The Iron age of host–microbe interactions

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Abstract

Microbes exert a major impact on human health and disease by either promoting or disrupting homeostasis, in the latter instance leading to the development of infectious diseases. Such disparate outcomes are driven by the ever-evolving genetic diversity of microbes and the countervailing host responses that minimize their pathogenic impact. Host defense strategies that limit microbial pathogenicity include resistance mechanisms that exert a negative impact on microbes, and disease tolerance mechanisms that sustain host homeostasis without interfering directly with microbes. While genetically distinct, these host defense strategies are functionally integrated, via mechanisms that remain incompletely defined. Here, we explore the general principles via which host adaptive responses regulating iron (Fe) metabolism impact on resistance and disease tolerance to infection.

Keywords anemia of chronic disease; disease tolerance; heme; iron; macrophage; nutritional immunity; tissue damage control

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See the Glossary for abbreviations used in this article.

Introduction

The pathogenic outcome of host–microbe interactions is countered by a number of evolutionarily conserved host defense strategies [1]. These include avoidance, a behavioral-based strategy that limits exposure of the host to potentially pathogenic microbes [2]. Physical barriers in the form of different epithelia provide an additional defense strategy that prevents microbes from gaining systemic host access [3]. In the event of an infection, when microbes become systemic, immune-driven resistance mechanisms exert a negative impact on microbes that limits their pathogenicity. Resistance to infection relies, in most cases, on the expression of cytotoxic molecules that target pathogens. However, there are also resistance mechanisms that rely on the expression of molecules that while not cytotoxic *per se* prevent pathogens from accessing metabolites and/or nutrients that are essential for their survival and/or proliferation. This defense strategy, termed nutritional immunity [4], encompasses

mechanisms that restrict microbes from accessing iron (Fe) [5–7] as well as other trace metals such as zinc, manganese, or copper [8,9]. That nutritional immunity is a central defense strategy against infection is supported by the realization that several “infection resistance genes” act via a mechanism controlling host Fe metabolism.

While essential to confer host protection against infection, some resistance mechanisms, including those involved in nutritional immunity, can compromise host homeostasis [10]. This trade-off is countered by an additional host defense strategy termed as tissue damage control [1,11], which confers disease tolerance to infection [1,12,13]. The term disease tolerance, as used herein, refers to the same phenomenon identified originally in host–microbe interactions in plants [14]. It limits the impact of infection on host integrity and fitness without interfering with the host’s pathogen burden [14]. This is distinct from immunological tolerance [15], and while the two may be functionally linked, they act via distinct mechanisms.

Here, we explore how adaptive responses regulating host Fe metabolism impact on resistance and disease tolerance to infection. We will put forward the notion that pathogen class-specific mechanisms regulating Fe metabolism evolved to confer protection against intracellular versus extracellular pathogens. Other recent reviews focusing on related aspects of Fe metabolism, in the context of infection, are cited throughout the manuscript [6,7,16–18].

Regulation of Fe metabolism during infection

Host–microbe interactions evolved in a manner that is often linked to the emergence of host adaptive responses that regulate Fe metabolism and impact on Fe availability for microbes [5]. These adaptive responses carry a trade-off such that modulation of Fe metabolism can only occur within narrow limits, compatible with host homeostasis [10]. The general principle being that adaptive responses supplying Fe to microbes increase, in most cases, their pathogenicity while those withholding Fe from microbes limit their virulence. In support of this notion, host Fe overload is associated with poor clinical outcomes in a number of infectious diseases such as AIDS (i.e., co-infections resulting from HIV-driven immunodeficiency), malaria, and tuberculosis [19–22], while dietary Fe supplementation exacerbates the overall rate of mortality in areas endemic for such infectious diseases [23–27]. Of note, systemic Fe chelation yields inconsistent clinical outcomes, as illustrated for malaria or invasive fungal infections [20,28], reflecting the complex interplay

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Glossary

AIDS	acquired immunodeficiency syndrome	NADPH	nicotinamide adenine dinucleotide phosphate
ALA	δ -aminolevulinic acid	NALP3	NACHT, LRR, and PYD domains-containing protein 3
ALAS1/2	δ -aminolevulinic synthase	NETs	neutrophil extracellular traps
CO	carbon monoxide	NF-IL6	nuclear factor for IL-6 expression
CPOX	coproporphyrinogen oxidase	NFκB	nuclear factor κ B
2,5-DHBA	2,5-dihydroxy-benzoic acid	NK cells	natural killer cells
DMT1	divalent metal transporter-1	NOX2	NADPH oxidase
Fe	iron	NO	nitric oxide
FECH	ferrochelatase	iNOS/NOS2	nitric oxide synthase
FTH	ferritin H (heavy/heart) chain	NRAMP1	natural resistance-associated M ϕ protein 1
FTL	ferritin L (light/liver) chain	Nrf2	nuclear factor erythroid 2-related factor 2
FPN	ferroportin-1	ONNO⁻	peroxynitrate
Epo	erythropoietin	PDGF-BB	platelet-derived growth factor subunit B
Erfe	erythroferrone	PBBS	catalyst porphobilinogen synthase
G6PD	glucose 6 phosphate dehydrogenase	PGBS	catalyst porphobilinogen synthase
GDF15	growth differentiation factor 15	PMN cells	polymorphonuclear cells
HAMP	hepcidin gene (hepcidin antimicrobial peptide)	PPIX	protoporphyrin IX
HFE	hemochromatosis protein gene	PPOX	protoporphyrin oxidase
HIF1α	hypoxia-inducible factor 1 α	PRR	pattern recognition receptor
HIV	human immunodeficiency virus	RBC	red blood cell
HMBS	hydroxymethylbilane synthase	ROS	reactive oxygen species
HO-1	heme oxygenase-1	Rbx1	RING-box protein 1
HP	haptoglobin	RING	really interesting new gene
HPX	hemopexin	RIPK1	receptor-interacting serine/threonine kinases 1
HRG1	heme-responsive gene-1	RNS	reactive nitrogen species
IL	interleukin	STAT	signal transducers and activators of transcription
IFNγ	interferon γ	TCR	T-cell receptor
IRP	Fe regulatory protein	Tf	transferrin
IRE	Fe-responsive element	Tfr	ferritin and transferrin receptor
JNK	c-Jun N-terminal kinase	T_H1	T helper type 1 cells
Keap1	Kelch-like ECH-associated protein 1	T_H2	T helper type 2 cells
Lcn2	lipocalin	TLR	Toll-like receptor
LPS	lipopolysaccharide	TNF	tumor necrosis factor
MHC	major histocompatibility complex	UROD	uroporphyrinogen III decarboxylase
Mϕ	monocytes/macrophages	UROS	uroporphyrinogen III co-synthase

between host Fe metabolism and the pathogenesis of infectious diseases.

