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Brief Reviews

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Innate Nutritional Immunity

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Iron (Fe) is an essential micronutrient for both microbes and their hosts. The biologic importance of Fe derives from its inherent ability to act as a universal redox catalyst, co-opted in a variety of biochemical processes critical to maintain life. Animals evolved several mechanisms to retain and limit Fe availability to pathogenic microbes, a resistance mechanism termed "nutritional immunity." Likewise, pathogenic microbes coevolved to deploy diverse and efficient mechanisms to acquire Fe from their hosts and in doing so overcome nutritional immunity. In this review, we discuss how the innate immune system regulates Fe metabolism to withhold Fe from pathogenic microbes and how strategies used by pathogens to acquire Fe circumvent these resistance mechanisms. *The Journal of Immunology*, **2018, 201: 11–18.**

Iron accounts for an estimated one third of the Earth's mass, and presumably for this reason it was co-opted early through evolution as the divalent metal of choice for catalysis of a range of redox-based life-supporting reactions. The biologic importance of Fe resides in its intrinsic ability to shift between ferrous (Fe^{2+}) and ferric (Fe^{3+}) states, as well as higher oxidation states, via reversible exchange of electrons with electrophile or nucleophile molecules, respectively (1). Most forms of life evolved strategies to acquire Fe from their immediate environment, a tactic of particular relevance in the context of host–microbial interactions, where competition for limited Fe resources emerged as an evolutionary conserved host defense strategy against infection in plants (2) and animals (3–5) as well as a microbial virulence factor (6).

In mammals, the large majority of Fe exists in the form of heme, a hydrophobic Fe-based compound in which four methane-bridged pyrroles form a tetrapyrrole ring that binds Fe through nitrogen atoms (7, 8). Heme is used as a prosthetic group of proteins (i.e., hemoproteins) where Fe is deployed to exchange electrons and catalyze essential biochemical processes, such as the transport and storage of gaseous molecules, microbicidal activity, or energy metabolism. The major pool of heme is contained in hemoglobin (Hb) in RBC, a prime target for pathogens in search of this essential resource.

Although essential to support life, Fe can be deleterious when allowed to exchange electrons in an unrestrained manner with hydrogen peroxide (H_2O_2) (9), leading to the production of hydroxyl radicals and hydroxide ions via Fenton chemistry. This eventually leads to the production of hydroxyl radicals, which oxidize proteins, lipids, and DNA as well as cellular organelles, leading to oxidative stress and cellular damage. This potentially damaging process is controlled via cell-autonomous and systemic mechanisms that regulate the relative rate of cellular Fe import versus export as well as Fe subcellular localization (5). These regulatory mechanisms are critical to decouple Fe withholding from pathogens from Fe toxicity and cellular damage.

Several evolutionarily conserved mechanisms are operational in mammals to withhold Fe from pathogens and confer resistance to infectious diseases, a defense strategy termed "nutritional immunity" (10, 11). Although similar mechanisms exist to limit the availability of other micronutrients to pathogenic microorganisms, this review focuses on how innate immunity restricts Fe supply to pathogens and how pathogens overcome this resistance mechanism.

Macrophage control of systemic Fe homeostasis

Infections are initiated in most cases at epithelial barriers, encompassing the physical transition of microbes into the body of their hosts. This is associated with a major constraint, in that Fe is required to support microbial expansion, and, as such, infection relies strictly on the capacity of pathogenic microorganisms to acquire this essential nutrient from their hosts. Presumably, this explains why most pathogens evolved a variety of strategies aimed at diverting Fe from their hosts into their own metabolic pathways, whereas hosts coevolved strategies to restrict pathogens from accessing Fe (3–6). This fierce competition for scarce Fe availability dictates to some extent the establishment, progression, and outcome of infections.

