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Transition Metals and Virulence in Bacteria

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Abstract

Transition metals are required trace elements for all forms of life. Due to their unique inorganic and redox properties, transition metals serve as cofactors for enzymes and other proteins. In bacterial pathogenesis, the vertebrate host represents a rich source of nutrient metals, and bacteria have evolved diverse metal acquisition strategies. Host metal homeostasis changes dramatically in response to bacterial infections, including production of metal sequestering proteins and the bombardment of bacteria with toxic levels of metals. Presumably, in response, bacteria have evolved systems to subvert metal sequestration and toxicity. The coevolution of hosts and their bacterial pathogens in the battle for metals has uncovered emerging paradigms in social microbiology, rapid evolution, host specificity, and metal homeostasis across domains. This review focuses on recent advances and open questions in our understanding of the complex role of transition metals at the host-pathogen interface.

Keywords

host-pathogen interface; metal acquisition; metal homeostasis; nutritional immunity; transition metal

INTRODUCTION

Metals are essential for all forms of life. Metal cofactors serve structural and catalytic roles in biological processes, including precursor biosynthesis, DNA replication, transcription, respiration, and responses to oxidative stress. Most organisms require the first-row transition metals manganese, iron, cobalt, nickel, copper, and zinc. Excepting zinc, these metals have unfilled *d*-orbitals and thus are redox active. Their ability to easily cycle between oxidation states contributes to both their catalytic properties and their toxicity. Their divalent cations bind ligands similarly, but each metal has different properties such that they are only occasionally functional substitutes. Thus, protein mismetallation often ablates function, necessitating exquisitely sensitive metal homeostasis systems. In bacterial pathogens, homeostasis strategies include complex metal-responsive transcriptional regulatory

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networks, as reviewed elsewhere (20, 62, 78, 130). Understanding how metal homeostasis proteins recognize and respond to the correct metals is an active area of research in bioinorganic chemistry with implications for bacterial and host metal metabolism during infection.

In the context of infection, bacterial and host metal metabolism determine disease pathogenesis with three emerging principles. First, although the host represents a rich nutrient metal source for bacteria, the host immune system can block bacterial metal acquisition in a process termed nutritional immunity. Second, hosts barrage bacteria with toxic concentrations of metals. Finally, dysregulation of host metal homeostasis—through genetic mutation or nutrition—alters susceptibility to infection. This review summarizes findings, highlights recent work, and discusses questions to be pursued in the study of metals at the interface of bacterial pathogens and their hosts.

BACTERIAL METAL ACQUISITION AND HOST NUTRITIONAL IMMUNITY

In order to obtain their required nutrient metals, bacteria have evolved three main classes of metal acquisition systems: elemental metal import, extracellular metal capture by siderophores, and metal acquisition from host proteins (including metal piracy from host nutritional immunity proteins). In response, mammalian hosts have evolved strategies to restrict bacterial metal acquisition. The protein transferrin was first reported to inhibit microbial growth by iron chelation in egg whites and human plasma in the 1940s, and lactoferrin was later shown to have similar properties at mucosal surfaces (105, 120, 121) (**Figure 1**) (see sidebar, Nutritional Immunity Protein Moonlights as an Antimicrobial). Thirty years later, Eugene Weinberg popularized the term nutritional immunity to describe the phenomenon, which has since been expanded to include host restriction of zinc, manganese, and nonmetal nutrients such as amino acids (65, 140, 149). Growing evidence suggests that this battle for metals represents an interface for coevolution of hosts and bacteria. As posited by the Red Queen hypothesis in evolutionary biology, with each development in nutritional immunity defense, pathogens evolve a new offense (11, 133). The following sections discuss our understanding of nutrient transition metals at the host-pathogen interface, focusing on iron, zinc, and manganese.

Iron and the Host-Pathogen Battle: The Vanguard in Understanding

Iron is the most widely used transition metal in biological systems, presumably because it is the most abundant element on Earth, making up 80% of the Earth's core. Bacterial pathogens require iron for essential processes, including the pentose phosphate pathway, DNA replication, transcription, and cofactor biosynthesis. For example, biosynthesis of the cofactor heme (Fe^{2+}-protoporphyrin IX) is important in *Brucella abortus* systemic infection and *Staphylococcus aureus* systemic and bone infection (5, 57, 58). Additionally, the iron-sulfur cluster chaperone Nfu was found to be important in mouse and moth models of infection by *S. aureus* and *Acinetobacter baumannii*, respectively (86, 150). Iron is incorporated into cofactors in its soluble ferrous form (Fe^{2+}). Many bacteria import Fe^{2+} with the ferrous transporter Feo, which is important for colonization and/or virulence in a number of bacteria, including the gastrointestinal pathogens *Helicobacter pylori*, *Salmonella*

enterica, and *Campylobacter jejuni* (77) (**Figure 2*a***). However, Fe^{2+} is largely unavailable for bacterial pathogens due to host restriction, and the physiochemical properties of iron make Fe^{3+} the predominant form in the host environment, as describe below.

Two-thirds of iron in humans is sequestered in erythrocytes as heme bound to the oxygen-carrying protein hemoglobin (24). Under oxic conditions and at physiological pH in serum, free iron is insoluble as Fe^{3+}. In vertebrates, nearly all Fe^{3+} is bound by transferrin in serum, by ferritin in cells, or by lactoferrin in milk, saliva, tears, mucus, and neutrophil secondary granules. These factors combined result in a low free-iron concentration in human serum, which is further restricted in response to bacterial infection (24). Bacteria have evolved mechanisms to circumvent host iron restriction. For the simplest solution, the Lyme disease pathogen *Borrelia burgdorferi* evolved a complete lack of iron requirement by exploiting the interchangeability of transition metals and replacing iron with manganese in many metalloenzymes (110). Pathogens that have not weaned themselves off iron often rely on cheating, thievery, and piracy to acquire iron in spite of nutritional immunity.

Siderophores, Evasion of Host Interference, and Cheating

Many bacterial pathogens use siderophores to overcome iron limitation in the host environment. Siderophores are low molecular weight, high-affinity Fe^{3+}-binding compounds secreted and imported by bacteria for iron acquisition. Siderophores have exceptionally high-iron binding affinity, which allows some siderophores to steal iron from host proteins. For example, enterobactin produced by Enterobacteriaceae binds Fe^{3+} (K_d 10^{-51}–10^{-49} M) more tightly than transferrin (K_d 10^{-24}–10^{-20} M), thus outcompeting transferrin chelation (23, 81). In Gram-negative bacteria, siderophores are transported across the outer membrane by a TonB-dependent system, then trafficked through the periplasm and imported into the cytoplasm by ATP-binding cassette (ABC) transporters (93) (**Figure 2*a***). Gram-positive bacteria import Fe^{3+}-siderophores with lipoprotein substrate-binding proteins and ABC transporters (93) (**Figure 2*b***).

Iron is released from the siderophores by one of two main mechanisms. Siderophores with high Fe^{3+} binding affinity, such as enterobactin or bacillibactin produced by *Bacillus anthracis*, are hydrolyzed by specific esterases before Fe^{3+} is reduced to Fe^{2+}. The fate of the hydrolyzed siderophores is not well understood; they may be further degraded or recycled (93). For other siderophores, ferric reductases reduce Fe^{3+} to Fe^{2+} that is then released due to its lower binding affinity or by competitive sequestration (93). Deferrated siderophores can then be recycled. *Pseudomonas aeruginosa* uses a dedicated periplasmic export system to recycle siderophores (66). Recent studies in *Mycobacterium tuberculosis* determined that one system exports both de novo synthesized and recycled siderophores from the cytoplasm and is required to prevent cytoplasmic siderophore accumulation and poisoning (69) (**Figure 2*c***).

Siderophore iron acquisition and host interference illustrate an evolutionary arms race in bacterial pathogenesis. The vertebrate host produces the siderophore-binding protein lipocalin 2, also known as siderocalin or neutrophil gelatinase–associated lipocalin, during the innate immune response to bacterial infection to sequester siderophores such as enterobactin or bacillibactin (1, 44). In response, bacteria evolved to elaborate stealth

siderophores that evade lipocalin 2 binding, including the highly glycosylated salmochelin produced by pathogenic enterobacteria and petrobactin produced by *B. anthracis* (1, 43). Remarkably, expression of the salmochelin biosynthetic locus confers virulence to a nonpathogenic *Escherichia coli*, implying stealth siderophore production is a key virulence determinant (43).

