Iron is a vital nutrient for virtually all forms of life. The requirement for iron is based on its role in cellular processes ranging from energy generation and DNA replication to oxygen transport and protection against oxidative stress. Bacterial pathogens are not exempt from this iron requirement, as these organisms must acquire iron within their vertebrate hosts in order to replicate and cause disease.

Vertebrates Sequester Iron from Invading Pathogens

One of the first lines of defense against bacterial infection is the withholding of nutrients to prevent bacterial outgrowth in a process termed nutritional immunity. The most significant form of nutritional immunity is the sequestration of nutrient iron [1]. The vast majority of vertebrate iron is intracellular, sequestered within the iron storage protein ferritin or complexed within the porphyrin ring of heme as a cofactor of hemoglobin or myoglobin. Further, the aerobic environment and neutral pH of serum ensures that extracellular iron is insoluble and hence difficult to access by invading pathogens. This difficulty is enhanced by the serum protein transferrin, which binds iron with an association constant of approximately 10^{36} [2]. Taken together, these factors ensure that the amount of free iron available to invading bacteria is vastly less than what is required to replicate and cause disease (Figure 1A).

The importance of nutritional immunity as it pertains to iron is exemplified by the increased susceptibility to infection of individuals with iron overload due to thalassemia and primary iron overload is perhaps best demonstrated by the enhanced transferrin-mediated iron sequestration [2]. The impact of this iron overload is perhaps best demonstrated by the enhanced susceptibility of hemochromatosis patients to *Vibrio vulnificus* infections [4]. Whereas *V. vulnificus* is killed by normal blood and rarely causes infection in healthy individuals, it grows rapidly in blood from patients with hemochromatosis, leading to a high risk of fatal infections in this cohort [4]. Moreover, the administration of excess iron increases the virulence of numerous pathogens in animal models, further highlighting the protection provided by nutritional immunity [2,5].

Many Bacterial Pathogens Sense Iron Depletion as a Signal That They Are within a Vertebrate Host

Vertebrates are devoid of free iron, ensuring that all bacterial pathogens encounter a period of iron starvation upon entering their hosts. In keeping with this, bacterial pathogens have evolved to sense iron depletion as a marker of vertebrate tissue. This sensing typically involves transcriptional control mediated by the iron-dependent repressor known as Fur (ferric uptake regulator) [6]. Fur binds to target sequences in the promoters of iron-regulated genes and represses their expression in the presence of iron. In the absence of iron, Fur-mediated repression is lifted and the genes are transcribed. Fur orthologs have been identified in numerous genera from both Gram-negative and Gram-positive bacteria and contribute to the virulence of both animal and plant pathogens [7].

A number of genes encoding for proteins involved in iron utilization have been reported to be positively regulated by Fur during iron-replete conditions [8]. This positive regulation occurs through Fur-mediated repression of a small RNA that represses genes encoding iron utilization proteins. This second level of regulation prevents the use of iron by non-essential enzymes during times of iron starvation. RNA-dependent regulation of iron utilization is a conserved process that has been identified in multiple bacterial pathogens, including *Vibrio* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, and *Bacillus subtilis* [8].

Many high G+C content Gram-positive bacteria express an additional iron-dependent repressor belonging to the DtxR family. The DtxR family was named for its founding member, the diphtheria toxin repressor. In fact, one of the first iron-dependent virulence factors described was diphtheria toxin produced by *Corynebacterium diphtheriae* [2]. DtxR family members negatively regulate genes involved in processes ranging from iron acquisition to virulence factor expression [5].

In addition to sensing alterations in iron levels, bacterial pathogens can also sense heme as a marker of vertebrate tissue. Heme-responsive activators have been identified in *Serratia marcescens*, the pathogenic *Bordetella*, *C. diphtheriae*, *Bacillus anthracis*, and *Staphylococcus aureus* [5,9,10,11]. Heme-sensing systems presumably alert bacterial pathogens when they are in contact with vertebrate tissues rich in heme, triggering the expression of systems involved in heme-iron acquisition and metabolism.