Microbes evolved strategies to acquire Fe from their hosts [5–7,17]. These share as a common denominator the expression of siderophores that elute Fe from host Fe-binding proteins [29,30] as well as other Fe uptake systems [6,31]. Of note, deletion of genes regulating the expression of siderophores or other Fe uptake systems is often associated with reduced pathogen virulence, as illustrated for bacteria, for example, *Staphylococci* and *Salmonella*, or fungi, for example, *Aspergillus*, infections [6,7,32,33]. Along this line, polymorphisms in host genes encoding Fe-binding proteins that counter microbial siderophores or other microbial Fe uptake systems enhance resistance to bacterial infections [34].

The majority of bioavailable Fe in mammals exists in the form of heme (Fig 1). It is therefore reasonable to assume that microbes evolved strategies to extract Fe from this tetrapyrrole ring structure [7,30]. Examples are provided by Gram-positive bacteria such as *Staphylococcus aureus*, which expresses a specific hemoglobin receptor (IsdB), promoting heme extraction from hemoglobin [35]. This is also the case for Gram-negative bacteria, such as *Escherichia (E.) coli* that express evolutionary conserved heme receptors, which bind and promote heme degradation [36]. The same may hold true for protozoan parasites such as *Plasmodium*,

which can incorporate host heme into their own metabolic pathways [37].

Host heme metabolism is controlled by several genes [38], including some that might restrict Fe access to microbes. This is probably the case for haptoglobin (HP) and hemopexin (HPX), two acute-phase proteins that scavenge extracellular hemoglobin and labile heme in plasma, respectively [39]. Moreover, heme catabolism by heme oxygenase-1 (HO-1), a stress-responsive enzyme that converts labile heme into equimolar amounts of Fe, carbon monoxide (CO), and biliverdin [40], should also contribute to deprive microbes from accessing Fe contained in heme. That these genes modulate the pathogenesis of infectious diseases in humans is supported by the association of polymorphisms in the human *HP* genes with an unfavorable course of co-infections resulting from HIV-driven immunodeficiency [41], malaria [42], or tuberculosis [43]. This also holds true for polymorphisms in the human *HMOX1* gene coding for HO-1, as illustrated for HIV infection [44] and malaria [45,46]. More recently, an association between plasma levels of HO-1 and active versus latent or treated pulmonary tuberculosis has been established [47]. This suggests that host adaptive responses regulating heme metabolism and depriving microbes from accessing this source of Fe can exert a major impact on the outcome of infectious diseases.

Figure 1. Interrelationship of Fe and Heme metabolism.

More than 80% of the bioavailable Fe in mammals exists in the form of heme contained in hemoproteins [241]. The most abundant pool of Fe in mammals are the prosthetic heme groups of hemoglobin in red blood cells (RBC), followed by the heme groups of myoglobin in muscle cells and those of cytochromes and other ubiquitously expressed hemoproteins in all cells [38,54]. The Fe required to sustain hemoglobin synthesis is made available by hemophagocytic Mø as these clear senescent RBC by erythrophagocytosis (top right) [49]. The heme contained in hemoglobin is transported by heme-responsive gene-1 (HRG1) into the cytoplasm where Fe is extracted by heme-oxygenase-1 (HO-1), exported via ferroportin (FPN), and delivered to transferrin (Tf) in plasma. Surplus cytoplasmic Fe in Mø is incorporated and stored within ferritin. Tf transports and provides Fe to the erythropoietic compartment via TfR, where it is used for heme synthesis (bottom right). Heme synthesis occurs via eight successive enzymatic reactions that take place back and forward in the mitochondria (brown) and the cytosol (blue). For details, see Box 1.

Regulation of Fe metabolism by macrophages during infection

Monocytes/macrophages (Mø) play a central role in the maintenance of Fe homeostasis, delivering approximately 95% of the Fe required to support *de novo* heme/hemoglobin synthesis during erythropoiesis [48,49] (Fig 1). Hemophagocytic Mø engulf senescent and damaged erythrocytes, digest their hemoglobin content in phagolysosomes, and extract the prosthetic heme groups from hemoglobin (Fig 1). Labile heme is transferred to the cytoplasm via a mechanism assisted by the heme transporter heme-responsive gene-1 (HRG1) [50,51] (Fig 1, top right). Once heme reaches the cytoplasm, it can be secreted from Mø via different cellular heme exporters [49] or degraded by HO-1, which extracts Fe from the tetrapyrrole ring of heme. Labile Fe is then excreted by the

transmembrane protein ferroportin-1 (FPN) [52,53], the only known cellular Fe exporter (Fig 1, top right). Alternatively, labile Fe is stored in Mø by ferritin, a multimeric protein composed of 24 heavy/heart (FTH) and light/liver (FTL) subunits [54,55]. Ferritin can store and, through the ferroxidase activity of FTH, convert about 4,500 atoms of Fe^{2+} into inert Fe^{3+} [54,55] (Fig 1, top right). Heme catabolism by HO-1 induces the expression of ferritin, via a mechanism involving the production of labile Fe, which inhibits the binding of Fe regulatory proteins (IRP) to the 5'UTR of *FTL* and *FTH* mRNA, hence promoting their translation and ferritin expression [56].

Fe export from Mø is controlled systemically by hepcidin, a 2.8-KDa acute-phase peptide secreted by hepatocytes, which binds FPN and triggers its proteolytic degradation [17]. This results in sustained inhibition of Fe cellular export, leading to intracellular Fe

Box 1. Heme synthesis

Heme synthesis occurs via eight successive enzymatic reactions that take place back and forward in the mitochondria and the cytosol. The first consists in the condensation of glycine and succinyl-CoA into δ -aminolevulinic acid (ALA), catalyzed by the mitochondrial δ -aminolevulinic acid synthase (ALAS1/2). ALA translocates to the cytosol where it reacts with pyridoxal phosphate to form a porphobilinogen, via a reaction catalyzed by porphobilinogen synthase (PBGs). The resulting four porphobilinogen molecules are condensed by hydroxymethylbilane synthase (HMBS) to generate hydroxymethylbilane, which is converted to uroporphyrinogen I and then to coproporphyrinogen I by a non-enzymatic and an enzymatic reaction catalyzed by uroporphyrinogen III co-synthase or isomerase (UROS). Decarboxylation of uroporphyrinogen III forms coproporphyrinogen III, via a reaction catalyzed by uroporphyrinogen III decarboxylase (UROD). Coproporphyrinogen III is then transported to the mitochondria where coproporphyrinogen oxidase (CPOX) catalyzes its oxidative decarboxylation into protoporphyrinogen IX, subsequently oxidized to protoporphyrin IX (PPIX) by protoporphyrin oxidase (PPOX). The final step consists in the insertion of the Fe, originating from hemophagocytic M ϕ and provided via transferrin, into newly synthesized protoporphyrinogen IX, via an enzymatic reaction catalyzed by ferrochelatase (FECH) [242]. See also Fig 1.

accumulation and hypoferremia [57,58]. Hepcidin expression is regulated mainly at the level of transcription of the hepcidin (*HAMP*) gene, being induced in response to: (i) hyperferremia, that is, higher than normal levels of circulating Fe, (ii) cytokines, for example, interleukin (IL)-1, IL-6, and IL-22, or (iii) recognition of bacterial lipopolysaccharide by Toll-like receptor 4 (TLR4) [59–61] and repressed in response to: (i) hypoferremia, that is, lower than normal levels of circulating Fe, (ii) anemia, (iii) tissue hypoxia, and (iv) hormones, for example, growth and differentiation factors 15, erythroferrone, platelet-derived growth factor-BB (PDGF-BB) and testosterone [62–65].

