Iron is essential to all microorganisms. To obtain iron from the very low concentrations present in their environment, microorganisms have developed sophisticated mechanisms such as the siderophore system. As a primitive defense mechanism, humans have developed mechanisms to withhold iron from microorganisms. Iron-binding proteins such as transferrin, ferritin, and lactoferrin have a central role in human ferrokinetics. These iron-binding proteins also participate in the process of decreasing iron availability for the microorganisms. They do so by decreasing iron reutilization.

Anemia of inflammation (previously called anemia of chronic disease) is seen in the setting of infectious, inflammatory, and neoplastic diseases. It results, in part, from changes in the intracellular metabolism of iron. Alterations of iron physiology seen in many clinical circumstances make excess iron available to microorganisms, thus enhancing their pathogenicity. Understanding the molecular basis of iron withholding by the human host, both in the absence of and during infection, and that of iron acquisition by microorganisms may provide us with new and innovative antimicrobial agents and vaccines.

Iron is vital for microorganisms [1–3]. There is abundant iron in the human body, most of which is bound to hemoglobin, myoglobin, and the cytochromes. Most of the iron that is not integrated to these essential proteins is bound to the iron-binding proteins (transferrin, lactoferrin, and ferritin). These iron-withholding mechanisms make the amount of iron available to bacteria extremely small, on the order of $10^{-15} M$. Because the iron requirements of microorganisms are on the order of $10^{-6} M$, as an evolutionary response, they have developed different mechanisms to obtain it. The human host uses iron withholding as a form of nonspecific immunity to discourage infection, and this sequestration is involved in the pathogenesis of anemia of inflammation (AoI). When excess iron becomes available under certain clinical circumstances (e.g., hemolysis, trauma, hemochromatosis, the use of desferrioxamine, iron supplementation, or transfusions), it becomes possible for certain microorganisms to proliferate and cause disease.

Iron Needs of Microorganisms

The fact that microorganisms can multiply in vivo and cause infections despite the virtual absence of freely available iron in body fluids suggests that they have the capacity to adapt to the iron-restricted environment by the development of sophisticated mechanisms to overcome iron scarcity. The most important of these adaptive mechanisms is the siderophore system. Microorganisms have developed high-affinity iron-bind-

Iron withholding is mediated by a series of iron-binding proteins: ovoalbumin, transferrin, lactoferrin, and ferritin [8]. The binding and saturation characteristics of these iron-binding proteins result in concentrations of free iron on the order of $10^{-15} M$. Moreover, under normal circumstances, there is an excess of unoccupied binding sites in plasma transferrin, which are capable of binding extra iron. This extra iron-binding capacity will serve to keep levels of free iron constant during periods of rapid entry of the metal into the circulation.

Ovoalbumin

Ovoalbumin (conalbumin) was the first protein discovered that suggested an important role for iron in host defense mechanisms. The first observations of the antibacterial properties of iron-binding proteins were made rather serendipitously by Shade and Carolin [9] in 1944.
Transferrin

Schade and Caroline [10] reasoned that an iron-binding protein with functions similar to that of conalbumin must be present in humans. They demonstrated such a protein, initially called siderophilin, and showed its antimicrobial capacity in human serum. Later on, Holmberg and Laurell [11] suggested the name transferrin. Transferrin’s affinity for iron is pH-dependent (the lower the pH the lower the affinity). This binding characteristic of transferrin has been implicated in the pathogenesis of rhinocerebral mucormycosis in patients with diabetic ketoacidosis [12, 13], in whom acidemia increases free iron (by decreasing transferrin’s binding affinity). The zygomycete will use this excess iron to its advantage, leading to invasive rhinocerebral mucormycosis. Lambert and Hunter [14] demonstrated the role of unsaturated transferrin as a predictor of survival in cases of pneumococcal infections.

Lactoferrin

Lactoferrin is a 78-kD cationic protein present in mammalian milk (where it was originally identified, hence the name), certain mucosal secretions, and polymorphonuclear (PMN) leukocytes (where it is a component of the specific [secondary] granules). Using sonicated extracts of rabbit PMN leukocytes, Bullen and Wallis [15] found that lactoferrin present in PMN leukocytes was 14%–40% saturated with iron. Thus, lactoferrin has the capacity to bind relatively large amounts of free iron. Lactoferrin synthesis and release from the secondary granules is increased by IL-1, as part of the acute-phase reaction (APR) [16]. Release of apolactoferrin (iron-free lactoferrin) by PMN leukocytes into an area of bacterial growth leads to its in situ combination with iron.

In contrast to conalbumin and transferrin, lactoferrin’s affinity for iron increases as pH decreases. Thus, lactoferrin is an efficient iron scavenger and/or trap at sites of infection or inflammation, where the pH has been lowered by the release of lactic acid and other by-products of the leukocytes and/or microbial metabolism. Several reports have attested to the importance of lactoferrin in host defenses by showing that patients with lactoferrin-deficient PMN leukocytes have an increased susceptibility to infections [17–20].

