonas
Salmonella
Shigella
Vibrio
Yersinia
Gram-positive bacteria
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Listeria
Staphylococcus
Streptococcus
Acid-fast bacterium
Mycobacterium
Protozoa
Entamoeba
Leishmania
Naegleria
Plasmodium
Toxoplasma
Trypanosoma
Fungi
Candida
Cryptococcus
Histoplasma
Paracoccidioides
Pneumocystis
Pythium
Rhizopus
Trichosporon

[a] Adapted from reference 81.

immune response from antibody-mediated and opsonin-dependent phagocytosis (19, 36, 44). For example, one study demonstrated that a hemochromatosis patient with *Listeria* meningitis showed a diminished capacity to phagocytose *S. aureus*, a phenomenon which was reversible by repeated iron-reducing phlebotomies. This finding has been confirmed in a number of different cases of iron overload (50, 77, 78). Iron, for example, has a direct inhibitory effect on vital gamma interferon-mediated pathways in macrophages, such as NO formation, TNF-α formation, major histocompatibility complex class II expression, and ICAM-1 expression (59, 86). As a consequence, gamma interferon pathways become ineffective at destroying intracellular pathogens in iron-overloaded macrophages. This has been shown to detrimentally affect the immune response to *Legionella*, *Listeria*, *Ehrlichia*, and some viruses, where NO is critical (83)—with iron blocking the transcription of inducible NO synthase (7). Most recently, appreciation of the subtle yet critical relation between the immune response and iron has been exemplified by the identification of NRAMP-1 (natural resistance-associated macrophage protein 1)—which both is involved with modulation of iron metabolism in macrophages and plays an important role in early-phase macrophage activation and therefore in host innate immunity (90).

HYPOTHESIS: HEPCIDIN IS THE HOST DEFENSE AGAINST *V. VULNIFICUS*

It is thus clear that the well-recognized association between the risk factors and invasive infection has been the subject of intense speculation. However, despite an increased appreciation of both the host and the pathogen axes, particularly with respect to the crucial role of iron acquisition as manifested by the extensive scavenging apparatus and the impact of iron on the immune response, a unifying explanation that adequately explains the striking susceptibility of the specific patient groups to specific bacteria remains elusive. For example in addition to iron overload, liver disease has been shown to be the underlying predisposing factor in *V. vulnificus* primary septicemia in up to 80% of those infected (65). Although it has been suggested, for example, that liver dysfunction may under some circumstances increase levels of readily available non-transferrin-bound iron and, independently of iron overload may predispose the patient to infection through aberrant immune responses (3, 42), perturbed complement activity (28), failure of appropriate Kupffer cell opsonization/uptake (27, 47, 60), and aberrant porto-systemic delivery due to portal hypertension, a unifying hypothesis relating liver disease, iron overload, and infections has been lacking. This may simply reflect the lack of a common final path—with disease susceptibility being distributed over a number of critical and independently modifiable immunological nodes—an assertion which must to some extent be correct.

Alternatively, to provide a unifying scheme integrating the role of liver disease and iron overload in disease, it might additionally be argued that the activity of a putative host antimicrobial peptide that was hepatically generated, that was augmented through hepatic modification, or that potentially facilitated bacterial clearance at least in part by the hepatic reticuloendothelial system might be reduced or impaired by liver dysfunction. Although potentially designed to reduce available essential iron, the same factor would itself be vulnerable to sequestration or modification and/or inactivation by iron, such that hyperferric states (including liver disease and chronic renal failure, where non-transferrin-bound iron is plentiful) would reduce antimicrobial activity. I propose hepcidin to be this factor.