Regulation of M ϕ intracellular Fe content by the hepcidin/FPN axis impacts on M ϕ polarization and presumably therefore resistance to infection [6,66,67]. Specifically, the intracellular Fe content of M ϕ modulates their response to interferon γ (IFN γ) [68,69], a cytokine produced by activated natural killer (NK) cells and T helper type 1 (T_H1) cells, which plays a central role in driving M ϕ microbicidal activity [70]. Moreover, accumulation of intracellular Fe in T cells mitigates the activation, differentiation, and proliferation of T_H1 cells while fostering T_H type 2 (T_H2) responses, as illustrated in the context of *Candida albicans* infection in mice [69,71]. Reduced cellular Fe content, resulting from blockage of Fe cellular import by the transferrin receptor in T cell, also inhibits T_H1 cell responses, whereas T_H2 cells are less sensitive to this effect, presumably due to their higher intracellular Fe content [72]. While perhaps unfavorable in the context of infections by intracellular pathogens, which are cleared by T_H1 responses [69,73], Fe-driven T_H2 responses might promote host resistance against large extracellular parasites, for example, helminthes, but this remains to be tested experimentally.

The microbicidal activity of activated M ϕ depends critically on the phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2/gp91^{phox}) and the inducible form of nitric oxide (NO) synthase (iNOS/NOS2) [74,75]. Both NOX2/gp91^{phox} and iNOS/NOS2 are prototypical hemoproteins that use Fe-heme to catalyze the

production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), respectively [76]. While NOX2/gp91^{phox} [77] expression and iNOS/NOS2 [78] expression are regulated essentially at the transcriptional level, via a mechanism involving the activation of the nuclear factor kappa B (NF- κ B) family of transcription factors, their activity requires the insertion of a heme prosthetic group into these apoproteins. This argues that expression of these enzymes must be functionally integrated with mechanisms regulating Fe-heme metabolism as to support their activity and hence M ϕ microbicidal activity. The underlying mechanisms remain elusive.

In addition to their intrinsic cytotoxicity, ROS and RNS regulate Fe metabolism in a manner that also contributes to the overall microbicidal activity of activated M ϕ . Both ROS and RNS have high affinity toward Fe²⁺ in the prosthetic heme groups or Fe-sulfur clusters of a variety of proteins [79,80]. These include IRPs, to which NO can bind and modulate their stability as well as activity [81,82], resulting in IRE-mediated regulation of Fe metabolism [58,66,81–86]. ROS also regulate IRP activity indirectly, via kinase/phosphatase signaling pathways [58,66]. The physiologic relevance of this mechanism was recently underpinned by the description of hepcidin-independent hypoferremia in mouse models of inflammation [87], and the fact that tumor necrosis factor (TNF) causes sustained hypoferremia in mice, irrespective of hepcidin [88]. Whether ROS or RNS contribute to hepcidin-independent hypoferremia has not been established.

ROS and RNS are also reactive toward thiol groups in the cysteines of redox-regulated proteins, such as the Kelch-like ECH-associated protein 1 (Keap1) [89–91]. This adaptor for the Cullin (Cul)3–RING (really interesting new gene)-box protein (Rbx)1 ubiquitin ligase complex targets constitutively the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) for proteolytic degradation by the 26S proteasome [91]. When Keap1 Cys¹⁵¹ is targeted by RNS, the tertiary structure of Keap1 is altered, impairing its ubiquitin ligase activity [89–91]. This allows newly transcribed Nrf2 to undergo nuclear translocation and drive the transcription of target genes containing DNA-antioxidant-responsive elements in their promoter [91]. These genes include HO-1, which generates CO via heme catabolism. This gasotransmitter compels bacteria to produce and release ATP, which is then sensed by the P2 $\times$ 7 purinergic receptor expressed by M ϕ [92]. This modulates a K⁺ efflux pump which activates the NACHT, LRR, and PYD domains-containing protein 3 (NALP3), leading to caspase 1 activation, pro-IL-1 β cleavage, and IL-1 β secretion by M ϕ [92]. This process of “microbe metabolic sensing” appears to be required to promote bacterial clearance by activated M ϕ [92]. Some intracellular pathogens, such as *Mycobacteria*, can subvert this sensing system, going into a state of dormancy in response to CO [93].

Taken together, these observations argue that regulation of M ϕ intracellular Fe content impacts on resistance to infection. This effect goes beyond secluding Fe from invading pathogens, encompassing the modulation of innate and adaptive immune responses against those pathogens.

Pathogen class-specific regulation of Fe metabolism

Pathogen class-specific mechanisms evolved most probably to limit Fe availability to extracellular versus intracellular pathogens, while

enhancing resistance mechanisms [17,94,95]. Induction of hypoferremia, via systemic inhibition of extracellular Fe export coupled to increased Mø Fe/heme uptake and intracellular retention [5,6], reduces the growth and virulence of extracellular pathogens, as illustrated for *Vibrio vulnificus* infection in mice [96,97]. However, this strategy can be detrimental against intracellular pathogens, enhancing their growth and proliferation, as illustrated for bacteria from *Chlamydia*, *Legionella*, *Salmonella*, and *Mycobacteria* spp. [98–102]. The impact of such pathogen class-specific mechanisms is particularly relevant in the context of co-infections, where restricting Fe access to one pathogen can be detrimental against co-infection by another pathogen. For example, heme catabolism by HO-1 [103–106] and subsequent intracellular Fe storage by ferritin [54,107] confers host protection against the blood stage of *Plasmodium* infection while increasing susceptibility to the intracellular bacteria *Salmonella* [108]. Whether this principle can be extrapolated to co-infections by other pathogens is likely, but remains to be established.

