Nutritional immunity: the battle for nutrient metals at the host–pathogen interface

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Abstract | Trace metals are essential micronutrients required for survival across all kingdoms of life. From bacteria to animals, metals have critical roles as both structural and catalytic cofactors for an estimated third of the proteome, representing a major contributor to the maintenance of cellular homeostasis. The reactivity of metal ions engenders them with the ability to promote enzyme catalysis and stabilize reaction intermediates. However, these properties render metals toxic at high concentrations and, therefore, metal levels must be tightly regulated. Having evolved in close association with bacteria, vertebrate hosts have developed numerous strategies of metal limitation and intoxication that prevent bacterial proliferation, a process termed nutritional immunity. In turn, bacterial pathogens have evolved adaptive mechanisms to survive in conditions of metal depletion or excess. In this Review, we discuss mechanisms by which nutrient metals shape the interactions between bacterial pathogens and animal hosts. We explore the cell-specific and tissue-specific roles of distinct trace metals in shaping bacterial infections, as well as implications for future research and new therapeutic development.

Mis-metalation
The association of metalloproteins with non-cognate metals, often abrogating enzymatic function.

Siderophore
A secreted low molecular-weight molecule that binds and sequesters iron (Fe), and potentially other metals.

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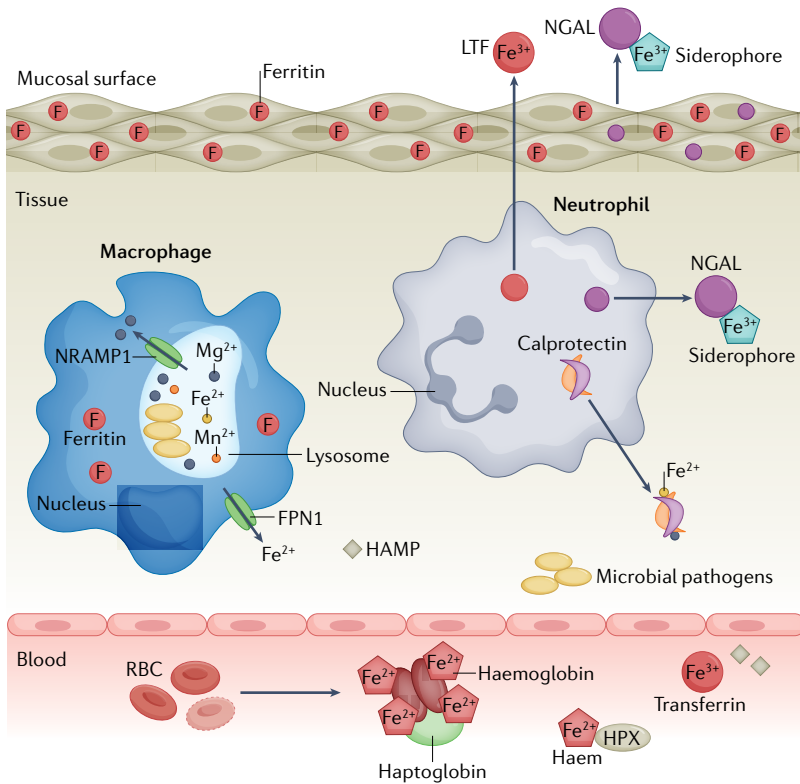
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Trace metals including zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) are essential for all forms of life. The importance of nutrient metals in vertebrate health is underscored by the fact that both metal deficiency and metal excess lead to the development of numerous pathologies and represent a major public health burden[1]. The interaction with metals alters the physico-chemical properties of proteins, thereby promoting catalysis of enzymatic reactions, stabilizing protein structure and/or facilitating electron transport[2,3]. These metal-associated proteins, referred to as metalloproteins, function in a diverse set of cellular processes, including respiration, transcription, signal transduction and proliferation[4]. Despite the necessity of trace metals in cellular activities, excess metals are toxic, likely due to the generation of redox-active molecules or from the mis-metalation of metalloproteins, which abrogates their enzymatic function. Organisms maintain appropriate physiological levels of metals by modulating uptake, use, storage and export at the cellular and systemic levels. Tissue metal levels in vertebrate hosts are mainly controlled by absorption from dietary sources in the intestinal tract, whereas bacterial pathogens acquire metal from the extracellular and intracellular environments of host tissues during infection[5].

Metal bioavailability exerts a strong selective pressure at the infectious interface giving rise to an evolutionary arms race between host and pathogen that shapes metal sequestration strategies[6]. Across all forms of life, metals such as Fe, Zn, Mn, magnesium, cobalt and molybdenum are required for enzymatic function of upwards of 36% of proteins in every enzyme class[7,8]. Given this vital requirement of metals for cellular function in both vertebrate hosts and bacterial pathogens, it is not surprising that vertebrates undergo drastic alterations in metal metabolism in response to bacterial infections. Vertebrates have developed numerous strategies to starve bacteria of nutrient metals via a process termed nutritional immunity[6,9,10]. Current research efforts are broadening the scope of nutritional immunology, with newly identified metal-binding molecules and metal transport systems in bacterial pathogens, as well as an appreciation of non-metal nutrients that dictate host–pathogen interactions. Moreover, knowledge of the effects of metal starvation on bacterial physiology is deepening with exciting discoveries in the areas of cell wall modifications and expression of virulence factors. Due to the essentiality of nutrient metal maintenance systems, understanding the role of trace metals in bacterial infections remains a critical area of research to identify novel therapeutic strategies. Recent developments in drug design include the emergence of siderophore-conjugated antibiotics, which harness bacterial metal uptake systems to aid in drug delivery. In this

Fig. 1 | **Host Fe-limitation strategies.** Following invasion by pathogenic bacteria, the host limits bacterial access to iron (Fe) through a systemic reprogramming of Fe homeostasis by sequestering Fe in macrophages, hepatocytes and enterocytes, while simultaneously reducing uptake of Fe from the diet. Within the tissue environment, Fe sources are limited through concerted action of host secreted factors including Fe-chelating molecules calprotectin, neutrophil gelatinase-associated lipocalin (NGAL) and lactoferrin (LTF) as well as intracellular Fe storage protein ferritin. In circulation, Fe is associated with haemoglobin in red blood cells (RBCs) or bound by transferrin. Lysis of RBCs results in release of haemoglobin and free haem–Fe, which is rapidly bound by haptoglobin and haemopexin (HPX) to prevent usage by bacterial pathogens. Secreted hormone hepcidin (HAMP) prevents host Fe export through binding and subsequent internalization of Fe transporter ferroportin 1 (FPN1). Intracellular pathogens within macrophages are starved of Fe through the action of natural resistance-associated macrophage protein 1 (NRAMP1) in the phagolysosome. Fe^{2+}, ferrous iron; Fe^{3+}, ferric iron.

Review, we discuss the elaborate mechanisms by which vertebrate hosts and bacterial pathogens maintain cellular metal homeostasis, providing a timely update to the Review previously detailing host–pathogen–metal interactions[9]. We examine the role of trace metals (Fe, Zn, Mn and Cu) in mediating host–pathogen interactions in cell-specific and tissue-specific host niches. Finally, we explore emerging concepts in nutritional immunity, including the paradox of metal starvation and intoxication at the host–pathogen nexus and novel mechanisms used for bacterial metal acquisition.

Fe in infection and immunity

Fe is a redox-active trace metal that has important roles in most biological systems, supporting vital processes such as DNA replication, transcription and central metabolism[9]. Although essential for life, levels of Fe must be tightly regulated, especially considering that ferric Fe participates in Fenton-type redox chemistry and can be detrimental to macromolecular function[11]. Under aerobic conditions, Fe typically exists in the insoluble ferric form (Fe^{3+}), whereas the soluble ferrous Fe (Fe^{2+}) exists under anaerobic or acidic conditions[11]. Fe can be kinetically trapped in molecules such as haem or associated with host metalloproteins and storage molecules. However, Fe sources can also be exchangeable whereby Fe is tightly bound but still labile. The requirement of Fe by both vertebrate hosts and bacterial pathogens has led to the development of sophisticated host and bacterial systems to liberate, sequester and scavenge Fe within host niches. Host strategies for maintaining Fe pools during infection have been recently reviewed[9,12] and, hence, these mechanisms are discussed only briefly below.