Mammals acquire Fe^{2+} from the diet via a mechanism assisted by the divalent metal transporter ion transporter 1 (DMT1) expressed by duodenum enterocytes (12, 13). Although sufficient to compensate for physiologic loss of Fe associated with epithelial shedding or bleeding, dietary Fe fails to match the amounts

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Abbreviations used in this article: EM, erythrophagocytic macrophage; Fe, iron; Fe^{2+}, ferrous; Fe^{3+}, ferric; Hb, hemoglobin; HMOX1, HO-1, heme oxygenase-1; HP, haptoglobin; HPX, hemopexin; IRP, Fe regulatory protein; Lcn2, lipocalin 2; Nramp1, natural resistance–associated macrophage protein-1; Nrf2, NF-E2–related factor-2; PRR, pattern recognition receptor; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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needed to support heme biosynthesis associated with erythropoiesis as well as other biologic processes sustaining homeostasis (14). This is circumvented by the recycling of the Fe contained in the heme groups of Hb inside RBC (14–16), achieved largely via the continuous engulfment and digestion of senescent or damaged RBC by erythrophagocytic macrophages (EM) in the red pulp of the spleen (15) (Fig. 1). This process allows for the Fe contained in the heme groups of Hb to be extracted and directed into the bone marrow to support erythropoiesis, where Fe is inserted in the last step of heme biosynthesis and incorporated into nascent Hb (15) (Fig. 1). With perhaps some exceptions these regulatory mechanisms controlling systemic Fe metabolism are prime targets for Fe hijacking by microbial pathogens in mammals (Fig. 1).

Innate immune control of Fe availability to extracellular pathogens

Once confined to the microenvironment of an infected host, pathogenic microorganisms depend critically on their capacity to redirect host Fe into their own metabolic pathways to survive and proliferate. This can be achieved via the expression of a variety of microbially encoded high-affinity Fe-binding molecules known as siderophores (17) (Fig. 1). These are coupled to specific microbial receptors that capture Fe–siderophore complexes and allow for microbial Fe acquisition (6) (Fig. 1). This microbial Fe acquisition strategy is countered by reducing Fe concentration in plasma, a host defense strategy termed hypoferremia (3, 5). Central to this resistance mechanism is the secretion of hepcidin, an acute-phase 25-aa peptide encoded by the *HAMP* gene (18, 19). Hepcidin binds and triggers ferroportin degradation, inhibiting cellular Fe efflux systemically (18, 20, 21). *HAMP* transcription is induced in hepatocytes in response to 1) Fe, 2) microbial sensing by pattern recognition receptors (PRRs), or 3) cytokines produced by innate immune cells, including IL-1β, IL-6, or IL-22 (19, 22–24). The central contribution of hepcidin for nutritional immunity against extracellular microbes is illustrated by the increased susceptibility of *Hamp*-deficient ($Hamp^{-/-}$) mice to infection by extracellular Gram-negative bacteria, such as siderophilic *Yersinia enterocolitica*, *Vibrio vulnificus*, or *Klebsiella pneumoniae* (25–27).

Another mechanism by which innate immune responses restrain extracellular pathogens from accessing Fe involves lactoferrin, a member of the transferrin family of Fe-binding proteins that contributes to the antimicrobial activity of breast milk (28, 29). This glycoprotein secreted by macrophages and polymorphonuclear cells at mucosal surfaces binds Fe^{3+} with high affinity (K_D of $\sim 10^{-20}$ M) and in so doing restricts the virulence of a variety of extracellular pathogens (29–31). Lactoferrin also modulates the microbicidal activity of innate immune cells against extracellular bacteria, as illustrated for *Staphylococcus aureus* (32, 33), while reducing mucosal bacterial colonization, as illustrated for *Streptococcus mutans* in carious lesions (34, 35). Pathogenic bacteria evolved strategies to recapture Fe from lactoferrin (36, 37), for example by importing Fe-loaded lactoferrin via lactoferrin-binding proteins (38, 39).