Two facts contribute to lactoferrin’s efficiency in iron sequestration in the reticuloendothelial system. First, because of its higher affinity for iron at lower than physiological pH, lactoferrin binds more iron than transferrin at sites of inflammation. Second, lactoferrin receptors, while present on the surface of macrophages, are not expressed on the surface of erythroid precursors. During the APR, the macrophages become activated. Activated macrophages exhibit an increase in the concentration of lactoferrin receptors on their surface, which further increases the internalization of iron (keeping it away from the microorganisms and the erythroid precursors). Thus, lactoferrin-bound iron is taken up by macrophages in preference to erythroid precursors. Within the macrophage, the lactoferrin-bound iron is transferred to the iron-storage molecule ferritin (vide infra), which traps it in a more stable form. Besides its role in iron withholding, lactoferrin has an intrinsic bactericidal capacity [21].

Ferritin

Although seldom saturated, a ferritin molecule can store up to 4,500 iron atoms. Under basal conditions, iron stores closely regulate the synthesis of ferritin. During inflammation, ferritin synthesis is increased under the influence of IL-1 and TNF [22, 23]. Cytokines can also induce ferritin synthesis indirectly by increasing iron uptake by hepatocytes. An expanded intracellular pool of iron in turn stimulates ferritin synthesis [24]. Following turpentine-induced inflammation, increased ferritin synthesis precedes the decrease in serum iron. Maximal reduction in serum iron occurs after 12 hours of inflammation [25]. Thus, both increased lactoferrin delivery of iron to macrophages and increased ferritin synthesis play central roles in the sequestration of iron as part of the pathogenesis of AoI (see below).

AoI

AoI became a nosologic entity following the seminal article by Cartwright and Wintrobe [26]. It is a very common type of anemia [27]. I agree with Schilling [28] that we should abandon the misnomer ‘‘anemia of chronic disease’’ and replace it with AoI, which better reflects our growing understanding of its pathogenesis. AoI is usually mild, with hematocrits ranging between 30% and 40% and hemoglobin levels of 9–13 g/dL. Nonetheless, in about 20% of cases, the hematocrit may drop below 25% [29]. A pathological process, such as chronic infections, inflammatory infections, or neoplastic diseases, is usually present at the time of detection of AoI. The underlying problem is usually easily identified. Occasionally, AoI is the first manifestation of such an underlying disease. The word chronic may also be misleading, because changes in hemoglobin levels and hematocrit can occur in <2 weeks [30].

Pathogenesis of AoI

AoI is a multifactorial and complex process [31, 32] whose many steps are mediated by cytokines such as IL-1, TNF, and IFN. Increased plasma concentrations of these cytokines have been described in patients with disorders associated with AoI [33–36]. In addition, the therapeutic administration of recombinant human TNF and IFN to patients has been reported to produce anemia [37, 38]. AoI is mainly the result of decreased RBC production secondary to decreased iron availability and erythropoietin (EPO) dysfunction.
Decreased iron availability is due to decreased iron reutilization and decreased iron absorption.

**Decreased iron reutilization.** In addition to the iron-binding proteins, reticuloendothelial macrophages play a central role in decreased iron reutilization. As part of the APR, macrophages increase their lactoferrin receptors, thereby enabling them to internalize more lactoferrin-bound iron. Iron released from the reticuloendothelial macrophage is reduced by inflammation [39, 40]. In patients with inflammatory conditions, ferritin synthesis is increased in the activated reticuloendothelial cells [41, 42]. This increase in ferritin synthesis precedes hypoferremia [25], thus suggesting that increased iron binding by ferritin may be the mechanism responsible for the reduction in iron output from the reticuloendothelial cell.

Iron kinetic studies have demonstrated the existence of a small labile pool of iron (compartment A in figure 1) with a rapid turnover rate [43, 44]. The iron in this labile pool is either released into the blood or transferred to a storage pool (compartment B figure 1). The size and capacity for iron release of the labile pool is the same for normal individuals and for patients with anemia secondary to rheumatoid arthritis. The storage pool, however, is greatly expanded in patients with rheumatoid arthritis. Thus, an increase in the storage pool will lead to iron withholding and play a central role in iron underutilization, thereby underscoring the importance of the macrophage storage capacity in the pathogenesis of AoI.

**Decreased iron absorption.** Decreased iron absorption plays only a minor role in hypoferremia in patients with AoI. Transferrin may play a role in these changes in iron absorption. Once synthesized in the liver, apotransferrin is secreted in bile into the gastrointestinal tract lumen. In the lumen, it binds iron and delivers it to the transferrin receptor located in the brush border of the mucosal cell [45]. Because transferrin synthesis is decreased during the APR, interference with iron absorption at this step may explain the decreased iron absorption in patients with chronic inflammation.

**Alterations in EPO Metabolism**

There are data suggesting that EPO disturbances are a central component of the pathogenesis of AoI.