Advances in our understanding of iron metabolism have relied on delineation of the genetics of inherited hemochromatosis. Five subtypes are now recognized (Table 2), the most common of which is caused by mutations in the HLA-linked *hfe* (hereditary hemochromatosis, or type 1) gene involved with

TABLE 2. Classification of different subtypes of inherited hemochromatosis detailing genetic loci and gene product function

Classification	Mutant gene product	Gene locus	OMIM	Function
Type 1 (most common)	Protein encoded by *hfe*	6p21.3	235200	Duodenal crypt body iron sensor
Type 2 (juvenile)	Unknown	1q21	602390	Unknown
	HAMP	19q13	606464	Hepcidin antimicrobial peptide
Type 3	TFR2	7q22	604250	Low-affinity transferrin receptor
Type 4	Ferroportin 1/IREG1/MTP1 (SLC11A3)	2q32	604653/606069	Intestinal iron exporter ferroportin 1
Type 5	H-ferritin	11q12-q13	134770	Ferritin heavy chain 1 (FTH1)

duodenal crypt iron sensing and uptake (8). Type 2 or juvenile hemochromatosis is a rare, autosomal recessive condition caused in one pedigree by a mutation in an unidentified locus on chromosome 1q. Recently, a genetic approach was able to associate mutations in the hepcidin antimicrobial peptide (HAMP) encoded on chromosome 19q13, a key regulator of iron metabolism, with type 2 juvenile hemochromatosis (62). The human gene encodes a precursor protein of 84 amino acids. The N-terminal 24-amino-acid endoplasmic reticulum signal sequence is cleaved to a 60-amino-acid propeptide, which is itself cleaved by propeptide tissue convertases at Arg^{59} (a consensus site for the subtilisin/kexin family of propeptide convertases) to an active C-terminal 25-amino-acid antimicrobial peptide (Hepc25) found circulating in blood and urine (56, 57). A number of variant remnants (e.g., 20- and 22-amino-acid peptides) and a proregion segment (Ser^{25}-Arg^{59}) have also been identified as containing a strained beta-pleated-sheet with a tight hairpin that is stabilized by eight cysteines disposed via four intramolecular disulfide bonds. This amphipathic hepcidin contains a motif of numerous cationic residues, well recognized in antimicrobial peptides that bind to negatively charged phospholipids on the cytoplasmic membranes of many microbes (bacterial, parasitic, and fungal). It has been speculated that hepcidin (originally termed liver-expressed antimicrobial peptide [LEAP-1]) might subsequently disrupt membrane function, penetrate cells in order to damage them, or excite an immune response through chemotactic properties. Operating downstream of potent cytokines and Toll-like receptors, hepcidin contains potential binding sites for transcription factors HNF3, C/EBP, and NF-κB in its regulatory region and is thus recruited by bacterial LPS and inflammation (35). Astonishingly hepcidin also regulates iron metabolism. In transgenic mice overexpressing hepcidin, it reduces systemic iron by reducing intestinal uptake, reducing the release from the reticulo-endothelium, and altering placental transport and results in lethal perinatal anemia (54). In most cases of primary and secondary hemochromatosis, its mRNA is significantly upregulated, attempting to compensate for the increased systemic iron (52). In one excellent clinical study confirming the dynamic role of hepcidin in an almost-Kochian manner, a glycogen storage disease type1a patient expressing hepcidin from a hepatic adenoma suffered from refractory anemia that resolved on resection of the adenoma (84). Finally, the patients with mutant hepcidin and type 2 juvenile hemochromatosis approximate to the targeted disruption of the upstream *USF2* transcription factor gene, which causes a reduction of hepcidin production and results in hepatic and/or pancreatic iron accumulation (53).

Intriguingly, hepcidin is also found in sea bass of the *Morone* genus and is activated by organisms such as *Streptococcus iniae*, another highly invasive marine organism (20, 66). Although the majority of existing studies have focused on the susceptibility of gram-positive bacteria to hepcidin, hepcidin is also known to have a potent effect on gram-negative bacteria, as confirmed by its potency in relation to *Neisseria cinerea* (35). *N. cinerea* is a common commensal of the oropharyngeal flora which only rarely causes infection; nevertheless, it interestingly expresses the HpuAB bipartite hemoglobin receptor system, attesting its dependence on iron. Furthermore, in those patients that have been noted to suffer disease, a suggestion for the role of complement deficiency and renal failure (potentially with iron overload) has been noted (24). There is thus a parsimonious elegance about the concept of a highly conserved vertebrate antimicrobial acute-phase hepatic peptide that increases in response to inflammation both by neutralizing the offending organism and that has been exapted by evolution to interact with the machinery of iron regulation to reduce the circulating iron so vital to pathogens while there is active infection (note: this effect of hepcidin is probably why chronic diseases cause anemia) (23). However, it might at first glance seem paradoxical that in hemochromatosis (except HAMP-related hemochromatosis), while hepcidin levels are "appropriately" upregulated, the host is still perturbingly susceptible to *V. vulnificus*.