Although conferring resistance to extracellular pathogens, hypoferremia is associated with a high evolutionary trade-off in that it leads to Fe overload in macrophages and parenchyma cells, interrupting Fe supply to support erythropoiesis, resulting in the development of anemia of inflammation (5). Consistent with this notion, individuals with genetic Fe-overload disorders, such as hemochromatosis, are highly susceptible to infection (40–43), and anemia is a clinical hallmark of many types of infection (5). These pathologic trade-offs are countered by tissue damage control mechanisms that limit the deleterious effects of cellular Fe overload (5, 44) via different mechanisms that involve Fe storage and neutralization by ferritin (44, 45).

Ferritins are evolutionary conserved multimeric protein complexes composed of adjustable ratios of heart/H chain (FTH) and liver/L chain (FTL) proteins, encoded by two distinct genes (45, 46). Ferritins acquire and neutralize intracellular Fe^{2+} from the Fe-chaperone poly(rC)-binding protein 1 (PCBP1) (47). The ferroxidase activity of FTH is critical for the conversion of Fe^{2+} into Fe^{3+}, allowing the storage of 4500 Fe^{3+} atoms per ferritin complex (45, 46). Ferritin expression is highly induced, posttranscriptionally, in response to Fe overload (48) as well as transcriptionally through the activation of the NF-κB family of transcription factors downstream of PRRs or cytokine receptors (49, 50). Activation of the transcription factor NF-E2–related factor-2 (Nrf2), in response to oxidative stress, is another important mechanism regulating ferritin expression (51). Induction of ferritin expression is essential to sustain host survival of extracellular bacterial infections (52) as well as *Plasmodium* infection (53). This protective effect is not associated with modulation of host pathogen load, revealing that ferritin establishes disease tolerance to infection (44, 54, 55).

Innate immune control of Fe availability to intracellular pathogens

The general strategy to limit Fe availability to intracellular pathogens consists in reducing intracellular Fe^{2+} concentration via mechanisms 1) inhibiting extracellular Fe uptake, 2) promoting Fe^{3+} storage in ferritin, or 3) increasing cellular Fe^{2+} efflux (5). In macrophages, the main conduit of Fe^{2+} from endosomes to the cytosol is the divalent metal ion transporter, named originally as natural resistance–associated macrophage protein-1 (Nramp1; also known as Slc11a1) (56, 57). Macrophages lacking Nramp1 activity exhibit higher intracellular Fe content, which is associated with increased pathogen load in endosomes (57, 58). Nramp1 reduces Fe concentration in phagosomes, restricting the vacuolar growth of intracellular pathogens such as *Salmonella enterica* serovar Typhimurium (*Salmonella*), *Leishmania donovani*, and *Mycobacteriun bovis*, but not *Listeria monocytogenes*, that exhibit a cytosolic life style (56–60). Importantly, C57BL/6 mice carry a loss-of-function mutation in the Nramp1 gene (61), which should be considered when extrapolating from this widely used experimental model system into human disease.

Some well-established strategies used by innate immune cells to confer resistance against intracellular pathogens act indirectly via a reduction of intracellular Fe content and should therefore be included within the conceptual framework of nutritional immunity. For example, generation of (NO•) by inducible NO• synthase (iNOS/NOS2) is a well-established host defense mechanism that confers resistance to *Salmonella*, *Leishmania*, or *Mycobacteria* (62). Briefly, superoxide ($\bullet O_2^-$) generated by NADPH oxidase (NOX) 2 family member ($gp91^{phox}$) (63) reacts with NO• and generates peroxinitrate ($ONOO^-$), a stable form of reactive nitrogen species (RNS) that is cytotoxic to pathogens and confers resistance to infections. Aside from its intrinsic microbicidal activity, RNS also activate the transcription factor Nrf2, which regulates