Host-imposed Fe restriction during infection. Vertebrate tissues present an Fe-restricted environment for bacterial pathogens (FIG. 1). Most intracellular Fe is incorporated into metalloproteins or stored in association with ferritin, thereby protecting the host from Fe toxicity while restricting access to invading bacteria[12]. The majority of extracellular Fe is stored in the tetrapyrrole cofactor haem, which is complexed to haemoglobin in circulating erythrocytes and is responsible for oxygen binding. If haemoglobin or haem is released from erythrocytes, it is rapidly bound by host haemoproteins, haptoglobin or haemopexin (HPX), further preventing Fe use by pathogens[13]. Circulating ferric Fe that is not associated with haem is bound by the serum protein transferrin. The concerted action of these proteins renders host Fe pools largely inaccessible to bacteria that lack high-affinity Fe uptake pathways or systems[9,12,14]. In fact, humans with genetic defects in Fe-handling systems have a substantially increased risk for infectious disease, highlighting the critical requirement for Fe-dependent nutritional immunity in host protection against bacterial pathogens[15] (see BOX 1).

Immune cells, including macrophages and neutrophils, have important roles in restricting metal access to invading pathogens. In macrophages, the natural resistance-associated macrophage protein 1 (NRAMP1) is an antiporter localized to late endosomal or lysosomal membranes[16]. For intracellular pathogens including *Mycobacterium tuberculosis* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium, NRAMP reduces metal availability by redirecting storage of cellular Fe, Mn and magnesium from the phagolysosome to the cytoplasm[17–19]. Moreover, neutrophils secrete numerous Fe-scavenging proteins including lactoferrin (LTF), calprotectin and neutrophil gelatinase-associated lipocalin (NGAL; also known as lipocalin 2) to limit bacterial growth[20]. Notably, secretion of metal-scavenging proteins is not limited to immune cells. Host mucosal epithelial cells such as those found in the intestinal tract also secrete metal-scavenging proteins including NGAL, which can inhibit bacterial growth of enteric pathogens[21], thereby having an important role in host innate immunity.

Bacterial acquisition of Fe. Bacterial pathogens have evolved numerous strategies to acquire Fe in host environments (FIG. 2). The prominent strategies that bacteria use to acquire Fe include the secretion of siderophores

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Ferritin
An intracellular protein used in the storage of labile cellular iron (Fe).

Haptoglobin
A host protein that binds haemoglobin in circulation, preventing oxidative activity and use by pathogens.

Haemopexin
(HPX). A host serum protein that binds circulating haem with high affinity.

and the uptake of siderophore–Fe, haem–Fe or kinetically labile ferrous Fe (REF.[9]). The extremely Fe-limited host environment acts as a signal to bacterial pathogens, resulting in the transcriptional upregulation of Fe-acquisition machinery as well as many virulence factors that facilitate host colonization. These changes in gene expression are predominantly mediated by Fe-responsive transcription factors, such as the ferric uptake regulator (Fur) or diphtheria toxin repressor (DtxR)[22,23]. Yet little is known regarding the direct effect of metals on the activity of proteins involved in these uptake systems. Interestingly, some bacteria, such as *Lactobacillus plantarum* and *Borrelia burgdorferi*, have evolved elegant mechanisms to circumvent the need for nutrient Fe altogether via the incorporation of non-Fe metals such as Mn into metalloproteins[24,25]. The host also uses mechanisms to sequester nutrient Mn (see below). In some cases, obligate human bacterial pathogens, including *Neisseria* and *Moraxella* spp., exhibit species-specific binding to Fe-binding molecules such as transferrin and LTF through transferrin or LTF receptors, thus facilitating bacterial scavenging of host-restricted Fe pools[26,27].

Circulating haem makes up the largest potential reservoir of Fe for bacterial pathogens in the vertebrate host[13]. Haem consists of an Fe atom coordinated by a tetrapyrrole ring and interacts with host proteins, which makes haem unavailable to potentiate toxicity or be acquired by pathogens. Because haem is a valuable Fe source and some bacteria are unable to synthesize haem de novo, pathogens have evolved elaborate mechanisms to import, catabolize and release Fe from haem. To access haem, several pathogenic bacteria including strains of *Pseudomonas*, *Staphylococci*, *Streptococci* and *Escherichia* secrete haemolysins that integrate into erythrocyte membranes and result in osmotic lysis[28,29]. Bacterial pathogens capture liberated haem or haemoproteins using either cell wall-anchored receptors (Gram-positive bacteria), TonB-dependent receptors (Gram-negative bacteria) or haemophores, which are secreted proteins that complex haem[30]. Subsequently, Fe must be liberated from the tetrapyrrole ring of haem via the activity of haem oxygenases in the bacterial cytosol[30]. Haem oxygenases are classified into five different enzyme families including the HO-1 family, the IsdG family, ChuZ, ChuW and HutW[31,32].

Many Gram-positive bacteria use the well-characterized Fe-regulated surface determinant system (Isd) to scavenge host haem. The prototypical Isd system is described in *Staphylococcus aureus* and consists of ten genes encoding cell wall-anchored proteins (IsdABCH), a membrane transport system (IsdDEF), haem oxygenases (IsdG and IsdI) and a transpeptidase (SrtB). The *Bacillus anthracis* Isd proteins include IsdX1 and IsdX2, which are secreted haemophores that bind haemoglobin, haptoglobin–haemoglobin or haem[33–35]. More recently, additional Isd proteins have been identified including an autolysin, IsdP, that reorganizes the cell wall to improve haem acquisition in *Staphylococcus lugdunensis*[36]. Acquisition of host haem is necessary for full infection, as mutants lacking Isd components have reduced virulence[37]. Isd-independent haem acquisition systems in other species include the ECF transporter LhaSTA in *S. lugdunensis*[38] and HtsABC and HmuUV in *Corynebacterium diphtheriae* and *Streptococcus* spp.[39,40]. Bacterial haem acquisition permits bacterial survival amidst the presence of host Fe-chelating molecules. For instance, in the presence of the Fe-sequestering protein calprotectin, haem availability is essential for the survival of *S. aureus* and *Pseudomonas aeruginosa*, highlighting the importance of haem as an Fe source within the host[41]. The host counteracts bacterial haem scavenging using haptoglobin, which binds haemoglobin and reduces accessibility of haem while promoting clearance by host cells. Recent studies have provided insights into how pathogens can compete with host clearance of haptoglobin–haemoglobin complexes. Although host haptoglobin reduces IsdH haemoglobin binding[42], evidence suggests that IsdH can bind the haptoglobin–haemoglobin complex, preventing recognition by macrophage CD136 and, thus, blocking subsequent internalization and clearance[43]. The recognition of host haemoprotein complexes and bacterial mechanisms to subvert host haem recycling is a growing area of research.

In Gram-negative bacteria, haem uptake systems are more diverse as the outer membrane presents an additional barrier for haem import. Host haem or

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Box 1 | Host genetics and diet

Trace metals are obtained through dietary sources in vertebrate hosts. Following absorption in the gastrointestinal tract, cellular and systemic metal levels are regulated by numerous well-defined metabolic circuits. Both conditions of metal deficiency and metal excess lead to increased risk of infection in humans, and these perturbations in metal homeostasis can be the result of either dietary or inherited factors. However, the mechanisms by which dietary metals and host genetic impairments in metal metabolism affect critical factors including colonization resistance, bacterial virulence as well as immune responses to bacterial pathogens are not fully understood, representing a promising field of future research using animal models with genetic and environmental manipulation of metal levels.

Human genetic polymorphisms in genes required for maintaining iron (Fe) homeostasis often cause the Fe overload disorders β-thalassaemia and haemochromatosis and lead to increased susceptibility to infectious disease[199]. More specifically, β-thalassaemia leads to increased sensitivity to bacterial infection by *Enterobacteriaecia*, *Salmonella enterica* and *Listeria monocytogenes*[200,201]. Haemochromatosis leads to increased susceptibility of hosts to numerous opportunistic pathogens such as *Vibrio vulnificus* and non-pigmented *Yersinia pestis*[202,203]. Dietary changes that result in altered metal levels in the gut lead to substantial alterations in microbial ecology that may facilitate gut colonization by enteric pathogens such as *S. enterica* subsp. *enterica* serovar Typhimurium, *Citrobacter rodentium*, *Enterobacter faecalis* and *Clostridioides difficile*[5,204,205]. In fact, excess Fe can lead to pathogenic *Yersinia enterocolitica* colonization[206]. Similar to Fe, high levels of dietary zinc (Zn) can lead to expansion of distinct gut commensals and increased colonization by *C. difficile*[205]. Due to host restriction of other metals including manganese (Mn), increased dietary Mn is likely to also contribute to bacterial pathogenesis.