The paradox may simply be explained by the fact that hepcidin plays only a small part in the response to these organisms. A more interesting explanation, consistent with a key role for hepcidin in these infections, derives from an "evolutionary oversight"—namely, that hepcidin has evolved in an environment where its upregulation almost invariably leads to a decrease in the level of iron in serum. Hemochromatosis represents the teleologically unlikely eventuality of simultaneously high levels of circulating iron and hepcidin. As Fig. 2 demonstrates, there are a number of sites at which iron can impair hepcidin synthesis and action. The primary expression of hepcidin mRNA, although probably increased in the context of hepatocellular iron-repletion, may under specific circumstances potentially be exacerbated by infections, resulting in a failure of mRNA upregulation (52). An interesting though preliminary observation has, for example, indicated that *hfe* hemochromatosis patients may be hepcidin deficient, perhaps due to complex intrahepatic signaling defects; these patients

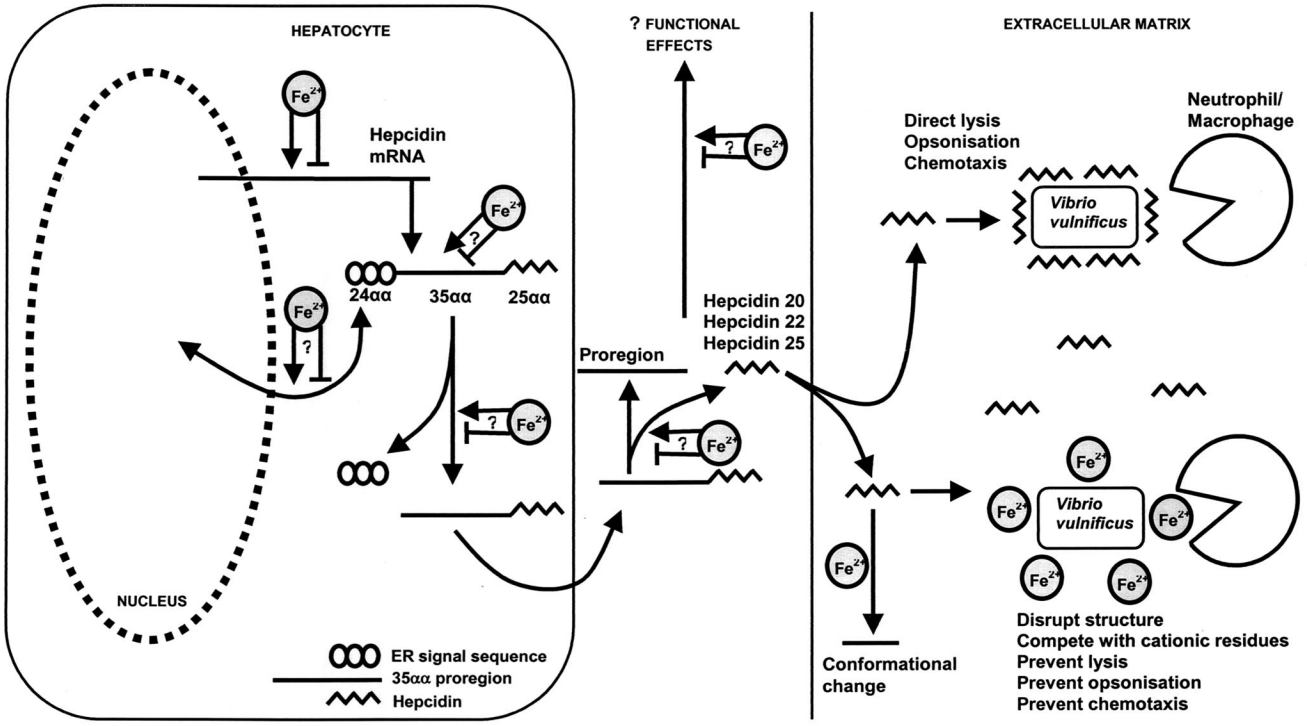

FIG. 2. Diagram of the possible role of iron in hepcidin processing. As detailed in the text, although as yet experimentally undetermined, iron may modify hepcidin mRNA expression and translation, the 84-amino-acid propeptide localization and intracellular transport, the endoplasmic reticulum (ER) signal sequence cleavage, the 60-amino-acid propeptide cellular extrusion and further cleavage, and the proportions of hepcidin remnants and 35-amino-acid proregion (these elements may together or in isolation have their own functional effects). Most importantly, iron may cause a hepcidin pseudodeficiency by electrostatically competing with the cationic hepcidin to bind to pathogens, altering hepcidin aggregation, preventing pathogen lysis and opsonization, altering chemotaxis, and preventing phagocytosis or the inhibition of the respiratory burst. αα, amino acid.

may be effectively hepcidin deficient (10). Additionally, although synthetic blocking or modification at the level of translation, signal sequence cleavage, or nuclear localization is unlikely to generally contribute to disease when systemic levels appear appropriate (52), the proportions of the different variants and of the proregion may vary under conditions of iron repletion. For example, the proregion (Ser^{25}-Arg^{59}) has antibacterial activity equally potent to that of Hepc20 and Hepc25, though under normal circumstances it cannot be detected and is thought to be susceptible to degradation (56). If the levels of these variants are low but critical to specific protection against *V. vulnificus*, then high iron levels may modify