Regulation of Fe metabolism as a host resistance mechanism against extracellular pathogens

The general strategy to restrict Fe access to extracellular pathogens consists in the retention of intracellular Fe by Mø leading to hypoferremia (Fig 2). This is achieved essentially by the induction of Fe import and retention mechanisms, coupled to suppression of cellular Fe export (Fig 2). Activated Mø can produce minute amounts of hepcidin in response to bacterial lipopolysaccharide (LPS) or cytokines, targeting FPN for proteolytic degradation [109,110]. However, while this may provide a fast acting strategy to limit Mø Fe export, sustained Fe retention requires additional inputs provided by cytokines such as IFN γ , which represses FPN transcription [6] (Fig 2). IFN γ also induces the expression of iNOS/NOS2 [78], via a mechanism regulated at a transcriptional level by the activation of the signal transducers and activators of transcription (STAT) family of transcription factors (Fig 2). As described above, the NO produced by iNOS/NOS2 regulates the binding affinities of IRP toward target IRE in genes such as transferrin receptor (TfR) and ferritin, inhibiting cellular Fe import and intracellular retention, respectively [58,81,82,111]. However, once intracellular Fe levels increase in Mø, iNOS/NOS2 transcription is repressed creating a negative feedback loop, which promotes intracellular Fe retention [85,111]. IFN γ also induces the expression of the divalent metal transporter-1 (DMT1) in Mø, promoting ferrous Fe uptake and intracellular incorporation by ferritin [112] (Fig 2). Cytokine-driven NF- κ B activation induces *FTH* transcription, supporting further retention of Fe in Mø [54,55] (Fig 2).

Other cytokines such as IL-4, IL-10, and IL-13 promote TfR-mediated Fe uptake and intracellular retention by ferritin [113]. Moreover, IL-10 and IL-6 also increase heme uptake from circulating hemoglobin-haptoglobin or heme-HPX complexes, via a mechanism involving the induction of the hemoglobin scavenger receptor CD163 and the HPX scavenger receptor CD91 [114,115], respectively (Fig 2). This is coupled to the induction of HO-1 in Mø [116], which extracts Fe from heme and promotes intracellular Fe storage by ferritin (Fig 2).

Another resistance mechanism against extracellular pathogens, based on regulation of Fe metabolism, involves the expression of

lactoferrin, a member of the transferrin family of Fe-binding proteins (Fig 2). Lactoferrin is expressed in many tissues and secretions where its primary function is to bind and restrict Fe delivery to extracellular microbes, a mechanism which accounts for its antimicrobial activity in breast milk [117]. Activated Mø express and release lactoferrin, which scavenges and restricts Fe availability to extracellular pathogens [6,117,118] (Fig 2). Lactoferrin also protects Mø from oxidative stress [117,119] and modulates Mø microbicidal activity, as shown in the context of *Staphylococcus aureus* infection [120,121]. The Fe-binding capacity of lactoferrin can also interfere with microbial virulence pathways such as biofilm formation [122]. Moreover, lactoferrin modulates the activation and proliferation of lymphocytes, NK cells, and Mø, via a mechanism mediated in part by lactoferrin receptors expressed by these cells [117,123]. Cellular internalization of Fe-loaded lactoferrin by Mø can lead to cellular Fe overload and cytotoxicity [124]. Of note, some bacteria evolved strategies to subvert the protective effects of lactoferrin extracting Fe from lactoferrin [125,126]. Although the exact mechanism by which bacteria can extract iron from lactoferrin remains to be determined, some Gram-negative bacteria can import lactoferrin following its binding to microbial transmembrane lactoferrin binding proteins A and B [127,128].

Lipocalin (Lcn2/NGAL) is a peptide produced by activated polymorphonuclear (PMN) cells and Mø, which captures Fe-laden bacterial catechole type siderophores, such as enterobactin, derived from Gram-negative bacteria (Fig 2). Lcn2 inhibits the acquisition of siderophore-bound Fe by bacteria, as illustrated for *E. coli* or *Klebsiella* spp. [129,130]. Lcn2 delivers bacterial as well as mammalian siderophore-bound Fe [131,132] to the Lcn2 receptor expressed by host cells, suggesting that it modulates Fe trafficking and hence Fe metabolism during infection (Fig 2). Lcn2 also promotes resistance to non-bacterial pathogens, as demonstrated for *Plasmodium* infection [133]. It should be noted, however, that Lcn2 exerts contrasting effects depending on the pathogen and tissue localization, improving or mitigating resistance to infection. For example, Lcn2 aggravates the outcome of *Streptococcus pneumoniae* infection [134] through an immunomodulatory effect possibly acting via regulation of Fe metabolism or another not yet fully elucidated pathway [135,136]. Of note, *E. coli* can use the mammalian siderophore, 2,5-dihydroxy-benzoic acid (2,5-DHBA) as Fe source [137]. Accordingly, Mø respond to bacterial challenge by downregulating 2,5-DHBA synthesis, leading to 2,5-DHBA depletion and enhanced resistance to *E. coli* infection.

Both ROS and RNS produced by activated Mø or PMN cells can elicit different forms of stress and damage to bystander red blood cells (RBC), eventually leading to the release of their hemoglobin content. Given the sheer number of RBCs ($\sim 2\text{--}3 \times 10^{13}$ in adult humans) and their extremely high hemoglobin ($\sim 3 \times 10^8$ molecules/RBC) and heme ($\sim 1.2 \times 10^9$ molecules/RBC) content, disruption of a relatively small numbers of RBCs is sufficient to release significant amounts of hemoglobin [49]. Extracellular hemoglobin exerts antimicrobial effects, driven by the peroxidase activity of the Fe contained in its prosthetic heme groups [138,139]. Furthermore, binding of extracellular hemoglobin to bacterial LPS [140] elicits conformational changes in the tertiary structure of hemoglobin [139,141], which enhance its peroxidase and microbicidal activity [138,139]. Hemoglobin also enhances the pro-inflammatory activity of LPS [141]. Overall, this suggests that extracellular

Figure 2. Regulation of Fe metabolism in response to extracellular pathogens.

(A) Immune responses to extracellular pathogens encompass the production of cytokines, for example, IL-1, IL-6, and IL-22, which induce the transcription of the hepcidin (*HAMP*) gene in hepatocytes. Cellular Fe retention by M ϕ can lead to the development of anemia of inflammation, which triggers the production of several hormones, such as Epo, Erfe, GDF15, PDGF-BB, or the activation of the HIF family of transcription factors, which reduce hepcidin expression. (B) Circulating hepcidin targets systemically the Fe export protein FPN for degradation, which inhibits Fe cellular retention in many cell types, including M ϕ , which are pivotal to the maintenance of Fe homeostasis. This is supported by the autocrine production of hepcidin by M ϕ . In addition, cytokines, such as IFN γ and TNF, also inhibit FPN transcription in M ϕ (not shown) promoting Fe retention and limiting further Fe availability to extracellular microbes. (C) Upon infection by extracellular pathogens, M ϕ can uptake extracellular Fe via different mechanisms involving Tf/TfR interaction, (D) the lactoferrin/lactoferrin receptor, (E) the Lcn2/Lcn2R, or (F) the divalent metal transporter DMT1. All of these contribute to scavenge extracellular Fe and prevent extracellular pathogens from accessing Fe. (G) In addition, M ϕ can engulf damaged RBC and prevent hemoglobin release or (H) scavenge extracellular hemoglobin/haptoglobin via the scavenger receptor CD163 as well as (I) heme/HPX complexes via the scavenger receptor CD91. (J) Uptake of heme/HPX, damaged RBC, or hemoglobin/haptoglobin by M ϕ is coupled to heme transport from phagolysosomes to the cytoplasm, by the heme transporter HRG1, and to heme catabolism by HO-1. These pathways are induced by several cytokines in the course of an infection.

hemoglobin acts both as a soluble pattern recognition receptors (PRRs) and as a cytotoxic effector molecule targeting extracellular pathogens [140].