Conversely, there are well-described links between deficiencies of Fe, Mn, copper (Cu) and Zn and bacterial infection. Zn is generally important in hosts for growth and immune development as well as in pathogen killing. Zn deficiency is associated with increased susceptibility to *L. monocytogenes*, pneumococcal pneumonia and infection by enteroaggregative *Escherichia coli*. Further, Zn deficiency results in increased expression of virulence factors of enteric pathogens including enteropathogenic *E. coli*[207,208]. Animals deficient in Zn uptake exhibit increased susceptibility to bacterial infection[209], as is seen in *Zip8*-knockout mice infected with *Streptococcus pneumonia*[210]. Reduced levels of Cu are associated with neutropenia and, therefore, increase infectious disease risks by pathogens such as *S. enterica* and *Mannheimia haemolytica* in animal models[211,212]. Although less studied, deficiencies in dietary Mn have also been linked to increased risk of infection by *Staphylococcus aureus*[213]. Collectively, these findings demonstrate that levels of dietary metals must be tightly regulated to prevent expansion of gut pathogens while maintaining a healthy immune response to invading bacteria.
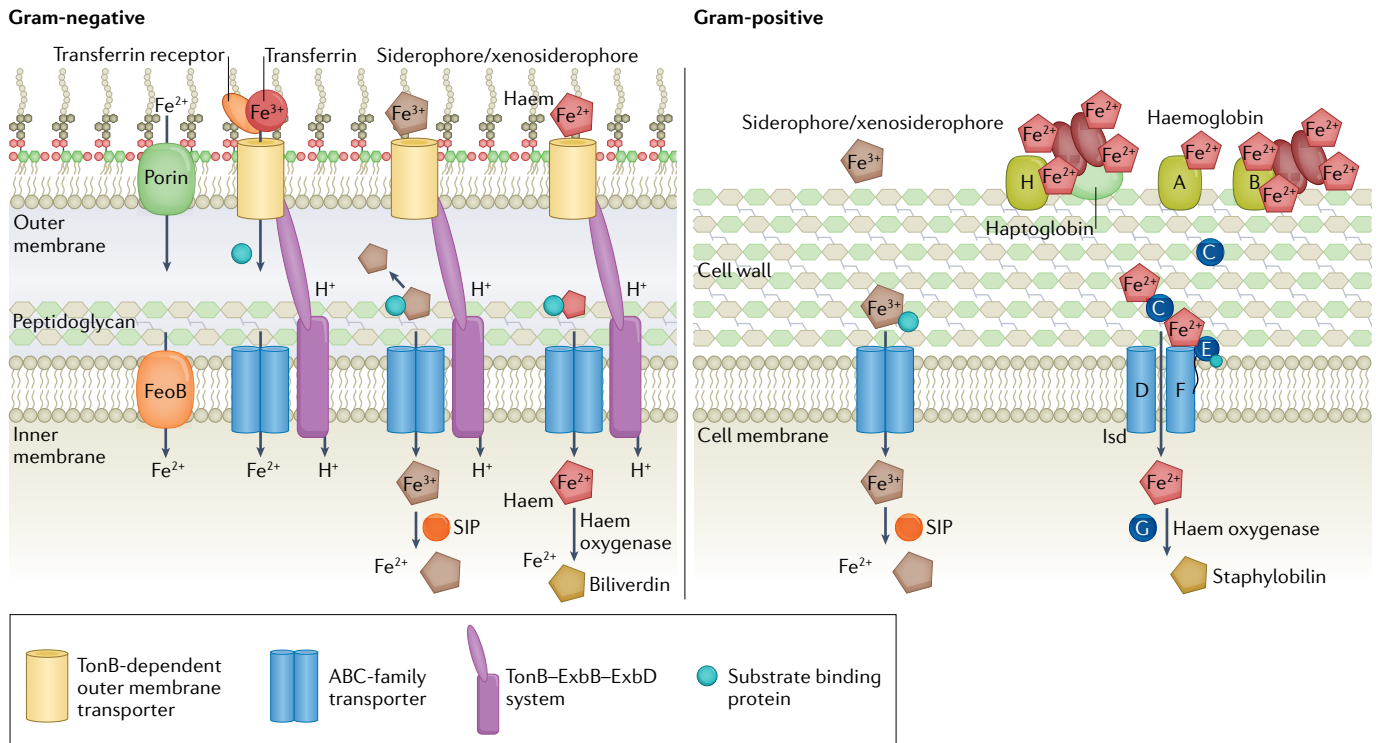
Gram-negative

Gram-positive

Fig. 2 | **Bacterial Fe homeostasis at the host–pathogen interface.** Bacterial pathogens can scavenge iron (Fe) through concerted action of secreted siderophores, uptake of host haem or Fe-containing molecules (transferrin and calprotectin) and uptake of ferrous Fe (Fe^{2+}). Gram-negative pathogens uptake Fe into periplasm via activity of porins or TonB-dependent outer membrane transporters. Periplasmic Fe is subsequently transported via FeoB (Fe^{2+}) or ATP-binding cassette (ABC)-family transporters (ferric Fe (Fe^{3+})) through inner membrane into cytosol. Gram-positive bacteria acquire Fe-bound siderophores or xenosiderophores using ABC-family transporters in the cell membrane and host haem–Fe via activity of Fe-regulated surface determinant system (Isd) transport systems. In the cytosol, siderophore-interacting proteins (SIPs) reduce Fe-loaded siderophores for liberation of Fe^{2+}. Iron is liberated from haem through activity of haem oxygenases, releasing biliverdin and staphylobilin as by-products in the cytosol.

haemoproteins are recognized by outer membrane receptors that bind haem directly or bind haem-bound secreted haemophores for trafficking into the periplasm. From the periplasm, haem is transported to the cytoplasm via ABC transporters within the inner membrane. Some Gram-negative pathogens encode multiple haem-acquisition systems such as the Phu and Has systems in *P. aeruginosa*[44], suggesting that multiple non-redundant haem uptake systems benefit bacterial virulence. In the nosocomial pathogen *Acinetobacter baumannii*, the haemophore HphA is recognized by the two-component system receptor HphR[45]. Moreover, recent findings demonstrate that haem uptake efficiency is dependent upon bacterial toxin production in *Vibrio cholerae*[46], raising the possibility that virulence factors may have a role in enhancing bacterial haem uptake in vivo.

Bacteria acquire non-haem Fe through the secretion of diverse low molecular-weight Fe-binding molecules called siderophores. To date, more than 500 distinct siderophores have been identified[47], and many pathogenic bacteria produce several types of siderophores with distinct molecular features, highlighting the importance of these molecules to access Fe within the host. Siderophores are released from the bacterial cell and bind the ferric form (Fe^{3+}) with remarkably high affinity,

often surpassing the affinity of host Fe-binding proteins including transferrin and LTF[48]. The production and utilization of siderophores give bacteria a competitive advantage in gaining Fe necessary for growth, while simultaneously impacting the host's ability to scavenge Fe for immune cells to generate reactive oxygen species (ROS)[49]. Siderophores are captured by dedicated bacterial uptake systems and release Fe within the cytoplasm or periplasm[50]. Conventional thinking has been that bacteria are uniformly Fe starved within the host. Recent work has demonstrated heterogeneous production of specific siderophores during infection, suggesting that either bacteria experience distinct Fe levels within host tissue or additional, as yet unidentified, regulatory strategies for siderophore production or secretion exist[51–56]. Additionally, the uptake of siderophores produced by other commensal microorganisms, termed xenosiderophores, are likely to contribute to Fe acquisition in bacterial pathogens in tissues with dense microbial communities such as the gastrointestinal tract or oral cavity. This is important because some bacterial pathogens, such as *Campylobacter jejuni*, are unable to produce siderophores, and rely instead on the uptake of xenosiderophores to colonize host niches[57–59]. Opportunistic pathogens may also rely on the use of host molecules such as neurotransmitters as siderophores

Biliverdin
A green pigmented by-product of haem catabolism by haem oxygenases.

Staphylobilin
A by-product of haem catabolism by IsdG family haem oxygenases.

to promote proliferation in Fe-depleted tissues[60]. To counteract bacterial siderophore-mediated Fe acquisition, the host secretes the siderophore-binding protein NGAL (also known as lipocalin 2 or siderocalin) from neutrophils and epithelial cells. The full repertoire of siderophore-binding molecules produced by the host is unknown and serves as a promising area for future research, as new host proteins are being described to target bacterial siderophores[61,62]. To evade NGAL binding of siderophores, certain bacterial species have evolved structurally unrecognizable 'stealth siderophores'. For example, *B. anthracis, Salmonella* spp. and *Klebsiella* spp. express stealth siderophores such as petrobactin, aerobactin, salmochelin and yersiniabactin.