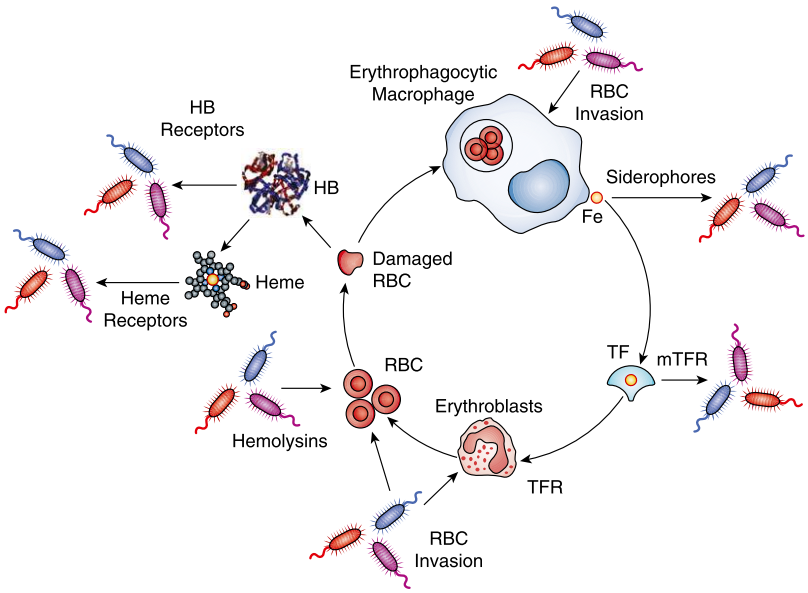

FIGURE 1. Microbial manipulation of heme-Fe metabolism. EM are generated via a lineage-specific genetic program controlled by the heme-responsive transcription factor SPI-C (100, 101). SPI-C regulates the expression of several effector genes coupling RBC sensing and engulfment with the breakdown of Hb and other RBC components, while sparing heme, which is transported to the cytosol by HRG1 (101, 102). Heme is degraded by heme oxygenase-1 (HMOX1 or HO-1), an inducible heme catabolizing enzyme constitutively expressed by EM (15). This allows for Fe extraction from heme and Fe transport via the cellular Fe exporter solute carrier family 40 member 1 (SLC40A1/ferroportin) (103–105). Once secreted, Fe is captured in plasma by transferrin (TF) and delivered via the transferrin receptor-1 (TFR) to erythroblasts in the bone marrow, where Fe is used in the last step of heme biosynthesis and incorporated into nascent Hb (15). Pathogenic microbes evolved several mechanisms that subvert these regulatory mechanisms of Fe metabolism. They can, for example, invade EM to access their heme-Fe content, use siderophores to capture Fe from plasma, acquire Fe bound to TF via microbial transferrin receptors (mTFR), or access heme-Fe by invading RBC. Pathogens can also lyse RBC via hemolysins to access their heme-Fe, or acquire Fe from extracellular Hb or from heme, using microbial Hb and heme receptors, respectively.

ferroportin expression, promoting Fe cellular export and reducing intracellular Fe content (64, 65). Importantly, the mechanism by which NO• confers resistance to *Salmonella* infection in mice (62) relates functionally to the activation of Nrf2 and the induction of ferroportin, withholding Fe from *Salmonella* (65, 66). To what extent this mechanism contributes to resistance to other intracellular pathogens remains to be established (67).

Although hepcidin-mediated downregulation of ferroportin is probably the major mechanism leading to hypoferremia in response to infection (19), ferroportin expression is inhibited independently of hepcidin in the context of *Salmonella* infection (68). Whether other intracellular pathogens (67, 69) use similar mechanisms to inhibit ferroportin expression and gain access to Fe remains to be determined.

Other defense strategies reducing intracellular Fe content rely on posttranscriptional regulation of Fe-responsive genes, afforded by the concerted action of Fe regulatory proteins (IRPs) (59). IRPs sense intracellular Fe content and bind *cis*-regulatory Fe-responsive elements on target mRNAs, including transferrin receptor-1 (TFR1), ferritin, and ferroportin, regulating their stability (59). This regulatory system is important to orchestrate nutritional immunity against intracellular pathogens, as demonstrated by the enhanced virulence of *Salmonella* infection in mice lacking IRP expression specifically in macrophages (70).