Taken together, these observations argue that several mechanisms evolved to regulate host Fe metabolism in a manner that limits the proliferation capacity and virulence of extracellular

pathogens. Moreover, leakage of hemoglobin from red blood cells appears to be an ancestral and evolutionary conserved defense strategy that targets extracellular pathogens for heme-driven oxidative cytotoxicity. This defense mechanism was also retained in arthropods, in which the hemoglobin functional homolog hemocyanin displays similar antimicrobial functions [138].

Regulation of Fe metabolism as a host resistance mechanism against intracellular pathogens

The general strategy to restrict Fe access to intracellular pathogens consists in the induction of hyperferremia, driven by systemic induction of cellular Fe export coupled to the suppression of cellular Fe import systems from Mø as well as other cell types. This strategy is illustrated by the natural resistance-associated Mø protein 1 (NRAMP1, SLC11a1), identified originally for its ability to confer resistance to intracellular pathogens, such as protozoan parasites from *Leishmania* spp. as well as bacteria from *Salmonella* or *Mycobacterium* spp. [142,143]. NRAMP1 is a transmembrane transporter expressed in late phagosomes, where it exerts multiple functions including regulation of phagosome

acidification, which is, however, exerted mainly by v-ATPase [144] along with pH-dependent shuttling of divalent metals, including Fe, zinc and manganese, across the phagolysosomal membrane [145–147] (Fig 3). Murine Mø lacking functional Nramp1 have a higher intracellular Fe content, whereas functional Nramp1 decreases intracellular Fe content, conferring protection against intracellular infections [145,148,149], an effect traced back to modulation of FPN expression [150]. A similar mechanism may contribute to control trypanosomiasis, even though NRAMP1 has not been identified as a primary resistance gene [151]. NRAMP1 increases the production of NO and TNF in infected Mø, whereas IL-10 secretion is reduced, arguing again that modulation of intracellular Fe content regulates Mø antimicrobial effector function [152–155].

Figure 3. Regulation of Fe metabolism in response to intracellular pathogens.

Mø activation in response to intracellular pathogens is associated with a reduction of intracellular Fe levels. This occurs via different mechanisms. (A) Fe or heme is exported from phagolysosomes by NRAMP1 and FPN or by HRG1, reducing Fe and heme availability to pathogens residing within this subcellular compartment. Heme catabolism by HO-1 induces the expression of the Fe-scavenging protein ferritin that stores intracellular Fe in Mø, away from intracellular pathogens. (B) Mø activation in response to PRR and/or IFN γ R signaling activates the transcription factors NF- κ B and STAT1/2, which induce the expression of the Fe-binding proteins ferritin, lactoferrin, Lcn2, and the Fe transporter DMT1 as well as NOX2/gp91^{phox} and iNOS/NOS2. (C) IFN γ R signaling also inhibits Fe extracellular uptake via TfR. (D) The NO produced by iNOS/NOS2 induces, via Keap1, the activation of the transcription factor Nrf2, which induces the expression of FPN and hence Fe export from phagolysosomes and/or from the cytoplasm. (E) Lcn2 neutralizes the action of bacterial siderophores. (F) Lactoferrin binds Fe and limits its availability to bacteria.

There is at least one additional mechanism by which NRAMP1 confers resistance to intracellular bacteria such as *Salmonella*, namely via the induction of Lcn2 expression [156]. Of note, Lcn2 can control the proliferation of intracellular bacteria that do not produce siderophores, such as *Chlamydia pneumonia* [157] (Fig 3). Of interest, *Mycobacteria avium* can overcome Fe limitation by Lcn2 by residing in Rab11⁺ recycling phagosomes where the bacterium has access to transferrin Fe but avoids Fe restriction by Lcn2 [158]. Nonetheless, induction of Lcn2 expression is also involved in the mechanism that protects mice lacking the hemochromatosis protein (HFE) from *Salmonella* infection [159]. While *Mycobacteria* infection is also impaired in Mø from individuals with the hemochromatosis-associated mutation of the HFE gene, whether this is driven by Lcn2 expression has not been established [160].

Yet another mechanism via which Mø reduce their intracellular Fe content involves the activation of the transcription factor Nrf2 [73]. Namely, when produced by iNOS/NOS2 in activated Mø, NO activates Nrf2 and induces the transcription/expression of FPN, which reduces Mø intracellular Fe content and confers resistance to *Salmonella* infection [73] (Fig 3). Of note, impaired resistance to *Salmonella* infection in *iNos/Nos2*-deficient mice can be restored by pharmacologic Fe chelation, arguing that the microbicidal effect of NO acts, at least partly, via regulation of intracellular Fe content [73]. A similar resistance mechanism may be of importance for other infectious diseases, such as trypanosomiasis [161].

When expressed in phagosomes FPN can reduce Fe supply to pathogens in this subcellular compartment, as shown *in vitro* for *Mycobacteria* infection [101] (Fig 3). The pathophysiologic relevance of this mechanism is illustrated by the fact that individuals carrying mutations in the FPN gene, which is associated with intracellular Fe retention and secondary siderosis in Africa (also termed Bantu disease), are challenged with an increased incidence of *Mycobacteria* infection and higher mortality from tuberculosis [162]. Of interest, some intracellular bacteria can counterbalance this host response, as illustrated for *Salmonella* spp., which promotes the expression of hepcidin in the liver via the induction of estrogen receptor gamma-mediated cascade. Subsequent degradation of FPN favors intracellular Fe accumulation, thereby promoting pathogen growth [163].

Intracellular Fe accumulation in Mø represses IFN γ receptor signaling, most likely explaining why reducing intracellular Fe content promotes IFN γ -driven resistance pathways involving the induction of histocompatibility (MHC) class II, iNOS/NOS2 TNF, IL-6, or IL-12 expression [164–167]. The mechanisms underlying this effect have not been entirely elucidated, but involve the transcription factors hypoxia-inducible factor 1 α (HIF1 α) nuclear factor for IL-6 expression (NF-IL6) and STAT and likewise phosphokinase-controlled pathways [164–167]. This suggests that mechanisms reducing intracellular Fe availability in Mø have a dual role, limiting microbial access to intracellular Fe while boosting antimicrobial resistance mechanisms in response to cytokines such as IFN γ [6]. This is in line with the recent observation that IRP expression and their subsequent regulatory effects on cellular Fe homeostasis in Mø confer resistance to *Salmonella* infection by restricting Fe availability to the bacterium and by promoting antimicrobial immune effector mechanisms of Mø [168]. Finally, signaling via the IFN γ receptor also induces FPN expression while inhibiting

extracellular Fe uptake via the transferrin receptor [169] and inducing Lcn2 expression [135] in Mø (Fig 3).