Many pathogens encode multiple siderophore biosynthetic systems, suggesting that diverse siderophores allow context-specific replicative advantages. Studies on siderophore production in enterobacterial pathogens have provided insight into these fitness benefits. During *S. enterica* gastrointestinal infection, *S. enterica* uses salmochelin to gain a competitive advantage over commensals but is outcompeted for siderophores by the probiotic *E. coli* strain Nissle, which reduces *S. enterica* colonization (33, 113). Although enterobactin can be sequestered by lipocalin 2, it has a higher binding affinity for Fe^{3+} than stealth siderophores salmochelin and yersiniabactin. This high Fe^{3+} binding efficiency renders enterobactin essential in certain host niches. In the lung, enterobactin is not required for *Klebsiella pneumoniae* pneumonia but is required for invasion into surrounding tissue; its higher Fe^{3+} binding affinity enables *K. pneumoniae* to acquire iron from transferrin in the perivascular space (9). Counterintuitively, uropathogenic *E. coli* use enterobactin to resist lipocalin 2 in human urine, apparently by extracting Fe^{3+} from Fe^{3+}-catechol-lipocalin 2 complexes (122). Human urine varies in characteristics such as pH and aryl metabolite (catechol) content. In donors with high urine pH, lipocalin 2 complexes with catechols and Fe^{3+} to sequester iron from invading pathogens. Uropathogenic *E. coli* can overcome this sequestration owing to the exceptionally high Fe^{3+} binding affinity of enterobactin (9). Given the large number of siderophores identified thus far, understanding mechanisms driving siderophore diversity warrants further investigation.

In addition to selective pressures associated with maintaining multiple siderophore biosynthetic systems, siderophore production in *P. aeruginosa* serves as a model to understand how cheating drives evolution. Because siderophores are secreted, bacteria can import siderophores for iron acquisition without bearing the metabolic cost of production. In this way, siderophores are public goods. Experimental evolution in *P. aeruginosa* showed that relatedness and low competition selects for cooperation (i.e., siderophore production), whereas local competition selects for cheaters (53). Additional research found that the nutritional environment and regulatory cross talk between social behaviors (i.e., quorum sensing and production of multiple siderophores) further modulate the selection for cooperation versus cheating in *P. aeruginosa* (49, 117). Importantly, long-term *P. aeruginosa* infection in the cystic fibrosis lung was recently shown to select for siderophore cheating, eventually driving some populations to lose siderophore production and thus reduce fitness in the iron-limited host (7). The competitive dynamics leading to complete loss of siderophore production demonstrate that bacterial communities are susceptible to the tragedy of the commons, an economics term first applied to evolutionary biology by Garrett Hardin in 1968 to describe each individual seeking maximum benefit from common goods at the expense of population-wide fitness (60). The *P. aeruginosa* findings suggest that the intensity of competition in the host environment can drive bacterial populations to extinction.

Iron Piracy Subverts Nutritional Immunity

In addition to acquiring iron from heme, some pathogens have developed specialized systems to obtain iron from nutritional immunity proteins through a process termed iron piracy. The transferrin-binding proteins TbpA/TbpB that are present in species, including *Neisseria* spp., *Haemophilus influenzae*, and *Moraxella catarrhalis*, are TonB-dependent receptors that bind transferrin and extract Fe^{3+} for transport into the cell (96). Additionally, some pathogens express lactoferrin receptors, including pathogenic *Neisseria* spp., *Treponema pallidum*, *H. pylori*, and *Streptococcus pneumoniae* (34, 59, 96). With these systems, enterprising bacteria convert host proteins that evolved to restrict bacterial growth into nutrient iron sources.

Selective pressure at the receptor-ligand binding sites between host transferrin and bacterial transferrin-binding proteins has driven evolution at the host-pathogen interface. Like the hemoglobin-IsdB interaction, transferrin-binding proteins restrict host range for numerous pathogens. The obligate human pathogens of *Neisseria* spp. are host restricted based on their transferrin-binding proteins, a fact that has been exploited to develop murine models for meningitis by introducing or expressing human transferrin (102, 148). Structural analysis of TbpB from the porcine pathogen *Actinobacillus pleuropneumoniae* revealed a single conserved residue required for transferrin binding, whereas surrounding residues varied extensively, presumably due to host selective pressure (95). Elegant evolutionary work showed that transferrin genetic variation among apes is the result of rapid evolution to counteract bacterial iron piracy (10). This work emphasizes the utility of metal acquisition by bacterial pathogens as a model to uncover rapid evolution at protein interfaces between host and pathogen.

Iron Thievery: Bacterial Heme Acquisition

Because the majority of iron in the human body is sequestered in erythrocytes, many bacterial pathogens evolved mechanisms to access heme iron. These pathogens first lyse erythrocytes, bind hemoglobin or other host heme proteins, and then extract heme for direct use or degradation for liberation of free iron (27). Following erythrocyte lysis, mammalian hosts employ hemopexin and haptoglobin to bind and sequester free heme and hemoglobin, respectively, to protect against their oxidative activities. Bacterial heme acquisition systems have surface-associated receptors that bind heme or hemoproteins and pass heme through the periplasm and/or cell wall to a membrane heme transporter.

Heme acquisition systems are well described for many Gram-negative pathogens and rely on an outer membrane receptor such as HmuR that binds heme or hemoproteins, transporting heme through the periplasm by a TonB-ExbB-ExbD system (19) (**Figure 2*a***). Heme is then transported into the cytoplasm by an ABC transporter. Some bacteria additionally release hemophores, such as heme assimilation system HasA in *Serratia marescens* and *P. aeruginosa*, which extracts heme from hemoglobin with 10^{-11} M binding affinity and relays it to the outer membrane hemophore receptor HasR (76, 144). *M. tuberculosis* uses a secreted hemophore with a unique fold, demonstrating the diversity of these systems (131). Many Gram-positive pathogens use the iron-regulated surface determinant Isd heme uptake system with dedicated cell wall sortase and heme passage proteins to acquire iron from free

heme or heme complexed to hemoglobin or hemoglobin-haptoglobin (87) (**Figure 2*b***). *B. anthracis* additionally encodes secreted hemophores IsdX1 and IsdX2 that bind heme extracellularly for import but are not required for virulence, implying that there are functional redundancies in *B. anthracis* heme acquisition (84). The recent discovery of an iron-regulated autolysin in the opportunistic pathogen *Staphylococcus lugdunensis* suggests that Gram-positive bacteria remodel their cell walls to accommodate heme acquisition proteins (42). Heme acquisition is required for virulence in *Staphylococcus aureus*, *B. anthracis*, and *Streptococcus pyogenes* (27). In *Corynebacterium diphtheriae*, one of at least two redundant heme transport–associated Hta uptake systems is tethered to the cytoplasmic membrane rather than relying on cell wall sortases. The roles that the two Hta systems play in virulence remain unknown (4).

The importance of heme as an iron source during infection has driven evolution at the host-pathogen interface. *S. aureus* evolved to use hemoglobin as its preferred iron source in infection, and its IsdB hemoglobin-binding protein evolved to preferentially bind human hemoglobin over hemoglobin from other animals (109, 123). Additionally, *P. aeruginosa* lineages colonized in cystic fibrosis patients evolved to prefer heme over the siderophore pyoverdine as an iron source (85). These examples suggest that the interaction between bacterial heme acquisition proteins and host hemoglobin is also susceptible to selective pressure in the host-pathogen arms race. Therefore, bacterial heme acquisition may offer opportunities for further understanding host-pathogen evolution.

Zinc Turf Wars: Sequestration and Potential for Piracy

Following iron, zinc is the second most abundant transition metal cofactor. Zinc binding by proteins can be structural, regulatory, or catalytic, and zinc is required for many metalloproteases. Bacterial zinc uptake (Znu) systems are ABC transporters required for virulence in a variety of pathogens, including *S. pneumoniae*, *Acinetobacter baumannii*, *Listeria monocytogenes*, and *P. aeruginosa* (29, 36, 64) (**Figure 3*a***). Recent work uncovered two novel zinc acquisition systems in *Yersinia* spp. In *Yersinia pseudotuberculosis*, a type VI secretion system (T6SS) imports zinc by transporting a zinc-binding protein effector bound to zinc, and maximal virulence requires both the Znu and T6SS systems, thus expanding known roles for T6SS (139). In *Y. pestis*, Bobrov et al. (16) demonstrated that the siderophore yersiniabactin binds zinc and is transported into the cell using a dedicated Zn^{2+}-yersiniabactin importer, YbtX. Importantly, in a model of septicemic plague, *Y. pestis* displays a virulence defect only in strains lacking both the *znu* transporter and yersiniabactin biosynthesis genes. This pivotal finding established the importance of siderophore noniron metal acquisition in bacterial pathogenesis.