Although most Fe is taken up by pathogens in a chelated ferric form (Fe^{3+}), bacteria encode systems that enable the uptake of ferrous Fe (Fe^{2+}). Ferrous Fe predominates in environments that are highly acidic, reducing and/or anaerobic, making Fe^{2+} uptake systems critical for bacterial colonization of host tissue. The canonical *feo* operon encodes three proteins (FeoA, FeoB and FeoC) and is the archetypal Fe^{2+} uptake system in bacteria[63,64]. FeoB is a membrane transporter containing a soluble G protein domain and is predicted to transport Fe across the membrane dependent upon GTP hydrolysis. The roles of small soluble cytoplasmic FeoA and FeoC remain uncharacterized[63]. Mutations in FeoB result in decreased colonization and virulence of several pathogens, including *Legionella pneumophila*, *S.* Typhimurium, *Helicobacter pylori*, *P. aeruginosa* and *C. jejuni*[63,65,66]. However, not all pathogens rely solely on FeoB for Fe^{2+} uptake, suggesting alternative mechanisms for ferrous Fe acquisition. In *A. baumannii*, FeoB is required for survival in human serum and mouse RAW macrophages[67], but seems to be dispensable for bacteraemia in mice[68]. Rather, the Fe^{3+} transporter, TonB3, has been implicated as critical for virulence[67–69]. Moreover in *P. aeruginosa*, a double mutant of *feoB* and TonB-dependent Fe import gene, *tonB1*, demonstrates a more attenuated virulence phenotype[66]. These findings highlight that Fe import may have host-specific and tissue-specific consequences on pathogenicity, and that other FeoB-independent mechanisms for uptake of kinetically labile ferric or ferrous Fe may have important cooperative roles in pathogenesis[70]. To date, alternative ferrous Fe import systems have been identified, including ZupT, YfeABCD, FutABC and EfeUOB[71–76]. Moreover, in the Gram-negative pathogen *L. pneumophila*, the type IV effector MavN transports intracellular Fe, Mn, cobalt and Zn in macrophage vacuoles, suggesting that intracellular pathogens also have adapted the use of secretion systems for Fe uptake[77,78].

Zn and Mn at the infection interface

The essentiality of nutrient metals extends beyond Fe to encompass other critical nutrient metals such as Zn and Mn. Zn is the second most abundant trace metal in humans and is predicted to metalate 9–10% of eukaryotic proteomes and 4–8% of bacterial proteomes[79,80]. Similarly, Mn has important roles in protein function, including in bacterial superoxide dismutase which is required to detoxify ROS during infection[81]. In certain

bacterial species, Mn can replace Fe in metalloproteins, circumventing host-imposed Fe restriction and reducing oxidative damage to proteins[24]. Below, we discuss host and bacterial mechanisms of Zn and Mn sequestration.

Host sequestration of Zn and Mn by S100 proteins. The vertebrate host uses several strategies to regulate systemic Zn levels including modulating cellular uptake and secretion of Zn by families of transporters (ZIP importers and ZnT exporters) as well as extracellular scavenging of free Zn by secreted proteins[82] (FIG. 3). The vertebrate S100 protein family contributes to the extracellular chelation of metals including Zn and Mn. S100 proteins contain an EF-hand motif and bind Ca^{2+}, and they have been implicated in diverse cellular processes including proliferation, apoptosis and energy metabolism. A unifying feature of S100 proteins is their ability to dimerize, generating high-affinity metal-binding sites at dimer interfaces. A subset of S100 proteins are released extracellularly and have roles in infection and inflammation[83,84]. The S100A8 and S100A9 heterodimer, also known as calprotectin, is involved in the immune response to bacterial pathogens. Secreted calprotectin binds to and sequesters Zn, Mn, Ni and Fe in the extracellular milieu through the action of two metal-binding sites termed site I and site II that are formed at the S100A8–S100A9 interface[20,85,86]. Site I binds Zn and Mn with high affinity as well as Fe and Ni, whereas site II binds Zn and Ni[86,87]. Calprotectin exerts antimicrobial activity in vitro that can be alleviated by the addition of exogenous metals, illustrating that calprotectin prevents bacterial replication via metal sequestration. The protein is highly expressed in immune cells including neutrophils, macrophages and dendritic cells as well as epithelial cells[20]. Calprotectin comprises approximately 50% of the protein content in neutrophils and protects against an array of bacterial pathogens including *S. aureus, A. baumannii, Clostridioides difficile, M. tuberculosis, Aspergillus fumigatus* and *Candida albicans*[20]. Apart from infection, calprotectin has demonstrated roles in modulating the composition of the commensal bacterial community in the intestine and the development of the immune system[88].

Other S100 proteins, such as S100A7 and S100A12, are expressed predominantly in non-immune cells and have antimicrobial functions. S100A7 (also known as psoriasin) is secreted as a homodimer from keratinocytes and mucosal surfaces and has a high affinity for Zn. S100A7 displays antimicrobial activities against pathogenic bacteria using both metal-dependent and metal-independent strategies[89–91]. S100A12, also known as calgranulin C, is expressed in keratinocytes, monocytes and neutrophils, and forms homodimers that bind both Zn and Cu at the dimer interface. Recombinant S100A12 exhibits Zn-dependent antimicrobial activity against *P. aeruginosa, Escherichia coli* and *C. albicans*[20]. Although host molecules sequester metals from pathogens to prevent bacterial proliferation, metal limitation can lead to beneficial adaptations in pathogens. For instance, Zn-starved *M. tuberculosis* has increased resistance to ROS and increased proliferation in vivo,

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Superoxide dismutase
An enzyme that catalyses the formation of hydrogen peroxide from superoxide.

EF-hand motif
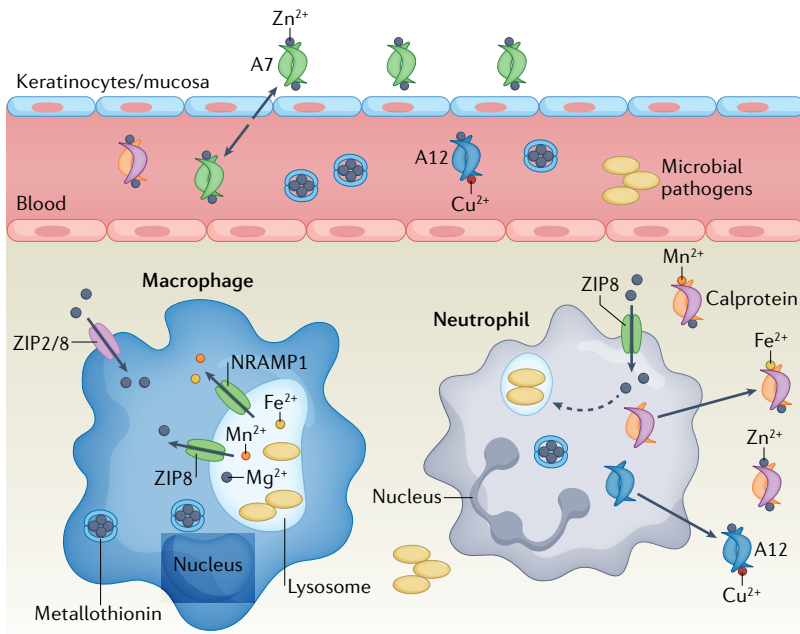A helix–loop–helix calcium binding structural domain present in S100 proteins.

Fig. 3 | **Host sequestration of Zn and Mn.** During infection, metals such as zinc (Zn) and manganese (Mn) are sequestered by host immune cells via concerted action of transporters and secreted molecules[177]. Zn is imported into innate immune cells by ZIP-family transporters, including ZIP2 and ZIP8. Within the cell and in circulation, metallothioneins bind and sequester Zn. Extracellular Zn is limited by secretion of Zn-chelating S100 proteins. S100A7 is secreted at epithelial surfaces and keratinocytes, whereas S100A12 and calprotectin (S100A8–S100A9) are secreted by innate immune cells such as neutrophils. S100A7 binds Zn, whereas S100A12 binds Zn and copper (Cu). Calprotectin functions to limit the extracellular availability of several transition metals and binds Mn, iron (Fe) and Cu intracellularly in macrophages, membrane protein resistance-associated macrophage protein 1 (NRAMP1) effluxes Mn from phagosome and ZIP8 effluxes Zn, limiting availability for pathogens. Fe^{2+}, ferrous iron.

suggesting that host Zn restriction primes bacterial cells for immune attack[92].

Bacterial acquisition of Zn and Mn. Zn and Mn uptake systems are critical for host colonization by bacterial pathogens[93–97] (FIG. 4). The mechanisms of Zn and Mn transport into the cytoplasm are well described in many pathogenic bacteria, yet the mechanisms of Zn and Mn trafficking across the Gram-negative outer membrane remain largely unknown. It was previously thought that Zn and Mn passively diffuse through non-selective porins in the outer membrane, yet evidence now suggests that specific transporters exist to traffic these metals such as the Mn-specific transporter MnoP identified in *Bradyrhizobium japonicum*[98], highlighting selectivity of membranes to trace metals.