these proportions detrimentally. Finally, elevated iron may act to cause pseudo-hepcidin deficiency by competing with the cationic residues on hepcidin for the pathogen, allosterically altering hepcidin's already-strained structure, sequestering the molecule, predisposing the molecule to its degradation, preventing localization or synthesis at the site of infection, or reducing opsonizing activity. Indeed, in the context of excess iron, hepcidin may actually promote bacterial virulence. There is already significant precedence for cations' interfering with small antimicrobial peptide function (25); for example, Fe^{2+} was capable of inhibiting the candidacidal activity of cysteine- or cation-rich rabbit leukocyte defensins and four unrelated cationic peptides, confirming the role of ions in peptide modification (37). Furthermore, the role of ionic conditions in modifying antimicrobial peptide activity has also gained momentum in the field of cystic fibrosis (CF). It has been suggested among other hypotheses that defects in the CF transmembrane conductance regulator protein result in an abnormally high level of NaCl in the airway surface fluid of lungs of patients with CF. Human β-defensins secreted by airway epithelia onto the airway surface are inactivated in this ionically abnormal milieu of CF in a dose-dependent fashion, resulting in diminished bactericidal activity against a broad group of pathogens (26). This observation also provides conceptual support for the role of ions (albeit in this case concentrations of monovalent ions) in modifying antimicrobial peptide activity.

CONCLUSION

Iron retrieval is clearly an essential component of bacterial pathogenesis and represents the battleground upon which the host and pathogen compete. Hepcidin is a newly discovered though ancient member of the host's armory, which I have proposed may be of great significance throughout phylogeny. If this assertion is correct, in addition to elucidating the role of a novel antimicrobial in bacterial pathogenesis, it provides yet another loophole for pathogens to utilize in manipulating ferrodynamics. This hypothesis has implications for the counseling and management of hemochromatosis and hepatically compromised patients with iron chelation or venesection. Fur-

thermore, although the effects of hepcidin may be most marked with specific patients in response to pathogens such as *V. vulnificus*, the role of iron and hepcidin variations (genetic polymorphisms and those that are acquired) may represent a significant, albeit less stark, axis in general host-pathogen interactions—for example, with other bacteria such as *Yersinia* spp. and a variety of other organisms.

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