Bacterial zinc acquisition systems are required during infection to counteract limitation by nutritional immunity. During the acute phase response to inflammation, serum zinc drops due to enhanced zinc uptake and sequestration by metallothionein in liver cells (55). Additionally, members of the helix-loop-helix (EF-hand type) Ca^{2+}-binding S100 protein family of the vertebrate innate immune system exert nutrient limitation through extracellular zinc chelation (**Figure 3*b***). The importance of metal chelation by S100 proteins for innate immunity was first established with the demonstration that S100A7 (psoriasin) protects

human skin from infection by zinc chelation (51). Psoriasin also contributes to mucosal immunity on the tongue and in the vagina (92, 94). Similarly, S100A12 (calgranulin C), which binds both Zn^{2+} and Cu^{2+}, is induced in *H. pylori* infection and limits bacterial growth through zinc chelation (56).

Molecular imaging by mass spectrometry demonstrated that *Staphylococcus aureus* liver abscesses are severely depleted of zinc and manganese and depletion depends on heterodimers of S100A8/S100A9 (calprotectin) (30). Calprotectin is an abundant neutrophil protein that can chelate Zn^{2+} and Mn^{2+} and efficiently competes away these nutrient metals from numerous bacterial pathogens in vitro (32). In addition to *S. aureus*, calprotectin zinc limitation is important for controlling infection by bacterial pathogens, including *L. monocytogenes*, *A. baumannii*, *K. pneumoniae*, and *H. pylori* (3, 47, 64, 147). Recent work showed that Fe^{2+} binding by calprotectin contributes to inhibition of bacterial growth in vitro, an activity that remains to be investigated during infection (98). Counterintuitively, calprotectin enhances pathogenesis of some bacteria by diverse mechanisms. For example, in pneumococcal pneumonia, calprotectin appears to protect *S. pneumoniae* from host-induced zinc toxicity in the lung (2). Calprotectin enhances *S. enterica* growth in the inflamed gut, presumably because *S. enterica* can outcompete the gut microbiota for limited zinc (79). Subinhibitory concentrations of calprotectin also increase *H. pylori* biofilm formation (46). In addition, calprotectin promotes pneumonia co-infection of *P. aeruginosa* and *S. aureus* by abrogating production of anti-staphylococcal factors by *P. aeruginosa* (137). This finding offers a potential explanation for co-infection by *P. aeruginosa* and *S. aureus* in cystic fibrosis patients, where calprotectin is abundant. The differential effects of calprotectin zinc sequestration likely reflect differences in host environments, bacterial zinc requirements, and acquisition systems.

Recent work identified the potential for bacterial zinc piracy from S100 proteins during infection. The *Neisseria meningitidis* outer membrane receptor CbpA was shown to bind calprotectin and extract zinc in a TonB-dependent manner (127). This finding suggests that bacteria can exploit host zinc nutritional immunity proteins analogously to iron acquisition from transferrin and lactoferrin. In this case, interactions between CbpA and calprotectin may also be susceptible to rapid evolution, establishing a new front in the study of the evolutionary battle for nutrient metals.

Manganese Restriction: A War in Two Theaters

Manganese is an essential trace element for all forms of life and serves as a cofactor for enzymes involved in oxidative stress, DNA replication, and central metabolism, including manganese superoxide dismutase and pyruvate kinase. Bacterial manganese import systems generally belong to the MntH/Nramp or ABC transporter families and are important during infection in a number of pathogenic bacteria, including *S. enterica*, *S. aureus*, *Neisseria gonorrhoeae*, *B. abortus*, *Streptococcus* spp., and *Yersinia* spp. (71) (**Figure 4*a,b***). In the Lyme disease pathogen *B. burgdorferi*, the BmtA manganese transporter is required for virulence. Curiously, BmtA is not related to bacterial manganese importers but, rather, to the ZIP (Zrt/Irt-like protein) family transporters in eukaryotes (106). The divergence of *B. burgdorferi* manganese importers from other pathogens may reflect its unique evolutionary

strategy to substitute its iron requirement with manganese. Interestingly, the requirement for manganese acquisition systems can depend on the mode of infection or the infected organ. For *S. aureus*, mutants lacking MntH and ABC transport systems have a lower burden in the liver during systemic infection but maintain their burden in the kidney (73). In *Y. pestis*, mutants lacking both transport systems display no defect in pneumonic plague but are attenuated in bubonic plague (108). These findings contribute to the understanding that the requirement for metal import depends on the specific host environment.

The host innate immune system has evolved both extra- and intracellular manganese restriction mechanisms (71) (**Figure 4*c***). The Mn^{2+}-binding activity of calprotectin limits available manganese for many bacterial pathogens (32). In *S. aureus*, calprotectin-mediated manganese restriction inhibits the bacterium's oxidative stress defense program by limiting manganese superoxide dismutase activity (72). In turn, the *S. aureus* high-affinity manganese import systems help combat calprotectin limitation during infection (73). In contrast to what occurs in the liver, *S. aureus* kidney abscesses remain manganese depleted in calprotectin-deficient animals, implicating additional host measures of manganese sequestration (73). During *S. enterica* infection of the gut, manganese acquisition is required for infection, where manganese restriction is IL-22 dependent but calprotectin-independent (35). Therefore, open areas for investigation include the role of calprotectin-dependent manganese sequestration in other infections and the remaining calprotectin-independent mechanisms of host manganese chelation.

Intracellular pathogens also face host manganese limitation in the phagosome. In response to infection, neutrophils and macrophages express the natural resistance–associated macrophage protein 1 (Nramp1), which depletes the phagosome of manganese and, to a lesser extent, iron (67, 142). Bacterial manganese acquisition systems are important for resisting Nramp1-mediated manganese restriction and may contribute differentially, such as in *S. enterica* infection of stimulated macrophages. The fact that polymorphisms in *nramp1* affect host susceptibility to infection emphasizes the importance of manganese limitation. The commonly used laboratory mouse strains C57BL/6 and BALB/c are Nramp1-deficient and rapidly succumb to *S. enterica* infection, in contrast to Nramp1-sufficient mouse strains (135). Human *nramp1* polymorphisms affect susceptibility to tuberculosis, meningococcal diseases, and leprosy (14). Additionally, *nramp1* expression in macrophages induces the host siderophore-sequestering protein lipocalin 2, uncovering a regulatory link between multiple mechanisms of nutritional immunity (45). Therefore, continued study of nutrient-limiting proteins may expose additional multimetal regulatory networks involved in nutritional immunity.

Cobalt, Nickel, and Copper: Neglected Transition Metals in Nutritional Immunity

Less is known about the roles of cobalt, nickel, and copper acquisition in bacterial pathogenesis. Nickel is well established as an essential cofactor for virulence factors, including nickel-iron hydrogenase and urease in *H. pylori*, where the NixA nickel transporter is required for virulence (41, 100). Urease is also expressed by *Proteus mirabilis* and *Staphylococcus saprophyticus*, other causative agents of urinary tract infections (21). Cobalt is an enzymatic cofactor for virulence determinants, including *S. pneumoniae*

GlcNAc deacetylase and *Helicobacter pylori* arginase (15, 89). Dedicated cobalt transporters have not been identified, but many bacterial pathogens encode nickel-cobalt transporters (115). In *S. aureus*, the nickel transporter Nik is required for urease activity and efficient kidney colonization, and the cobalt and nickel transporter Cnt is additionally required for infection in systemic and urinary tract infections in mice (63, 114). Recent work demonstrated that Cnt transports metals in complex with staphylopine, a nicotianamine-like metallophore capable of binding nickel, cobalt, zinc, copper, and iron (50). Characterization of staphylopine marks the first description of a nicotianamine-like molecule in bacteria and the first broad-spectrum metallophore.