All Bacterial Pathogens Can Circumvent Iron Withholding

In order to thrive within vertebrates, bacteria must possess mechanisms to evade nutritional immunity. Perhaps the most
Figure 1. A representative battle during the war for iron. (A) In a healthy individual iron is largely intracellular, sequestered within ferritin or as a cofactor of heme complexed to hemoglobin within erythrocytes. Any extracellular free iron is rapidly bound by circulating transferrin. Hemoglobin or heme that is released as a result of natural erythrocyte lysis is captured by haptoglobin and hemopexin, respectively. Taken together, these factors...
ensure that vertebrate tissue is virtually devoid of free iron. (B) During infection, bacterial pathogens are capable of altering the battlefield to increase the abundance of potential iron sources. Bacterial cytoxins damage host cells, leading to the release of ferritin while hemolytic toxins lyse erythrocytes, liberating hemoglobin. The resulting inflammatory response includes the release of lactoferrin from secondary granules contained with polymorphonuclear leukocytes (PMNs). Bacterial pathogens are capable of exploiting these diverse iron sources through the elaboration of a variety of iron acquisition systems. (C) Bacterial pathogens can acquire iron through receptor-mediated recognition of transferrin, lactoferrin, hemopexin, hemoglobin, or hemoglobin–haptoglobin complexes. Alternatively, secreted siderophores can remove iron from transferrin, lactoferrin, or ferritin, whereupon siderophore–iron complexes are recognized by cognate receptors at the bacterial surface. Analogously, secreted hemophores can remove heme from hemoglobin or hemopexin and deliver heme to bacterial cells through binding with hemophore receptors. Siderophore-mediated iron acquisition is inhibited by the innate immune protein sidocalin, which binds siderophores and prevents receptor recognition. This host defense is circumvented through the production of stealth siderophores that are modified in such a way as to prevent sidocalin binding.

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Targeting Bacterial Iron Acquisition as a Second Layer of Defense against Infection

A second layer of nutritional immunity employed by vertebrates is to combat siderophore-mediated iron acquisition through the production of sidocalin [18]. Siderocalin, also referred to as lipocalin-2 or neutrophil gelatinase-associated lipocalin (NGAL), is a protein that is secreted by neutrophils in response to infection. Siderocalin binds enterobactin, the primary siderophore of many enteric bacteria, and sequesters the siderophore–iron complex, preventing bacterial uptake. Mice lacking siderocalin exhibit increased sensitivity to enterobactin-expressing bacteria, demonstrating the pathophysiological relevance of this anti-siderophore defense system [19].

The requirement for iron by bacterial pathogens ensures that iron acquisition systems are expressed and surface exposed during infection. This fact has established surface-exposed iron receptors as viable vaccine candidates for the prevention of bacterial infection. The enterobactin receptor FepA from Neisseria meningitidis [20], the siderophore receptor IroN from Escherichia coli [21], the hemoglobin receptor HgbA from Haemophilus ducreyi [22], surface proteins of the S. aureus Isd heme uptake machinery [23], and a combination of E. coli iron acquisition proteins [24] are examples of iron utilization systems that have been proposed as candidate vaccines.

Bacterial Pathogens Are Leading the Arms Race for Nutrient Iron

Resistance to sidocalin is a conserved strategy across multiple pathogenic microbes. A primary bacterial defense against sidocalin involves the production of stealth siderophores. These molecules represent structurally modified enterobactin-type siderophores that are resistant to sidocalin binding. The Gram-positive pathogen B. anthracis produces the siderophore petrobactin, which incorporates a 3,4-dihydroxybenzoyl chelating subunit that prevents sidocalin binding [25]. Similarly, Salmonella Typhimurium produces salmochelin, a glycosylated derivative of enterochelin that is not targeted by sidocalin [26]. The production of stealth siderophores is the most recently uncovered layer in the arms race for nutrient iron during host-pathogen interactions. Undoubtedly, we have not yet discovered the complete armamentarium in this battle that has tremendous implications for the outcome of bacterial infections.

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