Unlike the passive transport of Mn across the membrane through MnoP, transport of Zn is an energy-dependent process mediated by the TonB–ExbB–ExbD systems in Gram-negative bacteria. Gram-negative pathogenic bacteria, including *Neisseriaceae*, *Acinetobacteriaceae*, *Bordetellaceae* and *Moraxellaceae*, use the TonB-dependent Zn transporter ZnuD for Zn uptake, with some strains encoding multiple copies[99,100]. Structural studies have illustrated that ZnuD takes up free Zn but do not exclude the possibility that it can transport chelated Zn. Zn acquisition facilitated by

ZnuD has been implicated in bacterial fitness by protecting against neutrophil killing and oxidative stress as well as Zn restriction from calprotectin[101]. ZnuD was initially mis-annotated as a haem uptake transporter as it shares structural homology with the haem uptake transporters HasR, ShuA and HmbR[102], but does not bind haem[101]. Expression of *znuD* is regulated by both Fur and Zur, suggesting that increased levels of Zn may be necessary under conditions of Fe starvation as endogenous haem biosynthetic enzymes require Zn[102].

Zn and Mn ions are transported through the inner membrane of Gram-negative bacteria and the cytoplasmic membrane of Gram-positive bacteria primarily by NRAMP-family transporters and ATP-binding cassette (ABC) importers (FIG. 4). NRAMP transporters are found across all kingdoms of life and are designated MntH in bacteria. ABC transporters have a cytosolic dimeric ATPase, a membrane-spanning dimeric permease and a monomeric substrate-binding protein (SBP). Notable Mn-specific ABC-type transporter systems include PsaABC of *S. pneumoniae* and the MntABC (SitABC) of *S. aureus*, whereas some FeoB orthologues can also transport Mn[103]. The widely conserved ZnuABC system encodes the high-affinity Zn uptake ABC transport system. Uptake of Zn and Mn by ABC importers is analogous to the uptake of haem and siderophores described above. Specialized proteins, such as ZinT or polyhistidine triad (Pht) proteins, aid in the capture of Zn by the ZnuABC system in numerous pathogenic bacteria such as *E. coli* and *S. enterica*[104–108]. Alternatively, some bacterial pathogens express the low-affinity Zn transporter ZupT[109–111] or acquire Zn through the action of inner membrane transporters such as ZevAB and ZurAM[112,113].

Bacterial pathogens can also use secreted small molecules to sequester Zn. Recent reports provide evidence of production and secretion of Zn-binding small molecules analogous to Fe siderophores as well as the assignment of Zn-binding properties to established siderophores, suggesting that some siderophores may be more accurately referred to as 'metallophores'. Bacterial Fe-chelating siderophores are well described, yet the specificity of siderophores to differing metals has not been studied in detail, leaving open the possibility that siderophores may function more broadly in scavenging trace metals. Specifically, siderophores including pyridine-2,6-bis (thiocarboxylic acid) (PDTC), pyochelin, micacocidin and yersiniabactin bind Zn with high affinity[114–120]. In fact, scavenging of Zn by yersiniabactin promotes *Enterobactericae* colonization in the inflamed gut, illustrating the importance of siderophore-mediated Zn acquisition in host colonization[121]. In addition to Zn-binding siderophores, secreted small molecules, termed 'zincophores', have been identified from numerous pathogenic bacteria. *Streptomyces coelicolor* produces a siderophore-like molecule, coelibactin, that is likely to function as a zincophore[122]. *S. aureus* produces and secretes staphylopine with broad metal chelating abilities[123,124], and *P. aeruginosa* encodes Zn-binding pseudopaline. The eukaryotic fungal pathogen *C. albicans* secretes a Zn-scavenging molecule, Pra1 (REF.[125]), and, finally, *Pseudomonas putida* produces the small Zn-binding siderophore PDTC[126,127]. Additionally, some

bacterial pathogens can use host Zn-sequestering molecules such as calprotectin and S100A7 to scavenge Zn[128–130]. The outer membrane receptor CpbA in *N. meningitidis* is expressed during Zn starvation[131] and capable of binding human calprotectin[128]. Homologues of CpbA

have been identified in *Neisseria gonorrhoeae*, suggesting that these Zn piracy mechanisms may be broadly conserved[128,130].

Some bacteria encode more nuanced strategies to acquire Zn. Type VI secretion systems (T6SSs) are multiprotein complexes that mediate the transfer of effector proteins into neighbouring cells in Gram-negative bacteria to facilitate host–bacteria and inter-bacterial interactions. Recent studies have illustrated that T6SSs can be co-opted for Zn and Mn acquisition in *Yersinia pseudotuberculosis* and *Burkholderia thailandensis*. The oxidative stress regulator, OxyR, and ZntR induce expression of T6SS4 in *Y. pseudotuberculosis*, thereby leading to the excretion of a Zn-binding molecule YezP that aids in Zn uptake[132,133]. Similarly, *B. thailandensis* secretes a Zn-binding protein, TseZ, via T6SS4 which is imported via the haem transporter HmuR to metallate CuZn superoxide dismutase enzymes. *B. thailandensis* also expresses a Mn-specific TonB receptor, MnoT, and a Mn-binding secreted protein, TseM[134]. Collectively these findings highlight the evolution of T6SSs in metal acquisition.

The assembly of T6SSs requires reorganization of the bacterial cell wall, highlighting the importance of cell envelope modifications during conditions of metal limitation[135]. To this end, in *A. baumannii* cell wall modifications through Zur-dependent expression of the endopeptidase *zrlA*, as well as increased outer membrane vesicle formation, have been noted in conditions of Zn deficiency[135–137]. Notably, cell wall reorganization is not specific to Zn but is also observed in conditions of Fe starvation, as is seen for IsdA and IsdB membrane localization to acquire haem–Fe in *S. aureus*[138]. These findings suggest that cell wall reorganization is necessary for bacterial fitness in metal-restricted host environments.

Once nutrient metals have entered the cell, specialized proteins, deemed metallochaperones, mediate the transfer of cognate metals to client metalloenzymes. G3E family P-loop GTPases are candidate metallochaperones for metals including Ni and Zn, and have demonstrated roles in bacterial metal homeostasis and virulence[139–141]. In bacteria, COG0523 proteins such as YeiR, ZigA and ZagA bind to Zn and hydrolyse GTP[141–143]. Bacterial COG0523 proteins are necessary to respond to conditions of Zn limitation, similar to that experienced in the host environment. In *A. baumannii*, ZigA facilitates the mobilization of Zn from histidine pools and is required for full bacterial virulence in a mouse model of pneumonia[141]. Despite these findings, metallochaperone client proteins and their impact on trafficking trace metals to bacterial enzymes during infection remains largely unknown.

Host-imposed metal intoxication

Nutritional immunity classically refers to host restriction of nutrient metals; however, vertebrates also use the toxic properties of metals to limit bacterial infection (FIG. 5). Although not discussed at length below, several metals including Mn and host metal-containing molecules such as haem can be detrimental to bacterial survival at high concentrations[31,144]. In *S. pneumoniae*, a riboswitch senses Mn levels and prevents toxicity of excess Mn
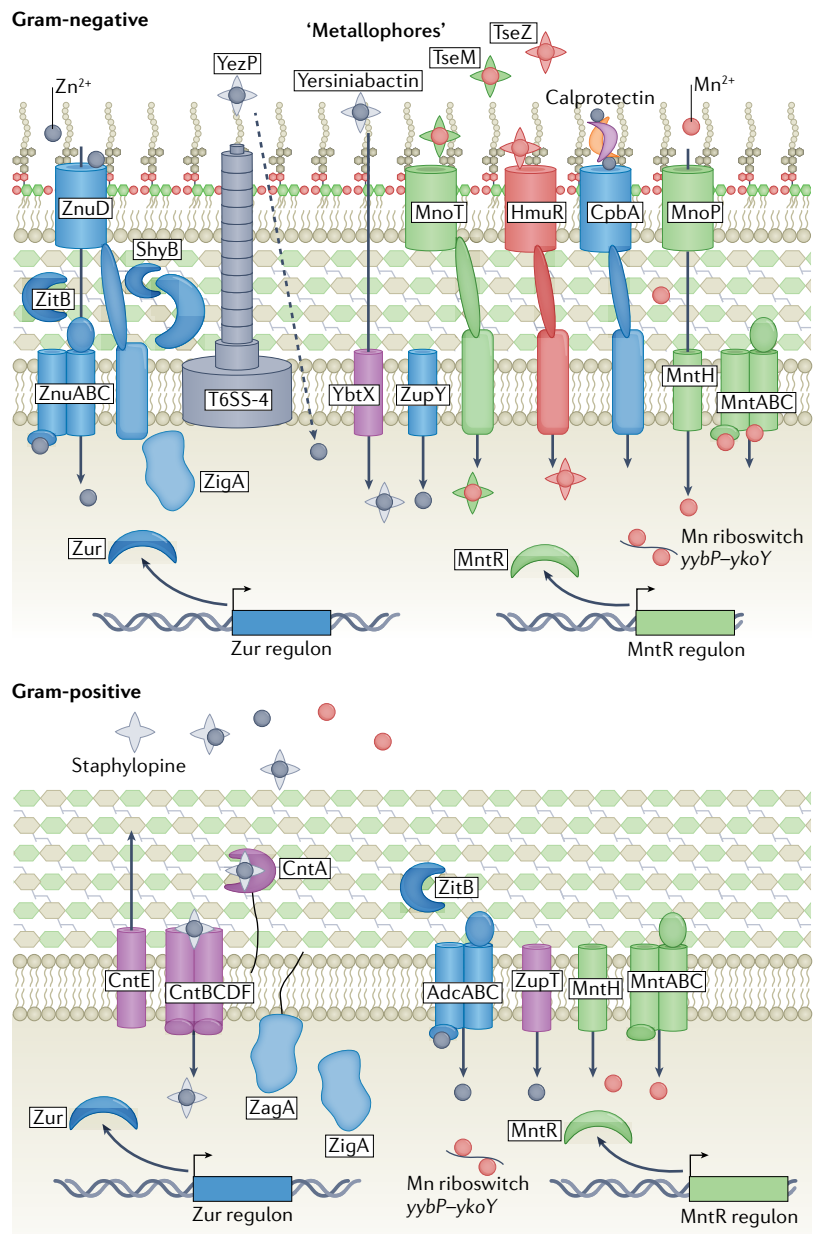