Withholding heme as a defense strategy against invading bacteria

Most of the current knowledge on host defense strategies limiting Fe availability to pathogens relates to mechanisms targeting elemental Fe. However, considering that each RBC contains 1.2×10^9 Fe molecules and that adult healthy humans carry ~2–3 $\times$ 10^{13} RBC, it is reasonable to envision why pathogens would evolve strategies to tap directly into this almost unlimited Fe resource (6) (Fig. 2). Perhaps the clearest example of such a strategy is the one co-opted by *Plasmodium* spp., which invade and proliferate inside RBC, accessing their heme-Fe content (71). Whereas *Plasmodium* spp. can synthetize heme, trypanosomatid protozoan parasites, such as *Leishmania* spp. and *Trypanosoma* spp., are natural heme auxotrophs that rely on host heme acquisition and transport for their survival. This occurs via different mechanisms that rely on heme transport by parasite-encoded heme-responsive gene 1 (HRG1/solute carrier family 48 member 1 or SLC48A1) or orthologous genes (72), as demonstrated for *Leishmania* spp. (73). and for *Trypanosoma* spp. (74).

Bacterial pathogens co-opted alternative strategies, which rely on the expression of hemolysins, damaging RBC and releasing their Hb content (Fig. 2). Extracellular Hb dissociates readily into dimers, which are prone to auto-oxidation, releasing their prosthetic heme groups and generating labile heme (75, 76) (Fig. 2). Gram-negative pathogenic bacteria, such as *Y. enterocolitica, Shigella*, *Escherichia coli* O157:H7, *Yersinia pestis*, or *Haemophilus influenzae*, express outer membrane Hb and/or heme receptors, whereas *Y. pestis*, *Pseudomonas aeruginosa*, and *Serratia marcescens* secrete hemophores that bind extracellular heme (3, 77). These are recognized by outer membrane TonB-dependent heme, hemoprotein, and hemophore receptors, respectively, which shuttle heme to the bacterial periplasm to be transported into the cytosol by specific ABC protein–dependent periplasmic permeases (3, 77). The cytosolic heme is used to