The role of copper in bacterial pathogenesis is an exciting area of current investigation. Copper is important during aerobic growth as a cofactor of cytochrome *c* oxidase and copper-zinc superoxide dismutase. It is unclear whether bacterial pathogens actively transport copper and whether copper is restricted by host nutritional immunity. In mycobacteria, porins in the outer membrane are known to be important for copper transport (124). Mounting evidence demonstrates that the siderophore yersiniabactin binds Cu^{2+} in physiologically relevant environments and that Cu^{2+}-yersiniabactin can be imported in uropathogenic *E. coli* using the TonB-dependent siderophore importer FyuA (26, 75). Copper acquisition by the fungal pathogen *Cryptococcus neoformans* is critical for meningoencephalitis virulence but not pneumonia, demonstrating that some host niches are copper limited (129). These findings raise questions as to the mechanism of copper limitation in these niches (e.g., cerebrospinal fluid). Although the host immune protein S100A12 binds copper in addition to zinc, its antimicrobial activity against *H. pylori* appears to be restricted to zinc chelation (56). Improved understanding of the importance of cobalt, nickel, and copper for bacterial pathogenesis will determine whether the host actively limits these metals through nutritional immunity and whether bacterial pathogens are affected by their limitation or can counteract host-mediated limitation.

HOST-IMPOSED METAL TOXICITY

Although the first-row transition metals are required trace elements for biological systems, they are also inherently toxic. One potential mechanism of toxicity is Fenton chemistry by the redox-active transition metals iron, copper, and manganese. In the Haber-Weiss reaction of Fenton chemistry, Fe^{2+} reacts with hydrogen peroxide to form Fe^{3+}, a hydroxyl radical, and hydroxide; Fe^{3+} can then react with hydrogen peroxide to regenerate Fe^{2+} in addition to a hydroperoxyl radical and a proton. These oxidative species inhibit cell growth by damaging proteins, DNA, and lipids. Another mechanism of toxicity is protein mismetallation. The prevalence of metalloproteins in the bacterial cell and their relatively compatible binding ligands necessitate tight control of metal homeostasis for continued metabolism. Complementary to nutrient metal limitation, hosts have evolved mechanisms to deploy toxic levels of metal ions for bacterial control, and bacteria use multiple metal exporters to resist host intoxication (20, 39, 54).

Copper Ions as Ammunition

Copper has long been recognized as antimicrobial and is often embedded in textiles and medical devices as a microbicide. Although copper can carry out Fenton chemistry, its toxicity is caused by mismetallation in bacterial species investigated thus far. The primary targets of its toxicity in *E. coli* and *N. gonorrhoeae* are iron-sulfur cluster proteins in branched chain amino acid and heme biosynthesis, respectively; in *S. pneumoniae*, the primary target is the manganese-containing aerobic ribonucleotide reductase (38, 68, 83) (**Figure 5*a***). In a possible twist on Fenton chemistry, copper was also found to potentiate nitrosative stress in *N. gonorrhoeae* (37).

Host bactericidal mechanisms take advantage of copper toxicity to create poisonous microenvironments for intracellular bacteria. During bacterial infection, interferon-γ induces expression of copper transport protein 1 (CTR1) to import copper from the environment to ATOX1, which then shuttles copper to the ATP7A transporter at the membrane of the phagolysosome for copper accumulation (74, 143, 145) (**Figure 5*b***). Accordingly, copper resistance is required for virulence of many intracellular pathogens. *N. gonorrhoeae*, *S. enterica*, and mycobacteria employ copper exporters and extracellular copper oxidation (39, 119) (**Figure 5*a***).

Investigation of copper resistance has uncovered surprising mechanisms. For example, in response to copper toxicity, mycobacteria produce the bacterial metallothionein MymT to sequester copper in the cytoplasm (52). Uropathogenic *E. coli* use Cu^{2+}-yersiniabactin to protect against copper toxicity and oxidative stress through at least two mechanisms: (*a*) binding to yersiniabactin prevents catechol-mediated Cu^{2+} reduction to Cu^{+}, which could participate in Fenton chemistry, and (*b*) Cu^{2+}-yersiniabactin exhibits extracellular and catalytic superoxide dismutase–like activity (25, 26). Our understanding of metal resistance beyond export mechanisms is in its infancy, but findings thus far suggest it may be fertile ground for discovery.

Zinc Toxicity Expands the Host Armory

Zinc cannot participate in Fenton chemistry and its mechanism of toxicity likely involves mismetallation. Zinc intoxication competitively inhibits manganese binding to an importer in *S. pneumoniae* and the glycolytic enzymes phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase in *S. pyogenes* (88, 104) (**Figure 6**). Like copper toxicity, zinc intoxication as a host defense mechanism was first described during mycobacterial infection. Transcriptional profiling of *M. tuberculosis* in human macrophages identified signs of metal intoxication in bacteria and in the host, revealing a burst of free zinc in macrophages and intraphagosomal zinc accumulation (18).

Zinc efflux by the cadmium-zinc-cobalt (Czc) family exporter was also found to be important for *S. pyogenes* in a mouse model of infection (103). Human neutrophils respond to *S. pyogenes* internalization by mobilizing zinc, apparently increasing free cytosolic zinc (103). Interestingly, an increase in cytosolic zinc due to the transporter ZIP8 is an innate immune signaling mechanism in macrophages and lung epithelial cells. Specifically, zinc accumulation modulates nuclear factor (NF)-κB activation and inflammation (80). It is

therefore unclear whether *S. pyogenes* zinc poisoning in the cytosol of neutrophils is an evolved mechanism of zinc intoxication or a side effect of the host using zinc as an immune signal. *S. pneumoniae* also likely experiences zinc intoxication during infection, based on the protective effect of zinc-chelating calprotectin and the increase in zinc/manganese ratios in the lung, brain, blood, and nasopharynx that occur during infection (2, 88). Further research may determine whether the rise in cytosolic zinc in neutrophils and macrophages is the main mechanism for zinc intoxication.

Potential Intoxicants: Manganese, Iron, Nickel, and Cobalt

There are currently no described mechanisms of host intoxication using the remaining first-row transition metals, but all are toxic at high levels and therefore could be harnessed by the host for intoxication. In a Mongolian gerbil model of stomach colonization, *H. pylori* requires resistance to the cadmium, zinc, and nickel (Czn) exporter, suggesting that *H. pylori* encounters metal stress in the host (125). In addition to elemental iron, Fe^{2+} in heme is toxic to bacterial cells (27). One could imagine host-imposed heme toxicity as a response to infection; consistent with this, *B. anthracis* induces heme efflux pumps during infection (126).

Manganese is often considered to be protective against toxicity. However, manganese efflux is important in multiple pathogens, revealing distinct metal niches in the host. For example, resistance to manganese toxicity by the manganese exporter MntX is conserved in *N. meningitidis* but not *N. gonorrhoeae*, suggesting that manganese homeostasis differs in their infectious niches (134). MntX appears to be important for maintaining the appropriate manganese/iron ratio, which is particularly important in low-iron environments (134). Similarly, the manganese efflux protein MntE is required for *S. pneumoniae* pathogenesis in murine nasal passages and dissemination to blood (116). *S. pyogenes* also requires MntE for virulence and resistance to manganese toxicity and hydrogen peroxide stress, apparently due to dysregulation of metal homeostasis (132). The maintenance of manganese efflux systems suggests that although the host may not impose manganese intoxication per se, it may manipulate metal ratios to disrupt bacterial metal homeostasis.

HOST METAL HOMEOSTASIS AND BACTERIAL INFECTIONS

Host metal status can dramatically affect susceptibility to bacterial infections. Changes in host metal homeostasis can cause increased metal availability for bacteria, which is thought to be particularly important for opportunistic pathogens that have not evolved the host-specific metal acquisition systems described above (141). Data establishing the link between host metal homeostasis and infection are often correlative and do not address bacterial mechanisms of increased pathogenicity; therefore, animal models of genetic and environmental variation in metal homeostasis offer potential avenues for molecular characterization. This section briefly reviews how genetic and dietary differences in host metal homeostasis can affect bacterial infection.