Fig. 4 | Bacterial acquisition of Zn and Mn. Pathogenic bacteria acquire zinc (Zn) using ATP-binding cassette (ABC) transporters ZnuABC (Gram-negative bacteria) and AdcABC (Gram-positive bacteria). Zn is transported through outer membrane using TonB-dependent transporter ZnuD in Gram-negative bacteria and ZupT in Gram-positive bacteria. Additional Zn acquisition systems include type 6 secretion system (T6SS)-secreted zincophore protein YezP in *Yersinia pseudotuberculosis*, transport of yersiniabactin–Zn in *Yersinia pestis* by TonB-dependent transporter MnoT, TseZ and TseM in *Burkholderia thailandensis* and outer membrane receptor CbpA that binds calprotectin in *Neisseria meningitidis*. Manganese (Mn) is taken up by outer membrane pore MnoP in Gram-negative bacteria and imported into cytosol by MntABC or MntH transporters. In Gram-positive bacteria, Mn is transported across cell membrane by MntH or MntABC transporters. The *yybP–ykoY* riboswitch family binds Mn with high affinity and modulates Mn homeostasis in bacterial pathogens including *Neisseria* and *Streptococcus* spp.

through expression of a Mn exporter[145]. Bacterial pathogens including the enteric pathogens *C. difficile* and *Enterococcus faecalis* are sensitive to increased haem levels in the gut and encode systems to export haem under these conditions[146–148]. Perhaps the most well-known examples of metal intoxication include host-induced excess of Cu and Zn by specific cell types, most notably immune cells, and will be further discussed below.

Cu toxicity in bacteria. Cu is an essential metal that is the most reactive metal in the Irving William series. Cu functions as a cofactor for enzymes that function in oxidative phosphorylation, pigmentation, superoxide dismutation

and Fe homeostasis. Notably, Cu can transition from two oxidation states Cu^+ and Cu^{2+}, which readily interact with biological ligands and therefore have important roles in redox reactions. Due to these properties, increased levels of Cu have potent antimicrobial activity against bacterial pathogens. Within macrophages, Cu accumulates in the phagolysosome of immune cells to combat infection by a defined pathway[149] (FIG. 5). Although the mechanisms of Cu toxicity in bacteria are incompletely understood, it is appreciated that excess Cu disrupts protein maturation and functions by disrupting Fe–S clusters, while also perturbing proper protein folding by catalysing the formation of non-native disulfide bonds[150].
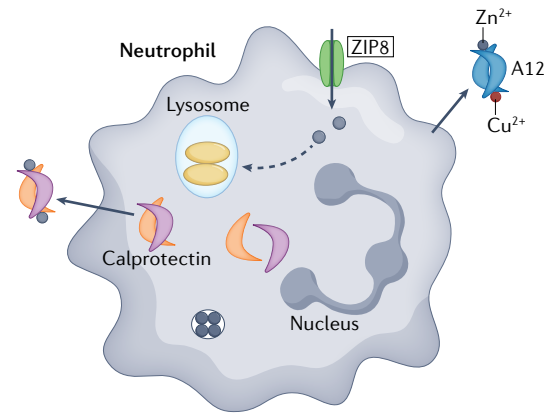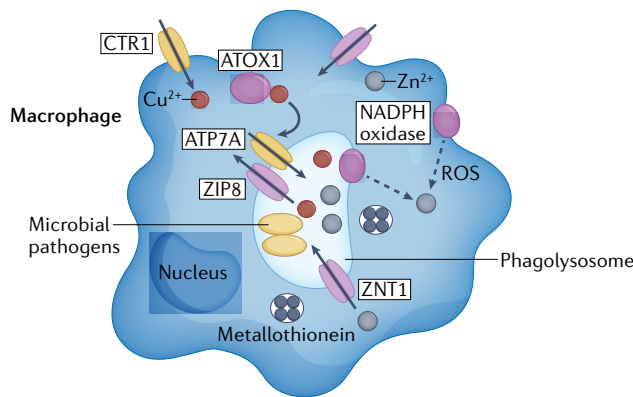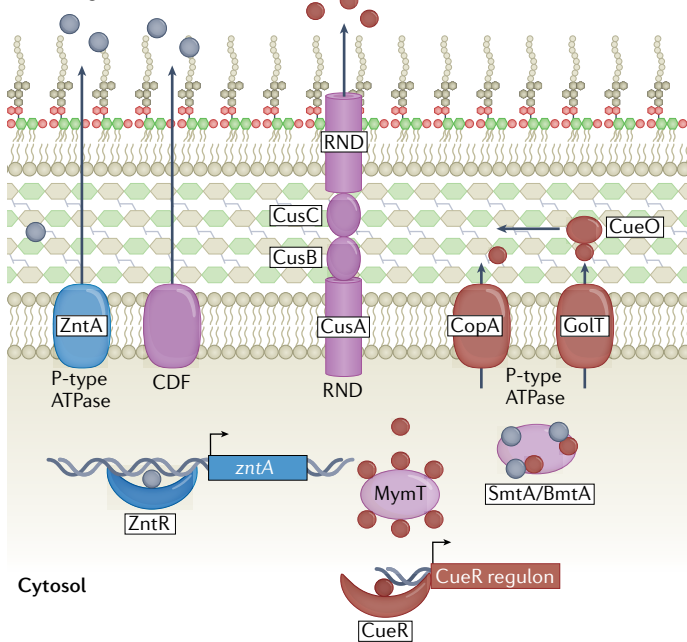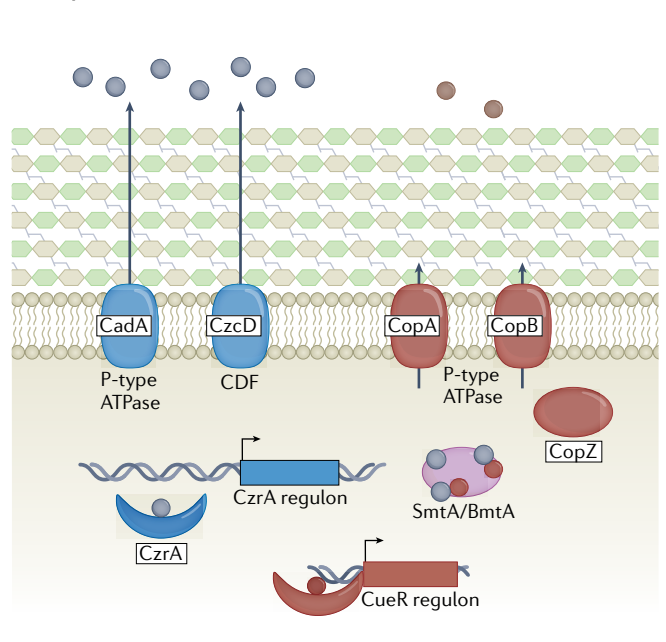

Fig. 5 | **Metal intoxication.** Metal intoxication is used by vertebrate hosts to combat bacterial proliferation. Following infection, innate immune cells accumulate zinc (Zn) in cytoplasm through ZIP8-mediated import and into phagolysosome via ZNT1. Zn accumulation induces generation of reactive oxygen species (ROS) by NADPH oxidase and NADPH oxidase may liberate Zn from host metallothionein. Copper (Cu) is imported into cytosol of phagocytic cells, including macrophages, by transporter CTR1. Cu is subsequently shuttled by ATOX1 to phagolysosomal membrane, where it is then transported into phagolysosome by ATP7A. Bacteria have evolved diverse mechanisms to withstand Zn and Cu toxicity, including efflux by cation diffusion facilitators (CDF), RND and P-type family ATPase transporters. Zn exporters ZntA, CadA and CzcD alleviate Zn toxicity in pathogenic bacteria. CopA and GolT export excess cytosolic Cu to prevent accumulation and reduce cellular redox stress. Bacterial metallothioneins including MymT in *Mycobacteria* and SmtA or BmtA bind and sequester cytosolic Cu (MymT, SmtA or BmtA) and Zn (SmtA or BmtA). Zn levels in cytosol are sensed by transcriptional regulator ZntR (Gram-negative bacteria) or CzrA (Gram-positive bacteria). Cytosolic levels of Cu are maintained at very low concentration and are typically regulated through transcriptional regulators including CueR. In *Escherichia coli*, periplasmic copper oxidase CueO is used to detoxify Cu.