Heme catabolism by HO-1 also supports resistance to intracellular bacteria, as suggested for *Mycobacteria* infection in mice [170,171]. While it is not clear whether this effect is mediated via CO and/or other products of heme catabolism, such as biliverdin and Fe, it is likely that the cytoprotective effect of CO [172] prevents infected Mø from undergoing programmed cell death by necroptosis [173]. This cytoprotective effect should support granuloma formation and hence resistance to *Mycobacteria* [170,171]. The recent finding that Mø necroptosis impairs granuloma formation and compromises resistance to *Mycobacteria* infection in zebrafish supports this notion [174]. Whether this cytoprotective effect is of importance for host resistance to other intracellular pathogens that evolved mechanisms to subvert Mø programmed cell death, such as *Listeria*, *Salmonella*, or *Shigella* spp., remains to be established.

It appears that several mechanisms evolved to reduce the intracellular Fe content of Mø infected by intracellular pathogens. This achieves several goals, namely to limit Fe access to intracellular pathogens while modulating Mø polarization and maintaining Mø viability, all of which concur to limit the virulence and eventually clear intracellular pathogens.

Regulation of Fe metabolism as a host disease tolerance mechanism

As discussed above, some immune-driven resistance mechanisms can encompass subtle alterations of host Fe metabolism characterized by a systemic reduction of circulating Fe levels, termed hypoferrremia. This is compensated by the expansion of Fe stores within the reticuloendothelial system, termed hyperferritinemia [175,176]. This dual effect contributes critically to host protection against infection by extracellular/circulating microbes, based on limiting Fe availability while regulating antimicrobial immune-driven resistance mechanisms. As a trade-off, however, immune-driven hypoferrremia and hyperferritinemia can become pathologic. Sustained hypoferrremia reduces Fe availability to erythropoiesis and can thus result in the development of anemia of chronic disease also known as anemia of inflammation [177]. This form of anemia is considered the second most frequent worldwide and is found in individuals suffering from infections or immune-mediated inflammatory diseases, such as cancer and systemic autoimmune disorders [177,178]. Anemia of inflammation is associated with reduced cardiovascular performance along with tissue hypoxia and overall impaired quality of life [179]. These clinical outcomes evolve irrespective of the host's pathogen burden likely reflecting impaired disease tolerance [1].

Anemia of inflammation, caused by extracellular pathogens, is driven by hepcidin- and cytokine-mediated reduction of Fe efflux from Mø as well as reduced Fe dietary absorption [113]. In contrast, intracellular pathogens like *Mycobacteria* or *Salmonella* trigger anemia of inflammation independent of hepcidin via a mechanism involving the induction of Lcn2 and NRAMP1 expression and enhanced FPN function in Mø [180,181]. This suggests that in a similar manner to resistance mechanisms, regulatory mechanisms controlling host Fe metabolism modulate disease tolerance in a pathogen class-specific manner.

Irrespective of pathogen class, anemia of inflammation is associated with the development of tissue hypoxia and hence with the activation of hypoxia-inducible factor (HIF). This family of transcription factors regulates the expression of several erythropoietic factors and hormones, which stimulate erythropoiesis. Erythropoietin (Epo), the major erythropoiesis stimulatory hormone, regulates Fe metabolism via the induction of TfR expression and Fe uptake into erythroid progenitor cells [182] as well as via stimulation of erythroferrone, a hormone that inhibits hepcidin expression, thereby increasing Fe availability for erythropoiesis [183]. Several cytokines driving immune resistance mechanisms can reduce Epo expression, blunting this adaptive response [177,184,185]. In some cases, this may be beneficial, because Epo exerts immunoregulatory effects that impair resistance to bacterial infections [186]. Other hematopoiesis-derived factors, such as the growth differentiation factor 15 (GDF15), can also reduce hepcidin expression, but their relative importance in the setting of anemia of inflammation appears to be limited [65,187]. Activation of HIFs can also inhibit hepcidin expression while inducing the expression of Fe transporters in the duodenum, thereby fostering Fe absorption from diet [188,189]. Moreover, hypoxia-induced hormones, such as PDGF-BB, also inhibit hepcidin transcription [64] (Fig 3). The interplay of all these factors with Fe homeostasis in the setting of anemia of inflammation and their impact on antimicrobial host immune responses during infection remains largely unknown.

Infection by extracellular pathogens can involve the development of tissue Fe overload, as observed for infectious diseases associated with more or less severe hemolysis. Accumulation of extracellular hemoglobin can exert pathogenic effects that foster the pathogenesis of infectious diseases, as illustrated for the development of severe sepsis in response to systemic polymicrobial infections in mice [190]. This is also the case for the development of severe forms of malaria in response to *Plasmodium* infection in mice [103–106,191,192]. These often-lethal pathologic conditions can be dissociated from host pathogen burden, suggesting that extracellular hemoglobin impairs disease tolerance to sepsis and malaria. The clinical relevance of this pathologic process is strongly supported by the finding that accumulation of extracellular hemoglobin is associated with higher risk of death from severe sepsis [193,194]. Moreover, reduced levels of the hemoglobin scavenger haptoglobin are also associated with increased malaria severity [195].

The mechanisms underlying the pathogenic effects of extracellular hemoglobin were associated originally to NO scavenging, reducing NO bioavailability, and impairing microvascular homeostasis [192,196] (Fig 4). This was suggested to contribute to the pathogenesis of severe forms of malaria in mice, without interfering with host pathogen burden [192], thus arguing that NO scavenging by extracellular hemoglobin impairs disease tolerance to *Plasmodium* infection. There are, however, other mechanisms contributing to the pathogenic effects of extracellular hemoglobin. When exposed to NO, the peroxidase activity of extracellular hemoglobin can catalyze the generation of cytotoxic peroxynitrate (ONNO^-) a highly toxic RNS fostering microvascular dysfunction. To what extent this impairs disease tolerance to malaria and/or other infectious diseases is not clear given that ONNO^- can induce the activation of the transcription factor Nrf2 and promote disease tolerance to malaria [191]. Moreover, extracellular hemoglobin

may also exert protective effects based on NO and ONNO^- detoxification [76,80,197].

When released from RBC, hemoglobin tetramers are readily dissociated into dimers. Sustained oxidation of extracellular hemoglobin dimers, such as driven by products of activated Mø and PMN cells, generates met(Fe^{3+}) and eventually ferryl(Fe^{4+})hemoglobin [198]. Electron transfer from Fe^{4+} can oxidize tyrosines in the globulin chains of hemoglobin, giving rise to dityrosine bonds that promote the generation of ferrylhemoglobin aggregates [198]. When deposited on the surface of vascular endothelial cells, these become vasoactive and pro-inflammatory [198], presumably contributing to the pathogenesis of systemic infections associated with hemolysis and vasculitis.