Iron Overload Lowers Defenses to Infection

Human genetic polymorphisms in iron homeostasis can confer increased susceptibility or resistance to bacterial infection. The hereditary blood disorders β-thalassemia and hemochromatosis can cause iron overload and are associated with increased rates of infection (141). In patients with β-thalassemia, which is caused by mutations in the hemoglobin β-chain, infectious disease is the second most common cause of death (>50% caused by *Enterobacteriaceae*) (138). Although iron overload is a consistent feature in β-thalassemia, its relationship to infection is muddied due to the prevalence of splenectomy and blood transfusion. However, in a murine model of β-thalassemia, the iron overload feature is isolated and confers increased susceptibility to infection by *S. enterica* and *L. monocytogenes* (6) (**Table 1**).

Hemochromatosis is also associated with increased infection by multiple, normally noninvasive pathogens. The importance of iron has been corroborated in mouse models of infection by *Vibrio vulnificus* and nonpigmented *Y. pestis* (plague vaccine strains) (112, 146). Until recently, it was unclear which aspect of hemochromatosis caused this increase in infection: liver injury, high basal iron overload, or the inability to reduce serum iron in response to infection. Using a mouse model of hemochromatosis by hepcidin deficiency, acute hypoferremia in response to infection was isolated as the critical mechanism of increased infection by *V. vulnificus* (8). Increased susceptibility could be partially rescued by dietary iron depletion or administration of hepcidin agonists, suggesting therapeutic potential to treat patients with hereditary iron overload and infection (8).

Excess iron can also develop from environmental exposure through occupational hazards and diet. For example, workers exposed to high levels of iron-containing dust have increased rates of respiratory tract infection (107). A cluster of pneumonia cases caused by the opportunistic pathogen *Acinetobacter calcoaceticus* was linked to iron inhalation by foundry workers (31). Excess iron can be induced by dietary iron overload, generally due to inappropriate iron supplementation. Dietary iron overload has caused shifts from commensalism to pathogenesis by *Yersinia enterocolitica*, and from latent to acute forms of tuberculosis (48, 91). The fact that zinc and manganese are similarly restricted by nutritional immunity suggests that dietary overload may also increase susceptibility to infection.

Dietary Metal Deficiencies and Infection

There are well-established links between infection and dietary deficiencies of iron, copper, and zinc. Iron deficiency is specifically associated with *H. pylori* infection, which is a leading cause of gastric cancer. Work in a gerbil model showed that iron restriction by host dietary deficiency or limitation in vitro enhanced *H. pylori* pathogenesis via increased expression and elaboration of virulence factors (101). *H. pylori* infection can also induce iron deficiency, highlighting the complex nature of trace metals at the host-pathogen interface.

In contrast, sufficient dietary copper and zinc are required to resist infection generally, which has been the subject of previous reviews (39, 55). Copper and zinc are important for immune development and can serve as bacterial intoxicants, and intracellular zinc levels serve as an

immune signal. Copper deficiency is associated with neutropenia and increases susceptibility to infection by *Mannheimia haemolytica* and *S. enterica* in animal models (70, 99). In addition to neutrophil development, dietary copper is important for phagosomal copper intoxication: Copper supplementation of guinea pig food led to increased *Mycobacterium tuberculosis* killing (145).

Approximately one-third of the world's population suffers from zinc deficiency, which is associated with increased rates of diarrhea and pneumonia (111). Prophylactic zinc supplementation was found to reduce incidence of persistent diarrhea by 33% and pneumonia by 41% in a pooled analysis; zinc as a therapeutic reduced prevalence of diarrhea by 34% but had no effect on pneumonia (13). Because zinc is critical for immune function, the role of enhanced bacterial virulence in a zinc-deficient host is unclear. Although rodent studies addressing the effect of zinc deficiency on pneumonia and diarrheal infection are limited, zinc deficiency was shown to increase susceptibility to *L. monocytogenes*, pneumococcal pneumonia, and diarrheal infection by enteroaggregative *E. coli* (EAEC), and to alter the time course of *Mycobacterium bovis* infection (17, 22, 90, 128). Notably, EAEC had increased expression of putative virulence factors in the cecal contents of zinc-deficient mice, suggesting that host zinc deficiency can alter bacterial pathogenesis (17).

CONCLUDING REMARKS

Transition metal biology at the host-pathogen interface is an active area of cross-disciplinary research spanning chemistry, biochemistry, microbiology, immunology, human genetics, and environmental and nutrition sciences. The field presents a number of opportunities for therapeutic potential. As secreted public goods, bacterial siderophores present a promising therapeutic target with reduced selective pressure for resistance, because the bacterium that evolves resistance does not necessarily benefit. For example, gallium (Ga^{2+}) quenching of *P. aeruginosa* siderophores reduced pathogenesis in a *Galleria mellonella* model and retained efficacy longer than the conventional antibiotics gentamicin and ciprofloxacin (118). If a bacterium were to evolve production of gallium-resistant siderophore, it would be secreted and available to the entire bacterial population. Therefore, the producer of the gallium-resistant siderophore would not gain a selective advantage. In this way, targeting bacterial public goods decouples resistance and selective pressure. The fact that gallium siderophore quenching takes advantage of its interchangeability with biologically relevant transition metals suggests that gallium may also be toxic to the host due to host protein mismetallation. Therefore, other methods to target siderophores present attractive therapeutic opportunities. For example, vaccines against bacterial siderophores could similarly decouple resistance and selective pressure without causing metal intoxication of the host.

Additional areas of interest include bacterial metal homeostasis, evolution of bacterial pathogenesis, the role of nutritional immunity in modulating bacterial social behavior, and the effect of host metal nutrition on bacterial virulence. Recent work defining a low molecular weight thiol as the labile zinc buffer in *Bacillus subtilis* has expanded our understanding of metal homeostasis in bacteria, but the role of metal buffering and metallochaperones in pathogenesis remains largely unexplored (82). Recent work on the *Acinetobacter baumanii* response to zinc starvation identified a putative Zn

metallochaperone and that intracellular histidine contributes to the labile zinc pool, expanding our understanding of zinc homeostasis in this opportunistic pathogen (97). We expect that additional targets of metal piracy will open new avenues for the study of rapid evolution at the host-pathogen interface. Additionally, polymicrobial infections can modulate the metal environment in the host. Infection by respiratory syncytial virus causes transferrin release, increasing available iron and promoting *P. aeruginosa* infection and biofilm formation (61). Future work will likely continue to uncover the role of metal homeostasis in bacterial pathogenesis and additional mechanisms by which metals influence coinfections.

The effect of host dietary metals on bacterial virulence also remains open for exploration. For example, future work could expand the effect of nutrient metals on gut microbial ecology and colonization resistance to pathogens. Transposon mutagenesis followed by selection and high-throughput sequencing of insertion sites could help determine whether bacterial pathogens can take advantage of altered nutrient metal landscapes in copper- and zinc-deficient hosts. Although the requirement of transition metals for enzyme catalysis likely predates life itself, the battle for metals at the host-pathogen interface remains a hotbed of evolution and continues to present opportunities for bettering human health.

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RELATED RESOURCES

Nriagu, JO.; Skaar, EP. Trace Metals and Infectious Diseases. MIT Press; Cambridge, MA: 2015.

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NUTRITIONAL IMMUNITY PROTEIN MOONLIGHTS AS AN ANTIMICROBIAL

Beyond its role in nutritional immunity, lactoferrin can inhibit bacterial growth by a variety of mechanisms including generation of antimicrobial peptides and binding of bacterial cell wall components (136). Bacterial pathogens have evolved systems to protect against these activities. Lactoferrin generates the antimicrobial peptides lactoferricin and lactoferrampin during bacterial infection. Recent work revealed rapid evolution in primates at the binding interface of lactoferricin and bacterial proteins that prevent lactoferrin-mediated killing (12). Additionally, the Staphylococcus aureus heme acquisition protein IsdA protects against lactoferrin-mediated killing by inhibiting lactoferrin's serine protease activity (28). Thus, resistance to a host nutritional immunity protein moonlighting as an antimicrobial agent relies on a bacterial metal acquisition protein moonlighting as a protease inhibitor. These evolutions/counter-revolutions exemplify the Red Queen hypothesis in bacterial pathogenesis.