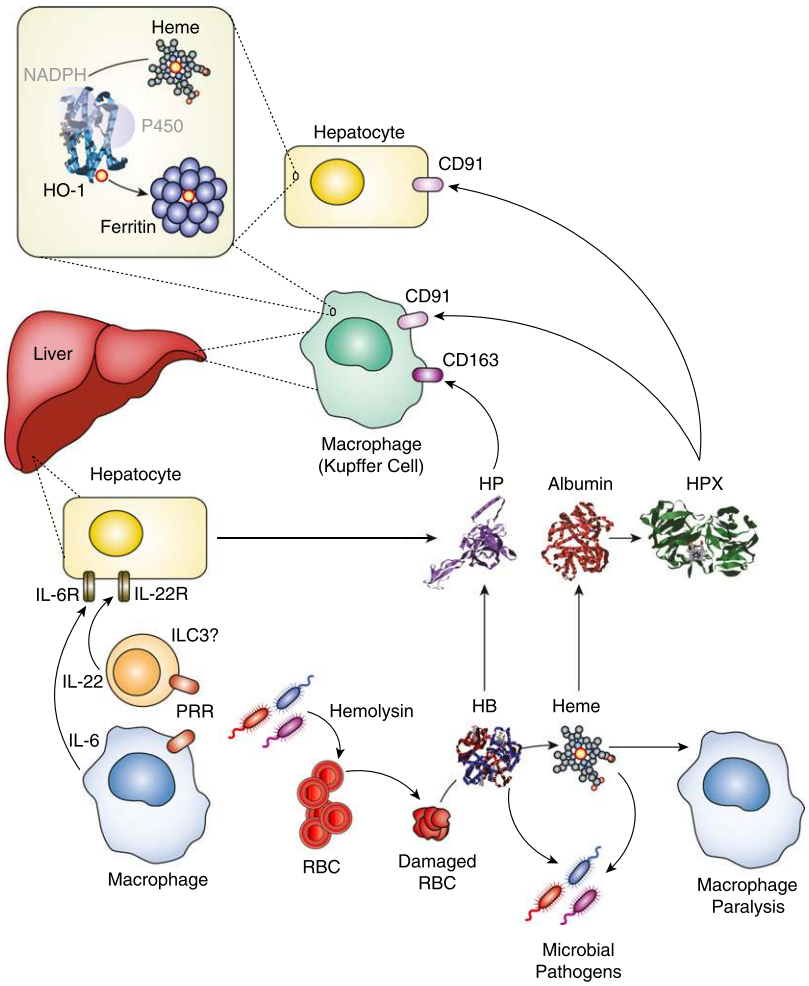

FIGURE 2. Heme-based nutritional immunity. Microbial sensing via PRRs, expressed by innate immune cells, triggers the secretion of cytokines such as IL-6 or IL-22, which induce the expression of HP or HPX in the liver. HP is an acute-phase glycoprotein that displays high affinity for Hb dimers (K_D of $\approx 10^{-12}$ M) (106–108) and prevents heme release from Hb dimers (109, 110). HPX is also an acute-phase glycoprotein, which displays high affinity for heme (K_D of $< 10^{-12}$ M). Albumin, the most abundant heme-binding protein in the plasma, displays affinity toward heme (K_D of $< 10^{-8}$ M) (111) and is thought to play a key role in heme scavenging, transferring labile heme in plasma to HPX (112). Although HPX was thought to act essentially to prevent the pathogenic effects of labile heme (79), it also acts as a host defense strategy against invasion by pathogenic and commensal bacteria (81). Hb–HP complexes are recognized by CD163 expressed in macrophages whereas HPX–heme complexes are recognized by the low-density lipoprotein receptor–related protein (LRP)/CD91 expressed in macrophages but also in hepatocytes (113). Upon binding to CD163, Hb–HP complexes undergo endocytosis and are targeted for lysosomal proteolysis, a process coupled to heme transport into the cytosol, and subsequent targeting of heme for catabolism by heme oxygenase-1 (HO-1). Upon recognition by CD91 in hepatocytes, HPX–heme complexes undergo endocytosis (113) and HPX is recycled, whereas heme is catabolized by HO-1 (113). In both cases, the Fe released via heme catabolism by HO-1 is stored by ferritin, away from microbial pathogens. Although RBC lysis by microbial bacteria is thought to act essentially as an Fe acquisition strategy (6), labile heme generated through this process can disrupt the cytoskeleton dynamics of innate immune cells impairing bacterial phagocytosis via a mechanism involving dedicator of cytokinesis 8 (DOCK8) (114). Similar mechanisms are likely to operate in the context of malaria to impair *Plasmodium*-infected RBC clearance.

extract Fe by heme oxygenases (3, 77). Gram-positive pathogenic bacteria, such as *S. aureus*, *Bacillus anthracis*, and *Corynebacterium diphtheriae*, also rely on heme acquisition systems to establish infection (78).

Acquisition of labile heme from plasma is countered by the acute-phase heme scavenger hemopexin (HPX) (Fig. 2). Although thought to act exclusively as part of a protective response limiting the pathogenic effects of labile heme (79, 80), HPX also acts as a component of nutritional immunity, limiting heme availability to extracellular bacteria, as demonstrated for *Citrobacter rodentium* and commensal *E. coli* (81). This defense strategy is orchestrated by IL-22, a cytokine produced by innate immune cells, that acts on hepatocytes, where it induces the expression of HPX (81) (Fig. 2). Whether Hb scavenging by haptoglobin (HP) also acts as a component of nutritional immunity is likely, but this remains to be established.