Extracellular methemoglobin can release its non-covalently bound heme [38,104,105,190,199–201], generating labile heme, that is, redox active heme that is loosely bound to plasma proteins or to other molecules (Fig 4). Labile heme plays a central role in the pathogenesis of severe forms of malaria as well as severe sepsis in mice [103,105,190]. The pathogenic effect of labile heme can be dissociated from the host's pathogen burden [103,105,190], suggesting that labile heme impairs disease tolerance to these systemic infections. The clinical relevance of this pathologic process is strongly supported by the recent finding that heme accumulation in plasma is associated with the development of severe forms of malaria in humans [202].

The mechanisms underlying the pathogenic effects of labile heme are probably diverse, but there is a growing body of evidence to suggest that labile heme acts as a pro-inflammatory agonist through the engagement of TLR-4 in Mø [203,204] and endothelial cells [205] (Fig 4). Free heme can also engage other PRR, as illustrated for NALP3 [206]. Given the central role played these PRR in the activation of innate immunity, labile heme is likely to tailor resistance to infections associated with more or less severe hemolysis. Labile heme also exerts chemotactic effects via the activation of G protein-coupled receptors expressed in PMN cells [207], while promoting the release of neutrophil extracellular traps (NETs) from activated PMN cells [208] (Fig 4).

Once released from hemoglobin, the redox activity of labile heme becomes pro-oxidant and cytotoxic [38,106,107,190] (Fig 4). Presumably, it is this cytotoxic effect that promotes tissue damage and hence compromises disease tolerance to malaria and severe sepsis [103,105,190]. Briefly, labile heme sensitizes parenchyma cells to undergo programmed cell death in response to pro-inflammatory cytokines such as TNF [106] as well as other agonists such as Fas as well as ROS and RNS [38,190]. The mechanism underlying heme-mediated cytotoxicity involves the sustained activation of the c-Jun N-terminal kinase (JNK) signaling transduction pathway [107] (Fig 4). Heme overrides the cytoprotective program set in motion by the transcription factor NF- κ B [209], which prevents sustained JNK activation and programmed cell death [210,211]. Presumably, this occurs via a mechanism driven via the Fenton reaction in which hydrogen peroxide (H_2O_2) oxidizes Fe^{2+} , either within the context of the tetrapyrrole ring or when Fe is released from heme, to produce highly reactive hydroxyl radicals ($\text{OH}^\cdot$) [38,212]. This pro-oxidant effect blunts the activity of redox-sensitive phosphatases controlling JNK activation [213,214], thus inducing programmed cell death in response to TNF [107,213,215]. The pathophysiologic relevance of this signal transduction pathway

Figure 4. Fe/heme metabolism and disease tolerance to infection.

RBC are present at high numbers throughout the body [49]. Cytotoxic products generated by pathogens can damage RBC, which are rapidly engulfed and cleared by MΦ. Alternatively, damaged RBC release hemoglobin (HB) into plasma, which can exert deleterious effects, compromising disease tolerance. For example, extracellular hemoglobin can scavenge NO, promoting vasoconstriction and eventually affecting microvascular circulation (top). Oxidized extracellular hemoglobin can also release heme, which acts as a pro-inflammatory agonist in endothelial cells and MΦ via TLR4, or via GPCRs in PMN cells. Labile heme sensitizes parenchyma cells to undergo programmed cell death in response to TNF, leading to tissue damage and compromising disease tolerance (right). These pathogenic effects are countered by tissue damage control mechanisms that confer disease tolerance to infection. The hemoglobin and heme scavengers HP and HPX, respectively, shuttle heme for degradation by HO-1 via the MΦ scavenger receptors CD163 and CD91 (middle). This is coupled to mechanisms regulating intracellular Fe reactivity. These include ferritin, which stores intracellular Fe and FPN, which delivers Fe to Tf and supports erythropoiesis. This prevents anemia and tissue hypoxia, an effect likely to promote disease tolerance. This heme/Fe detoxifying mechanism also operates in parenchyma tissues to prevent programmed cell death and tissue damage, presumably enforcing disease tolerance (right). One of the mechanisms relies on the Fe-scavenging capacity of ferritin, which acts in an antioxidant manner and prevents sustained JNK activation from triggering programmed cell death in response to TNF. Moreover, HO-1 enzymatic activity generates CO, which promotes the secretion of IL-10 in activated MΦ, impacting on MHC class II/TCR-driven activation of T_H cells and thereby contributing to tissue damage control and eventually to disease tolerance (middle). CO also binds to the heme groups of extracellular hemoglobin and prevents heme release, thus protecting tissues from the vasoactive, pro-inflammatory, and cytotoxic effects of labile heme. Finally, CO is a potent cytoprotective molecule that prevents tissue damage and as such can enforce disease tolerance to infection.

is supported by the finding that both Fe sequestration by ferritin and pharmacologic administration of antioxidants prevent sustained JNK activation and confer disease tolerance to malaria in mice [38,106,107].

Heme sensitization to programmed cell death is partially suppressed by pharmacologic inhibition of caspases [106,107], indicating that heme sensitizes cells to undergo apoptosis [216]. However, heme-driven programmed cell death is also suppressed by pharmacologic inhibition of the receptor-interacting serine/threonine kinases 1 (RIPK1), as well as by the deletion of the *Ripk3* gene (unpublished observation). This suggests that heme might also trigger necroptosis [217]. This is in line with the finding that sustained JNK activation shifts TNF-mediated programmed cell death from apoptosis to necrosis [217,218] and that heme can trigger necroptosis

in MΦ, via an autocrine loop in which heme triggers the production of TNF upon the engagement of TLR4 [173].

The pathogenic effects exerted by extracellular hemoglobin and labile heme are countered by adaptive responses that converge at the level of heme catabolism by HO-1 [38,219], providing tissue damage control and disease tolerance to systemic infections [103,105,106] (Fig 4). The emergence of several stress-responsive genetic programs culminating in the catabolism of labile heme is probably required to neutralize its pro-inflammatory [203,204,207], vasoactive [205], and cytotoxic [38,104–106] effects [219] (Fig 4). When coupled to other mechanisms regulating Fe homeostasis, HO-1 avoids tissue Fe overload and tissue damage while sustaining Fe supply to erythropoiesis to maintain Fe homeostasis during infection [38,54,219,220] (Fig 4). This notion is strongly supported

by the observation that deletion of the *Hmox1* allele in mice [221,222] and humans [223] is associated with impaired Fe homeostasis, revealed by the development of anemia, vascular damage, and Mø cytotoxicity [173,224]. Whether these effects are exerted when HO-1 is expressed by Mø and/or in parenchyma cells is not clear (Fig 4).

In the context of systemic infections, the protective effects exerted by HO-1 are assisted by FTH [54,107] (Fig 4). Mice in which the *Fth* allele is deleted develop tissue Fe overload and tissue damage succumbing to *Plasmodium* [107] as well as to polymicrobial infections (unpublished observation). The protective effects of FTH do not interfere with host pathogen burden, illustrating further how regulation of host Fe metabolism confers disease tolerance to infection [103,106,190].