SUMMARY POINTS

1. Although bacterial pathogens use vertebrate hosts as nutrient metal sources, hosts possess multiple mechanisms to restrict bacterial access to transition metals through nutritional immunity. Bacteria evade host metal sequestration through metal acquisition strategies, including stealth siderophores and metal piracy from host proteins. The coevolution of pathogens and vertebrate hosts at the nutrient metal interface illustrates the Red Queen hypothesis in evolutionary biology.
2. Vertebrate hosts barrage bacteria with metals during intracellular infection, exploiting the toxicity of high concentrations of copper and zinc. The host intoxicates intraphagosomal bacteria by transporting high levels of copper with ATP7A. The mechanism of zinc intoxication and its relationship to zinc innate immune signaling is still being elucidated. Resistance to copper and zinc toxicity is important for virulence in a number of pathogens.
3. Host metal dysregulation through genetic disease or dietary metal intake affects susceptibility to infection by many bacterial pathogens. In general, iron overload, zinc deficiency, and copper deficiency increase susceptibility to infection. During *H. pylori* infection, iron deficiency accelerates production of bacterial virulence factors and carcinogenesis, demonstrating that changes in dietary metals can affect bacterial virulence.
4. The interchangeability of transition metal binding requires all organisms, including bacterial pathogens and their vertebrate hosts, to maintain appropriate metal homeostasis to prevent toxicity and mismetallation. The role of metal homeostasis at the host-pathogen interface remains an important area of investigation.

Transition metals: *d*-block elements in rows 3–12 of the periodic table that generally have partially filled *d*-orbitals and produce many oxidation states

Siderophores: small molecules that bind Fe^{3+} with high affinity secreted by microbes to acquire iron from the environment

Transferrin: a protein that sequesters iron in blood, transporting iron to the rest of the body following absorption in the duodenum

Red Queen hypothesis: a concept referencing Lewis Carroll's *Through the Looking Glass* to describe evolutionary pressures on organisms in close association

Heme: Fe^{2+}-protoporphyrin IX, a cofactor for cytochrome oxidases used for respiration in bacteria and eukaryotes

ATP-binding cassette (ABC) transporters: a family of proteins that transport molecules across the cytoplasmic membrane via ATP hydrolysis

Public goods: a term originating from economics to describe resources that may be costly to produce but are freely accessible to all

Tragedy of the commons: refers to loss of a public resource from overuse, from economist William Forster Lloyd's phrase describing overgrazing of common lands

Hemopexin: a protein that binds free heme to protect against its oxidative effects and recycle the body's iron

Haptoglobin: a protein that binds free hemoglobin to protect against its oxidative effects; most hemoglobin-haptoglobin is then removed by the spleen

TonB-ExbB-ExbD system: a protein complex that harnesses the proton motive force for active transport across the outer membrane in Gram-negative bacteria

Type VI secretion system (T6SS): a system used by Gram-negative bacteria to transport effector proteins into host or bacterial target cells

Metallothioneins: a family of low molecular weight cytosolic proteins rich in cysteines that bind heavy metals on their thiol groups

S100 protein family: Ca^{2+}-binding proteins with a helix-loop-helix (EF-hand type) conformation that often bind additional metals and serve as nutritional immunity proteins

Natural resistance–associated macrophage protein 1 (Nramp1): a protein that exports iron and manganese from the phagosome and enhances resistance to intracellular pathogens

β-**thalassemia:** a hereditary blood disorder caused by defects in synthesis of the hemoglobin β-chain that can cause iron overload

Hemochromatosis: hereditary blood disorder caused by mutations in transferrin receptor–interacting genes and characterized by iron overload

Iron overload: an accumulation of iron in the body due to genetic diseases, repeated blood transfusion, or excess iron consumption

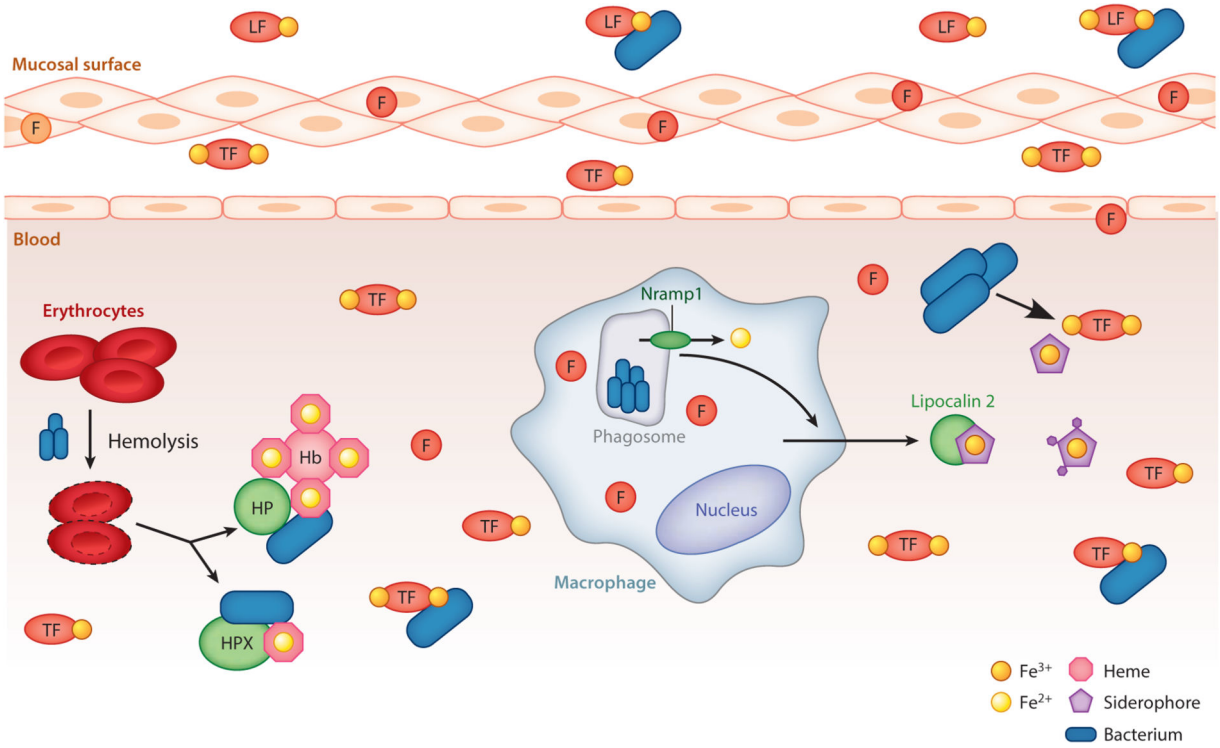

Figure 1.

Iron at the host-pathogen interface. In the host, Fe^{3+} is limited because of chelation by lactoferrin (LF) at the mucosal surface, transferrin (TF) in blood and tissue, and ferritin (F) in the cellular cytoplasm and at low levels in serum. Some bacteria can acquire iron from these host ferroproteins. In the bloodstream, bacteria can liberate iron from erythrocytes by hemolysis followed by extraction of heme from hemoprotein complexes, including hemoglobin (Hb), hemoglobin-haptoglobin (HP), and hemopexin (HPX). Intracellular pathogens are iron starved by Nramp1-mediated iron efflux from the phagosome. Nramp1 expression further induces lipocalin 2 production to bind and sequester some bacterial siderophores, whereas stealth siderophores evade binding.