Additionally, during protein maturation, Cu can mis-metalate metalloproteins due to its strong affinity for thiol ligands. To counteract mis-metalation, bacteria can induce the expression of metallochaperones that have higher specificity for cognate partners such as SufA in *E. coli*[151–153]. Cu excess can also result in several membrane and cell surface disruptions including defects in lipoprotein and peptidoglycan maturation. Notably, Cu accumulation can generate ROS in vitro under aerobic conditions through the Haber–Weiss reaction and Fenton-like chemistry[154], yet the contribution of Cu to generating ROS in vivo still remains unclear.

To avoid the toxicity of Cu, pathogenic bacteria have evolved mechanisms of Cu handling and detoxification to maintain a cytosolic environment free of Cu. The periplasm of Gram-negative bacteria contains the most numerous and diverse Cu-dependent enzymes, and thus is most at risk for Cu toxicity. To combat this pressure, bacteria have evolved multi-copper oxidases whose function is not completely understood. In *E. coli*, the multi-copper oxidase CueO is thought to convert Cu^+ to the less toxic Cu^{2+} and oxidize the precursor of enterobactin, thereby preventing the generation of toxic cuprous ions[155,156]. One means of relieving Cu excess is through Cu export. Cu is primarily exported from bacteria via P-type family ATPases, examples of which include CopA of *E. coli*, CopA1 and CopA2 of *P. aeruginosa*, CopA, CopB, CopL and CopZ of *S. aureus*, and CopA and GolT of *S. Typhimurium*[157–159]. Cu is delivered to these ATP-dependent pumps via soluble or more recently discovered membrane-bound Cu metallochaperones[160]. Another example of Cu exporters found in bacteria is the *E. coli* CusABC complex. Other mechanisms for preventing Cu toxicity includes Cu binding to cysteine-rich metallothioneins, such as MymT in *Mycobacteriaceae*[161] and CusF in *E. coli*[162]. Moreover, recent evidence demonstrates Cu^{2+} binding by Fe-scavenging siderophores, such as yersiniabactin[163]. This prevents Cu toxicity by restricting the formation of Cu^+ and protecting *E. coli* from ROS in macrophages[117]. In *M. tuberculosis*, production of the VapBC4 toxin activates stress survival pathways, which increases bacterial Cu resistance in macrophages[164], shedding light on the importance of toxin production on bacterial resistance to metal intoxication. The critical role of Cu detoxification strategies in bacterial pathogens is underscored by the fact that bacteria harbouring mutations in key Cu tolerance genes often display decreased virulence in an animal host and increased killing by host immune cells[165].

Zn toxicity in immune cells. Although Zn sequestration has demonstrated roles in host defence against bacterial infection, growing evidence supports a model whereby host-imposed Zn excess enhances intracellular bacterial killing within immune cells. Loss of Zn detoxification machinery in several bacterial pathogens, including Group A and B streptococci[166], *M. tuberculosis* and *S. pneumoniae*, results in decreased intracellular survival[167–170] or in vivo survival in mouse models of infection[169]. Zn levels increase in macrophages infected with mycobacteria[171], and Zn specifically localizes to phagosomes containing internalized *M. tuberculosis*

in human macrophages[167]. However, the molecular mechanisms by which Zn is trafficked to phagosomes within macrophages remain largely unexplored. It is speculated that Zn may be released from cytosolic Zn–metallothionein complexes by NADPH phagocyte oxidase prior to transport into endocytic compartments by an SLC30-family transporter or fusion with Zn-containing vesicles (zincosomes)[167]. Recent evidence suggests that Zn transporters such as SLC30a1 partially mediate intracellular Zn transfer in a lipopolysaccharide (LPS)-dependent manner in macrophages[172]. Zn accumulation has also been observed in neutrophil lysosomes and azurophilic granules in response to Group A *Streptococcus* (GAS) infection[169], and exposure of GAS to human neutrophils leads to an upregulation of Zn-efflux genes[170]. Interestingly, neutrophil expression of ZnT exporters remained unchanged in these conditions, suggesting that Zn-loaded granules fuse with the phagosome containing bacteria.

The mechanisms of Zn toxicity are not completely understood. Zn is a highly competitive metal for protein binding but is redox-inert due to a stably filled $3d$ orbital[173,174]. The focus of Zn toxicity has been placed on the ability of Zn to mis-metalate proteins, thereby disrupting enzymatic activity. Proteins such as topoisomerase I bind Fe and Zn, and evidence indicates that excess Zn can compete for binding for Fe in vivo[169], which raises the possibility that Zn and Fe may have similar binding sites in critical enzymes. Excess Zn inhibits glycolytic enzymes and phosphoglucomutase in GAS, resulting in attenuated growth and decreased capsule formation[175]. Furthermore, Zn excess in *E. coli* disrupts Fe–S clusters in dehydratases through inhibition of Fe–S cluster assembly proteins such as IscU, IscA and ferredoxin[176]. Due to the role of Fe–S cluster proteins in diverse cellular pathways ranging from DNA replication to energy metabolism, Zn toxicity through impairment of Fe–S protein biogenesis would have broad impacts on the cell, but these remain to be explored.

Intracellular levels of Zn are sensed by transcriptional regulators, and excess Zn is exported via the activity of P-type family ATPases, RND-family transporters and/or cation diffusion facilitators (CDF)[177]. In *E. coli*, the metalloregulator ZntR facilitates the response to Zn excess. In Gram-positive species such as *Bacillus subtilis*, the transporter CzcD is upregulated by CzrA to export Zn in conditions of Zn intoxication[177]. Additionally, Zn buffering in the cytosol is mediated by metallothioneins, histidine and low molecular-weight thiols[174]. *S.* Typhimurium subverts host Zn intoxication in phagocytes by evading Zn-containing vesicles through an unknown mechanism that is likely to be associated with effector molecules encoded from *Salmonella* pathogenicity island 1 (REF.[178]). Collectively, these strategies ensure that intracellular Zn abundance is maintained at appropriate levels to facilitate bacterial colonization and proliferation.

Nutritional immunity-based therapeutics

Due to the emergence of multidrug-resistant bacterial pathogens, the discovery of novel therapeutic strategies targeting bacterial pathogens is an urgent area of research (FIG. 6). Numerous drugs have been developed to

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P-type family ATPases
A class of autocatalytic ATP-hydrolysing transporters found across all kingdoms of life.

Metallothioneins
Low molecular-weight cysteine-rich proteins that bind metals such as zinc (Zn), copper (Cu) and cadmium.

Phosphoglucomutase
An enzyme that catalyses the transfer of phosphate between the C1 and C6 positions on glucose monomers.