Pathogen strategies to circumvent nutritional immunity

Some highly virulent bacterial pathogens deploy "Fe piracy" strategies, hijacking Fe or heme from host Fe- or heme-binding molecules and in so doing subvert nutritional immunity (82–84). For example, *Neisseria* spp., *H. influenzae*, and *Moraxella catarrhalis* deploy transferrin-binding proteins (TbpA/TbpB) that capture host Fe-bound transferrin (6). Other bacteria express lactoferrin receptors to capture Fe^{3+}-bound lactoferrin (6). Most bacterial pathogens secrete siderophores that bind Fe^{3+} at extremely high affinity and can (re)capture Fe from host Fe-binding proteins such as transferrin or lactoferrin (85). This strategy is perhaps best illustrated for

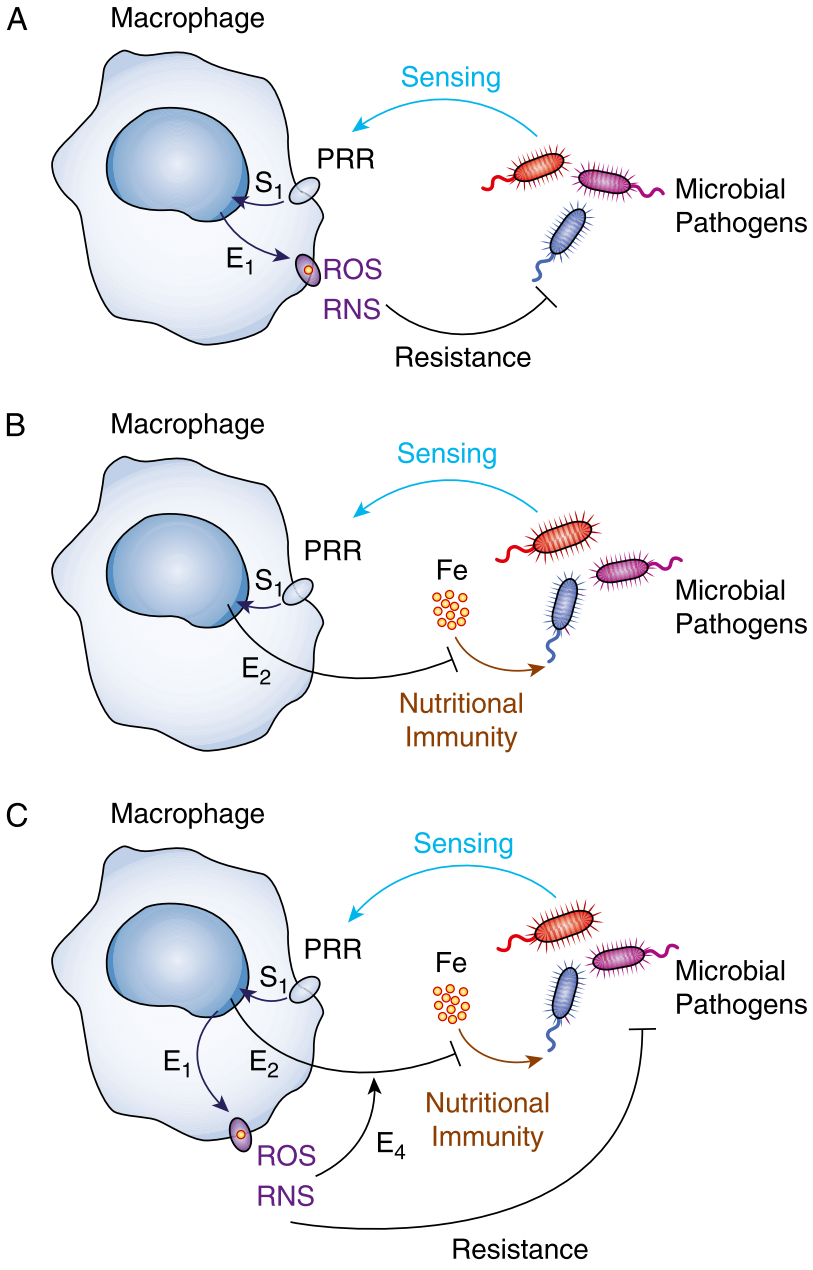

FIGURE 3. Integration of nutritional immunity as an innate resistance mechanism. Pathogen sensing via PRR signaling (S_1) activates the expression of effector genes conferring resistance to pathogens. These encode molecules (E_1) such as NOX2 and NOS2 that induce intrinsic microbicidal activity via ROS and RNS (**A**), as well as molecules (E_2) withholding heme-Fe from pathogens, such as ferroportin (15) (**B**). ROS and RNS can regulate ferroportin and nutritional immunity via activation of the transcription factor Nrf2 (E_3) to optimize resistance to infections (**C**).

enterobactin, a prototypical siderophore produced by Enterobacteriaceae (85). Siderophores such as enterobactin are essential for Fe acquisition by many bacterial pathogens such as *E. coli* or *Salmonell*a, a strategy coupled to the expression of siderophore receptors by these pathogens (85, 86).

Innate immune cells secrete molecules that impair the action of microbial siderophores, such as lipocalin 2 (Lcn2/neutrophil gelatinase-associated lipocalin; NGAL, siderocalin) (87). Lcn2 is a soluble peptide secreted by activated polymorphonuclear cells, monocytes/macrophages, and epithelial cells in response to the engagement of PRRs. Lcn2 binds siderophores such as enterobactin and delivers Lcn2–Fe–siderophore complexes to host cells via the Lcn2 receptor (88, 89), inhibiting bacterial uptake of Fe-laden siderophores (87, 90). This defense strategy is not specific to bacteria in that it also acts against protozoan parasites, as illustrated for example for *Plasmodium* infection (91). This defense strategy is countered by some pathogens via a mechanism that relies on the production of modified siderophores, such as, for example, the glycosylated enterobactin salmochelin or the production of structurally unrelated siderophores not bound by Lnc2 (92–94). The variety of siderophores produced by any given bacterial strain provides a high level of functional redundancy reflecting the enormous selective pressure imposed on pathogens to acquire Fe under the Fe-withholding conditions of the host (95–97).