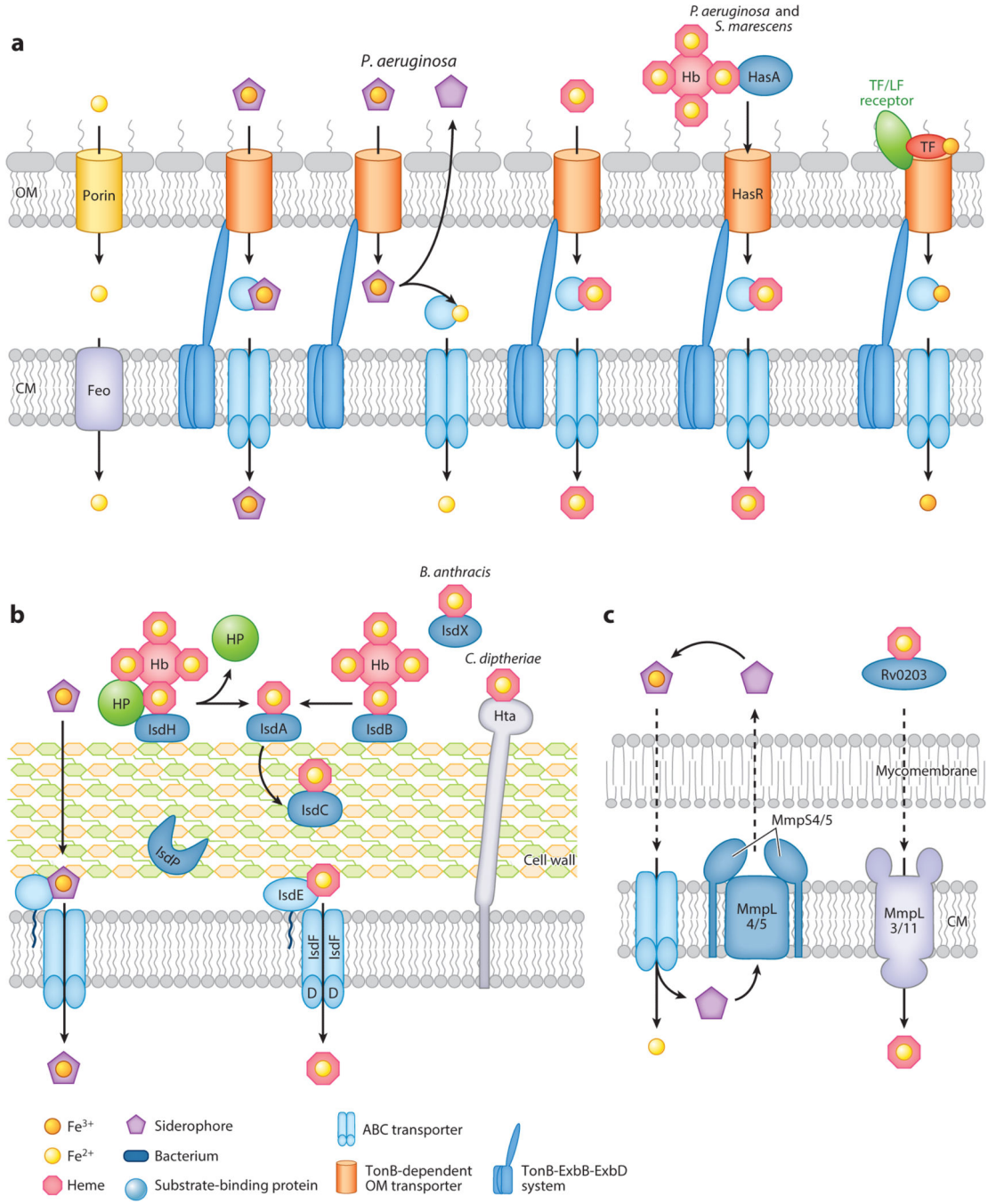

Figure 2.

Iron acquisition in bacterial pathogens. (*a*) Gram-negative pathogens use porins or TonB-dependent outer membrane (OM) transporters to mediate the passage of iron complexes to the periplasm and, subsequently, the Fe^{2+} transporter Feo or ABC transporters to transport iron complexes into the cytoplasm. *Pseudomonas aeruginosa* reduces Fe^{3+}-siderophores in the periplasm and exports deferrated siderophores from the periplasm via an ABC transporter (process not shown). *P. aeruginosa* and *Serratia marescens* use the secreted hemophore HasA and its receptor HasR for heme acquisition. Some bacteria also encode

transferrin or lactoferrin OM receptors to actively pirate Fe^{3+} from host nutritional immunity proteins. (*b*) Gram-positive pathogens acquire iron using siderophores or dedicated heme/hemoprotein uptake systems. Pathogenic *Staphylococcus* spp. and *Bacillus* spp. use the Isd system to mediate the passage of heme through the cell wall to a dedicated transporter. Additional species-specific features include the *Staphylococcus lugdunensis* autolysin IsdP and *Bacillus anthracis*–secreted hemophores IsdX1 and IsdX2. *Corynebacterium diptheriae* uses the lipoprotein heme transporters HtaA/HtaB. (*c*) Portions of the mycobacterial iron acquisition process have been elucidated. Mycobacteria couple Fe^{3+}-siderophore import with reduction and Fe^{2+} release; the deferrated siderophores can then be exported by the MmpL4/5-MmpS4/5 system for recycling. Heme import requires the secreted hemophore Rv0203 and the CM importers MmpL3/11. Abbreviations: ABC, ATP-binding cassette; CM, cytoplasmic membrane; Hb, hemoglobin; HP, hemoglobin-haptoglobin; LF, lactoferrin; TF, transferrin.

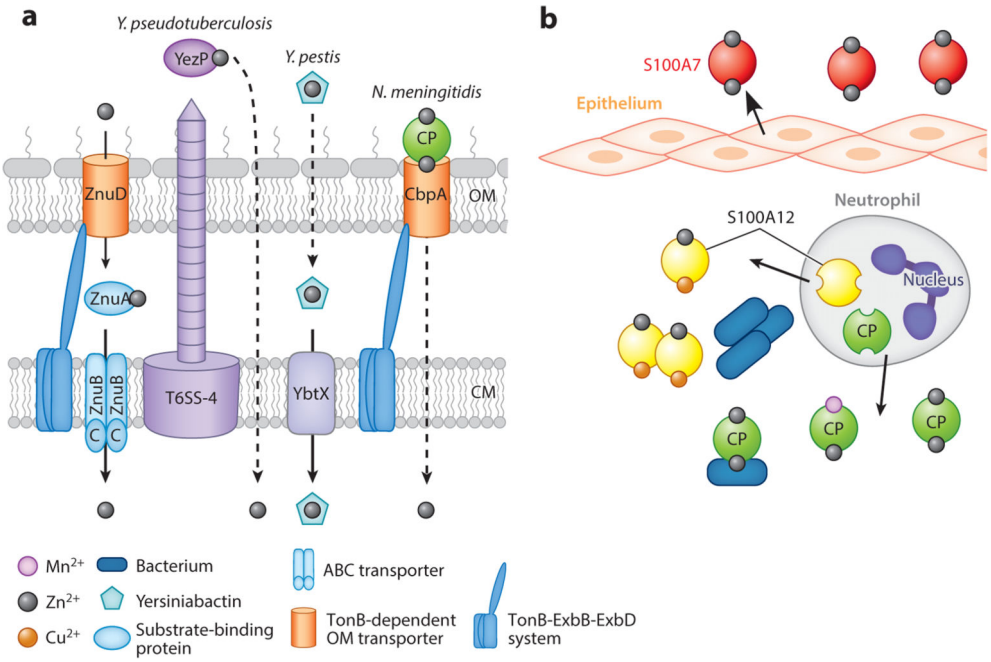

Figure 3.

Zinc sequestration and bacterial zinc import. (*a*) Pathogenic bacteria acquire zinc using ABC transporters ZnuABC (Gram-negative bacteria) and AdcABC (Gram-positive bacteria; *not shown*). Zinc is transported through the outer membrane (OM) using the TonB-dependent transporter ZnuD. Additional zinc acquisition systems include a T6SS-secreted zincophore protein YezP in *Yersinia pseudotuberculosis*, transport of yersiniabactin-Zn^{2+} in *Y. pestis*, and an OM receptor CbpA that binds calprotectin (CP) in *Neisseria meningitidis*. (*b*) During infection, zinc is limited by secretion of zinc-chelating S100 proteins: S100A7 is secreted at epithelial surfaces, whereas S100A12 and CP are secreted by innate immune proteins such as neutrophils. Some bacteria can acquire zinc from CP. Abbreviations: ABC, ATP-binding cassette; CM, cytoplasmic membrane; T6SS, type VI secretion system.