RND-family transporters
Gram-negative bacterial efflux transporters that span the inner and outer membrane and use the proton gradient to move substrates from the cytosol to the extracellular space.
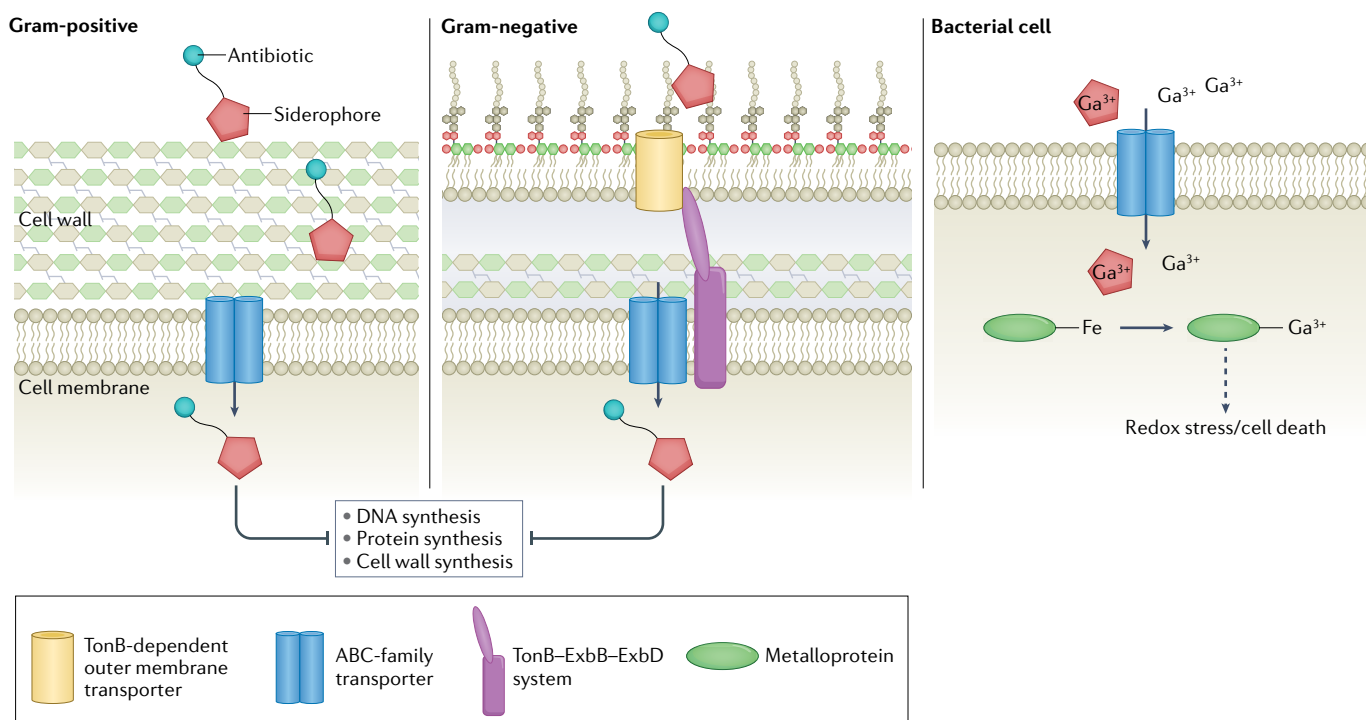
Fig. 6 | **Therapeutic interventions harnessing nutritional immunity.** Targeting of bacterial metal uptake systems through use of 'Trojan horse' antimicrobials presents a rapidly growing area of therapeutic development against infection. Conjugation of siderophores to antibiotics or alternative metals, such as gallium (Ga^{3+}), ensures uptake by bacterial metal transport systems. Once internalized, antibiotics prevent DNA, protein or cell wall synthesis. Gallium-conjugated siderophores result in iron (Fe) starvation of bacteria and uptake of free gallium results in mis-metalation of Fe–metalloproteins within the cell leading to redox stress. ABC, ATP-binding cassette.

target siderophore uptake systems to treat drug-resistant strains[179]. Another therapeutic strategy involves the use of non-ferrous Fe to target bacterial pathogens. Gallium (Ga^{3+}) can compete with ferric Fe for siderophore binding and can further arrest Fe-dependent cellular metabolic processes. Treatment of *S. aureus* and *A. baumannii* with Ga^{3+} results in antimicrobial activity in vivo and in vitro, potentially via binding to secreted siderophores or competing with Fe in important proteins, which leads to redox stress and cell death[180]. Ga^{3+}-conjugated siderophores, including Desferal (DFO) and pyochelin, are taken in by bacterial cells, thereby preventing uptake of Fe-conjugated siderophores and starving bacterial cells of Fe[181]. Siderophore-mimicking antibiotics have gained traction with the US Food and Drug Administration (FDA) approval of cefiderocol (Fetroja)[179], which exploits the natural bacterial siderophore uptake systems to increase efficacy of the antibiotic, signifying the feasibility of targeting metal uptake systems in bacteria to use as human therapies. The FDA approval and clinical use of cefiderocol represent a landmark event in microbiology as an example of how basic research into microbial metal metabolism can lead to the development of new therapeutic strategies to combat bacterial pathogens. Recent work has attempted to harness the physical and magnetic properties of metal to treat antibiotic-resistant bacterial biofilms. Biofilms treated with magneto-responsive gallium-based liquid metal droplets and exposed to a low-intensity rotating magnetic field resulted in rupturing of bacterial cells and biofilm matrix[182].

Due to the importance of the metal uptake machinery for bacterial growth in the host environment, metal transporters and trafficking proteins are promising drug targets for novel therapeutic development. Metal receptors are some of the most abundant proteins on the bacterial cell surface during infection, further adding strength to their utility as vaccine candidates and drug targets. Examples include screening strategies to identify small-molecule inhibitors of FeoB[183–185], or antibodies that target IsdA and IsdB and prevent *S. aureus* from binding to haem, which has led to protection in mouse models of infection[186,187]. Similarly, ZnuD is a promising target for vaccine development in several bacterial pathogens including *A. baumannii* and *N. meningitidis*[188,189]. However, these transporter proteins are often not homogeneously expressed in bacterial populations, which may present technical challenges in targeting these proteins. An alternative strategy to circumvent this issue is to manipulate levels of host proteins that bind nutrient metals with high affinity, such as transferrin, to starve invading bacteria of key resources[190,191]. Collectively, these avenues of new therapeutic development targeting metal homeostasis at the host–pathogen interface pose promising advances to improve human health by harnessing nutritional immunity.

Conclusions and future perspectives

Nutritional immunity shapes host–pathogen interactions, and the specific impacts of metal limitation on bacterial processes are becoming increasingly

appreciated. Mounting evidence suggests a much larger arsenal of host and bacterial factors involved in metal sequestration. Identification of additional host proteins beyond the S100 proteins and NGAL as well as identification of bacterial non-Fe-binding metallophores would greatly expand the field of bacterial pathogenesis and nutritional immunity. Our understanding of nutritional immunity and the ever-evolving battle for nutrient metals at the host–pathogen interface will vastly expand our knowledge of bacterial metal metabolism and provide novel therapeutic targets for future drug development. Host-imposed metal starvation prevents population of critical bacterial enzymes with necessary co-factors, thereby hindering bacterial growth. We speculate that metallochaperones such as ZigA in organisms such as *A. baumannii* and *S. aureus* have important roles during bacterial pathogenesis, potentially shuttling trace metals to essential client metalloenzymes required for host colonization and persistence[139–141]. The kinetics of metal allocation and the transcriptional and translational response to altered metal levels in bacterial pathogens are fruitful areas of study that will inform how pathogens prioritize and distribute metals to facilitate infection. To this end, sequential Fur-regulated and Zur-regulated responses to metal deprivation have been studied extensively in *B. subtilis*[192], and have revealed Fe-sparing responses and the molecular mechanisms of Zn mobilization during nutrient starvation[193].

Regulation of cellular metal concentrations is a delicate balance in both host and bacterial systems. Opposing strategies of both limitation and intoxication are used by vertebrates to control bacterial proliferation in host tissue environments. Fluctuations in relative metal availabilities encountered by bacteria in host tissues present a challenge in properly metalating metalloproteins. Moreover, the redox properties of metals, including Cu and Fe, shape host immune responses to infection by promoting the production of oxidants. Although methods of coordinating seemingly contradicting processes to mediate bacterial infection remain incompletely understood, cellular compartmentalization is emerging as a prominent factor in dictating metal-mediating killing. Toxicity of metals, such as Zn and Cu, is often restricted to phagosomes as intracellular killing strategies. This is likely to have evolved to protect host tissues while harnessing the toxic properties of metals to specifically target invading microorganisms. Metal starvation commonly occurs in routes of entry and systemic transfer such at mucosal barriers and in the blood, respectively.

Importantly, limitation of non-metal nutrients also has a critical role in restricting bacterial pathogenicity by stressing the metabolic needs of bacterial pathogens in host tissue environments. Numerous pathogens including *S. aureus*, enteropathogenic *E. coli* and *M. tuberculosis* rely heavily on host amino acids and fatty acids as essential nutrients to establish infection[194–197]. Recent studies identify the presence of metal storage organelles within bacteria, which could serve both as a means to prevent metal toxicity as well as to provide metals in conditions of nutrient starvation[198]. Such systems may be strategies to balance metal excess and limitation in bacterial pathogens. Further research is necessary to understand the intersection between metal and non-metal nutrient limitation in preventing bacterial infections and how these processes can collectively be harnessed to develop novel antimicrobial therapies.

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