Innate immune control of Fe availability as a resistance mechanism against infection

Pathogen sensing via PRRs activates resistance mechanisms in innate immune cells, which rely on the microbicidal activity of reactive oxygen species (ROS) and RNS (Fig. 3). Additionally, pathogen sensing via PRRs promotes resistance mechanisms in innate immune cells, which withhold heme-Fe from pathogens (15) (Fig. 3). Importantly, this defense strategy modulates cellular Fe content, which impacts the microbicidal activity of innate immune cells

such as macrophages (30, 98, 99). This argues for a tight integration between these two resistance responses, as suggested further by the observation that in some instances the microbicidal activity of ROS and RNS relies on the induction of effector mechanisms preventing pathogens from accessing Fe and possibly heme (65) (Fig. 3). This suggests that the microbicidal activity of innate immune cells involves a functional interplay between cytotoxic and Fe-depriving mechanisms.

Conclusions

Although the concept of nutritional immunity was proposed over 40 years ago (10), this defense strategy has not gained much attention among immunologists. This is paradoxical because one of the central resistance mechanisms deployed by innate immunity relies on starving pathogens from essential micronutrients required for their proliferation. This defense strategy is particularly relevant for Fe, a micronutrient essential for virtually all microbial pathogens. Given that the large majority of Fe in mammals is contained inside heme, microbial pathogens evolved to capture heme as the means to access Fe whereas hosts coevolved to withhold heme from microbial pathogens. This strategy is illustrated by the finding that some acute-phase proteins such as the heme scavenger HPX are a central component of nutritional immunity that confers resistance to bacterial infections. This defense strategy must be coupled to tissue damage control mechanisms that detoxify heme-Fe and contribute to establishing disease tolerance to infections. To what extent this coupling of nutritional immunity and disease tolerance acts as a universal host defense strategy against infections remains to be established.

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Disclosures

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