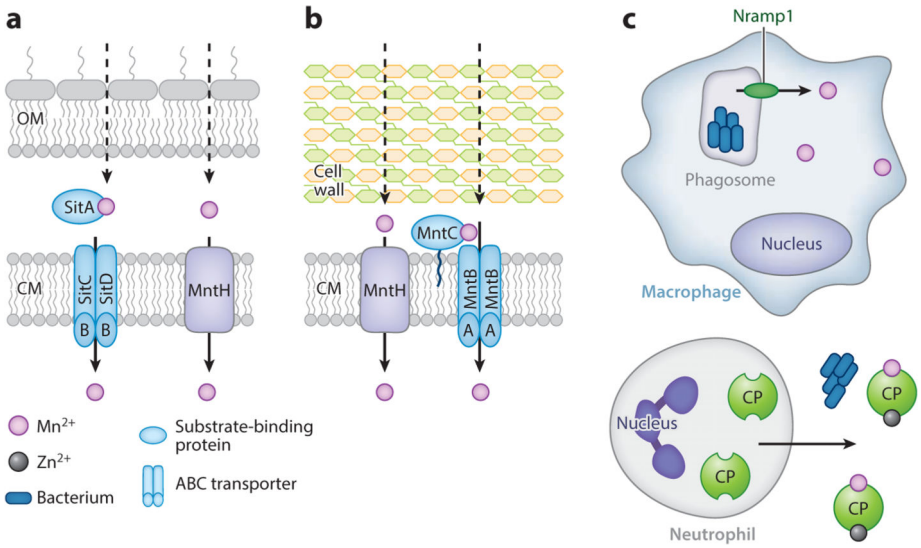

Figure 4.

Intra- and extracellular manganese restriction. (*a*) Gram-negative pathogens import manganese with the SitABCD or MntH transporters; the mechanism of transport across the outer membrane (OM) is unknown. (*b*) Gram-positive pathogens import manganese with the MntABC or MntH transporter. (*c*) The phagosome membrane protein Nramp1 effluxes manganese from the phagosome, limiting availability for intracellular pathogens. Extracellularly, manganese is sequestered by calprotectin (CP), which is secreted by innate immune cells, including neutrophils, in response to infection. Abbreviations: ABC transporter, ATP-binding cassette transporter; CM, cytoplasmic membrane.

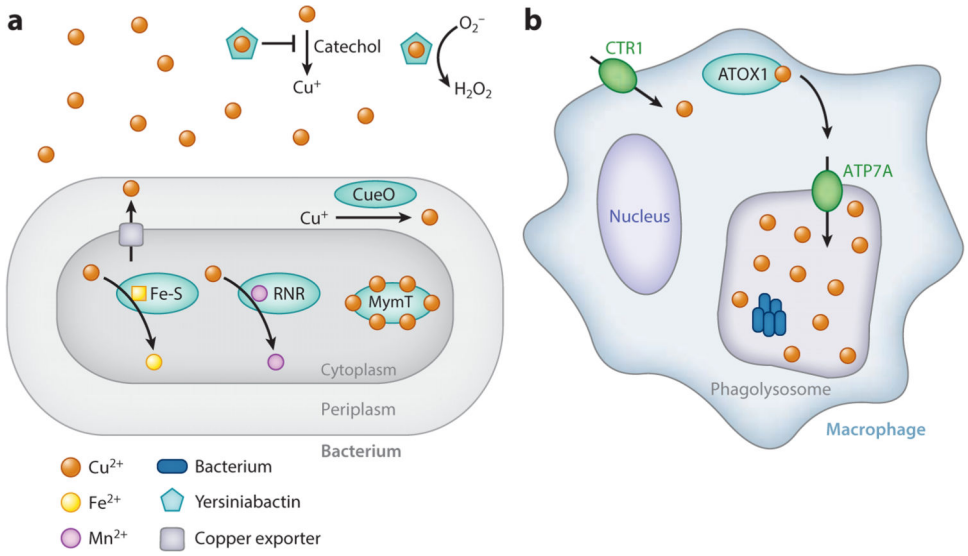

Figure 5.

Copper intoxication and bacterial resistance. (*a*) Evidence thus far suggests that copper is toxic because it displaces metal cofactors in certain proteins, including iron-sulfur clusters and manganese in ribonucleotide reductase (RNR). Bacteria have evolved diverse mechanisms to withstand copper toxicity, including copper efflux. In uropathogenic *Escherichia coli*, extracellular yersiniabactin-Cu^{2+} prevents copper reduction to the more reactive Cu^{+} and can convert superoxide to hydrogen peroxide. *E. coli* also utilizes the periplasmic copper oxidase CueO to detoxify Cu^{+}. Mycobacteria also produce a metallothionein, MymT, to sequester cytoplasmic copper. (*b*) In response to infection, phagocytic cells, including macrophages, import copper into the cytosol with the transporter CTR1. Copper is then shuttled by ATOX1 to the phagolysosomal membrane, where it is transported into the phagolysosome by ATP7A to intoxicate bacterial cells.

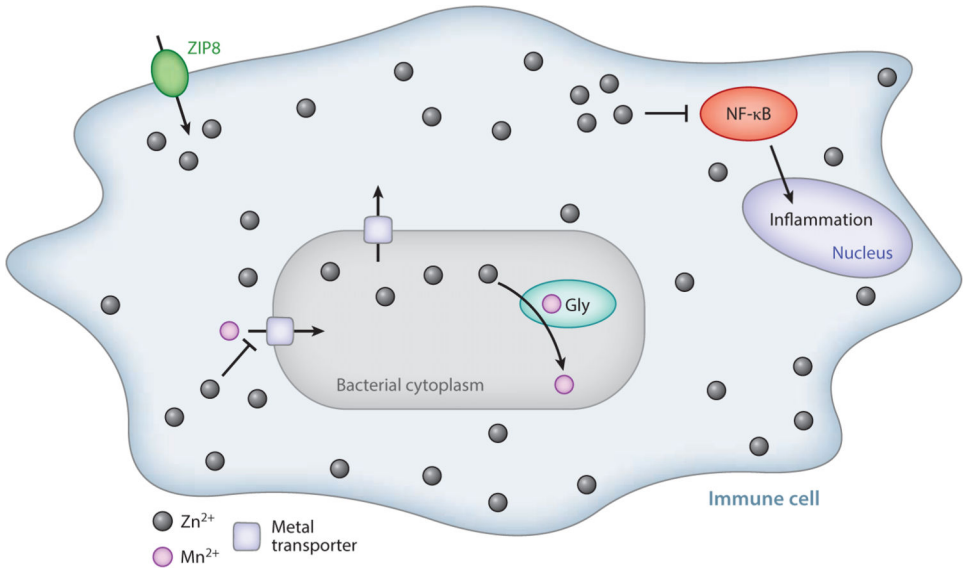

Figure 6.

Zinc intoxication in innate immune cells. In response to infection, innate immune cells accumulate zinc in the cytoplasm through ZIP8-mediated import and in the phagosome by an unknown mechanism. Zinc accumulation in the cytoplasm modulates nuclear factor (NF)-κB activation, dampening proinflammatory responses. Bacterial zinc poisoning inhibits manganese import and the manganese-utilizing glycolytic enzymes (Gly) phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase. Bacteria express zinc efflux proteins in response.

Table 1

Selected bacterial species with improved virulence in host environments with metal dysregulation

Bacterial species	Host metal dysregulation	Host	Effect on infection	Reference
Acinetobacter calcoaceticus	Occupational inhaled iron exposure	Human	Cluster of *A. calcoaceticus* pneumonia cases with two deaths	31
Enteroaggregative *Escherichia coli*	Dietary zinc deficiency	Mouse	Enhanced disease	17
Helicobacter pylori	Dietary iron deficiency	Gerbil	Enhanced bacterial burdens and carcinogenesis	101
Listeria monocytogenes	Iron overload from β-thalassemia	Mouse	Increased mortality and bacterial burdens	6
	Dietary zinc deficiency	Rat	Reduced immune response	22
Mannheimia hemolytica	Dietary copper deficiency	Mouse	Reduced lethal dose	70
Mycobacterium bovis	Dietary zinc deficiency	Guinea pig	Enhanced bacterial burdens early in infection	90
Mycobacterium tuberculosis	Dietary iron overload	Human	Shift from latent to active infection	48
Salmonella enterica	Iron overload from β-thalassemia	Mouse	Increased mortality	6
	Dietary zinc deficiency	Rat	Increased mortality	99
Streptococcus pneumoniae	Dietary zinc deficiency	Mouse	Increased mortality and bacterial burdens	128
Vibrio vulnificus	Iron overload by injection	Mouse	Reduced lethal dose	146
	Hepcidin deficiency	Mouse	Increased bacteremia and mortality, partially rescued by dietary iron depletion	8
Yersinia enterocolitica	Dietary iron overload	Human	Infection by commensal species	91
Yersinia pestis	Iron overload from hemochromatosis	Human, mouse	Restored virulence of attenuated vaccine strain	112