The gasotransmitter CO, produced via heme catabolism by HO-1, also confers disease tolerance to systemic infections, as demonstrated for malaria [105]. This salutary effect relies essentially on the intrinsic ability of CO to bind Fe^{2+} in the heme groups of hemoglobin [103–105,199]. While reputedly toxic when targeting hemoglobin in RBC, binding of CO to extracellular hemoglobin inhibits hemoglobin oxidation and the release of its prosthetic heme groups [103–105,199], thus preventing labile heme from partaking in the pathogenesis of severe forms of malaria [103–105]. In addition, CO modulates signaling by PRRs, as illustrated for TLR4, inhibiting TNF while increasing IL-10 secretion by activated Mø [225]. This immunoregulatory effect is reinforced by IL-10, which acts as a positive feedback loop to induce further HO-1 expression and CO production [116]. This suggests that CO regulates Mø polarization, in a manner that should limit the extent of tissue damage associated with Mø activation, in the context of infectious diseases, such as sepsis driven by polymicrobial infection [190] as well as severe forms of malaria [103–106], tuberculosis [47,170,226], or trypanosomiasis [227]. Moreover, CO acts *per se* as a cytoprotective molecule [172,228], which should also contribute to promote tissue damage control and disease tolerance to malaria. The finding that sickle hemizygous mutations confer protection against malaria via a CO-dependent mechanism supports its pathophysiological relevance [103–105]. Namely, sickle hemoglobin mutations induce the generation of labile heme, which triggers the activation of the transcription factor Nrf2 and the expression of HO-1 [103,104]. Production of CO by HO-1 targets the extracellular hemoglobin generated during the blood stage of *Plasmodium* infection and prevents the pathogenesis of severe forms of malaria [103–105]. This protective effect is not associated with modulation of host pathogen load, revealing that sickle hemoglobin mutations confer disease tolerance to malaria [103,104]. It is likely that a similar mechanism underlies the protective effect exerted by other chronic hemolytic conditions against malaria, including hemoglobin C [229,230], glucose 6 phosphate dehydrogenase (G6PD) deficiency in males [231], β - or α -thalassemia [230] as well as mutations involved in RBC cytoskeleton or membrane protein defects [232].

Targeting Fe/heme metabolism in the treatment of infectious diseases

There is an urgent need of alternative treatment strategies that can cope with the recent raise of microbial resistance to antimicrobial

drugs. Given the involvement of Fe in the control of microbial pathogenicity, it appears reasonable to propose that targeting Fe may offer therapeutic value. Presumably, pathogen class-specific mechanisms regulating host Fe metabolism should be targeted specifically when dealing with extracellular versus intracellular pathogens (Figs 2 and 3). Considering that physiologic mechanisms promoting Fe efflux from Mø confer resistance to intracellular bacteria [73,98,99,157], drugs that promote this process should enhance resistance to intracellular bacteria. This was demonstrated, as a proof of principle, using Nifedipine, a calcium channel blocker that promotes Fe efflux from Mø and enhances host resistance to *Salmonella* infection in mice [233]. In contrast, infection by extracellular pathogens should be better controlled by pharmacologic agents that reduce Mø efflux and decrease the levels of circulating Fe, such as hepcidin or hepcidin mimetics [95]. The proof of principle for this approach was provided for *Vibrio vulnificus* infection in mice [96]. There are other examples, indicating a beneficial effect of targeting Fe, as illustrated for the ability of lactoferrin to prevent bacterial biofilm formation [122]. Moreover, lactoferrin acts synergistically with antibiotics against *Staphylococci* infections and can be used to control bovine mastitis [234]. Delivery of Lcn2 by probiotic bacteria has also been shown to control *Salmonella* infection [235]. Systemic Fe chelation by desferrioxamine can improve the clinical course of malaria, without, however, providing an overt survival benefit [236]. In mucormycosis, an invasive mold infection, desferrioxamine shows adverse outcomes because mucorales can use desferrioxamine as a Fe source [237]. However, the Fe chelator desferasirox, which cannot be used by molds to acquire Fe, improves survival of infected mice, limiting Fe availability to the pathogen while enhancing host resistance mechanisms [238]. However, a small, multicenter clinical study using desferasirox as an adjunct treatment in patients that develop mucormycosis after allogeneic bone marrow transplantation resulted in a rather unfavorable outcome, for reasons that are not clear [28]. Finally, approaches which block microbial Fe acquisition pathways, including binding of host Fe proteins, may prove to be effective in limiting microbial proliferation and pathogenicity as shown for *Aspergillus* spp. [33].

Pharmacologic targeting of heme metabolism may also be used to modulate host resistance and/or tolerance to intracellular and extracellular infections. Perhaps the strongest argument in support of this strategy is provided by anti-malarial drugs, which target in most cases *Plasmodium* heme metabolism [239]. Pharmacologic induction of HO-1 has also been used experimentally to modulate resistance or tolerance to bacterial or protozoan infections in mice [220]. Another possible venue for intervention is to target heme release from extracellular hemoglobin, such as afforded by pharmacologic administration of CO, which may be used clinically [240] to confer disease tolerance to malaria [103–105]. The recent finding that HO-1 is central to support resistance to *Mycobacteria* infection in mice suggests that targeting heme catabolism may be used therapeutically against tuberculosis [170]. Whether pharmacologic use of hemoglobin or heme scavengers, such as haptoglobin or HPX, may be used therapeutically to avoid the deleterious effects of extracellular hemoglobin and labile heme, respectively, remains to be established. It is likely as well that interfering with heme cellular trafficking in pathogenic microbes or in the infected host may have therapeutic value.

Sidebar A: In need of answers

- 1 What is the impact of different pathogen class-specific mechanisms modulating host Fe/heme metabolism in the pathogenesis of infectious diseases?
- 2 What are the dynamic thresholds of host Fe/heme metabolism that provide optimal immune-driven resistance to infection and limit Fe/heme access to microbes while not compromising host homeostasis?
- 3 Is pharmacological modulation of host and/or microbial Fe/heme metabolism a viable therapeutic target against infectious diseases?
- 4 Are pathogen class-specific mechanisms modulating host Fe/heme metabolism a driving force in the pathologic outcome of co-infections?

Concluding remarks

The ever-evolving nature of host–microbe interaction relies on the establishment and continuous adjustment of an intricate network of mechanisms controlling the availability of essential metabolites and nutrients. Perhaps the best studied of these is Fe, a central and vital nutrient required for host as well as microbial survival. This relationship is complex and pathogen class-specific strategies evolved to control Fe and heme availability to intracellular versus extracellular pathogens. Pathogen virulence often emerges via natural selection of genes that favor Fe and/or heme uptake by microbes, suggesting that targeting such mechanisms may be of therapeutic value to combat infectious diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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