**Decreased EPO synthesis.** EPO release in response to anemia is blunted in patients with AoI [46]. This subnormal response may be the result of the action of several cytokines [47]. Serum and urine EPO levels are lower than expected for the level of anemia in patients with AoI [48–53].

**Impaired EPO action.** Hematocrit rises in response to EPO in patients with AoI, but the magnitude of the change is subnormal, thus suggesting decreased responsiveness of the erythroid precursors to EPO. This unresponsiveness may be the result of macrophage-derived factors [54–58].

**Decreased RBC survival.** RBC survival in patients with AoI is typically reduced to 60–90 days compared with the normal 90–120 days [59, 60]. Probably more important in decreased RBC life span in patients with AoI is the role of macrophages in RBC disposal. A normal function of the macrophages is removal of senescent RBCs from the circulation. Therefore, it is possible that macrophages activated by the inflammatory process will exaggerate this normal function. Senescent RBCs and those coated by Igs or immune complexes are cleared more efficiently by an activated phagocytic system [61]. Although fever per se has been reported to decrease RBC survival [62], not all patients with AoI are febrile.

Cartwright and Lee [63] speculated that AoI—given that the anemia is mild and thus of little, if any, deleterious effect to the host—could represent some appropriate homeostatic mechanism. I submit that the purpose of AoI is to deprive invading microorganisms of iron. This possible role of AoI could be integrated into the concept of "nutritional immunity" (figure 2). Iron withholding in patients with AoI may be construed as a host defense mechanism in that it is an attempt to deprive the invading microorganisms of iron as a nutrient. Fever (a component of the APR), independent of cytokine-induced hypoferremia, may also contribute to iron deprivation. Elevated temperature suppresses the synthesis of siderophores and their receptors [64, 65]. Thus, fever appears to have two synergistic effects aimed at decreasing bacterial growth rates:
fever, an acute and severe hemolytic disorder caused by *Bartonella bacilliformis* (a gram-negative bacterium found almost exclusively in Peru and Ecuador) [73]. If salmonella bacteremia occurs during the acute hemolytic phase of bartonellosis, the prognosis is worse than if it occurs following the abatement of the hemolytic process, which suggests that hemolysis (with the consequent increased iron availability) contributes to the severity of salmonella bacteremia.

**Nutritional Immunity**

Figure 2. IL-1 and other cytokines mediate both arms of nutritional immunity. By mediating fever, they decrease siderophore production by the bacteria. Through the acute-phase reaction (APR), they participate in the pathogenesis of anemia of inflammation (AoI). Both these effects lead to bacterial iron deprivation.

Hemochromatosis and Other Iron Overload States

Hemochromatosis, the epitome of iron overload, is associated with an increased incidence of certain infections. Serum from patients with hemochromatosis contains a pool of iron complex not present in healthy individuals from which bacteria can readily obtain iron [76]. *Vibrio vulniﬁcus* is one of the microorganisms seen in association with hemochromatosis. Although this bacterium is usually inhibited by human serum, it thrives in the presence of 1 mg of hemoglobin/mL, whether or not it is complexed with haptoglobin [77]. This microbe can grow rapidly in sera of individuals with hemochromatosis [78]. The growth of *V. vulniﬁcus* in sera of patients with hemochromatosis can be inhibited by the addition of human apotransferrin. Primary hemochromatosis was present in 50% of cases of multiple liver abscesses due to *Yersinia enterocolitica*, and other forms of iron overload (Bantu siderosis, thalassemia major, and iron therapy) were present in 13% [79]. The incidence of infections with *Yersinia pseudotuberculosis* in patients with hemochromatosis is also increased [80].

Patients with chronic renal failure frequently have multifactorial iron overload. Before the use of EPO, these patients received multiple transfusions (each unit of whole blood contained 200 mg of iron). These patients also received frequent parenteral iron preparations. Moreover, one or more alleles for hemochromatosis may be present that predispose patients undergoing dialysis to iron overload [81].

It is therefore of interest that 10 episodes of yersinia bacteremia (nine due to *Y. enterocolitica* and one due to *Y. pseudotuberculosis*) were detected in a cohort of Belgian patients undergoing dialysis [82]. Before having yersinia bacteremia, the patients had received a mean dose of parenteral iron ± SD of 24.9 ± 19.5 g; their mean ferritin concentration ± SD was 2.1 ± 2.125 ng/mL, and stained bone marrow specimens from five patients were strongly positive for iron. Two of these episodes
occurred within 4 months of the initiation of desferrioxamine therapy (vide infra). Data on invasive yersinia disease and iron status were obtained for 1,656 patients undergoing dialysis in Belgium. Yersinia bacteremia was described in five of 539 individuals with serum ferritin levels of >500 ng/mL, while no cases occurred in 882 individuals with serum ferritin levels of <500 ng/mL (P < .05).

**Therapeutic Use of Desferrioxamine**

Desferrioxamine is a siderophore produced by *Streptomyces pilosus*. It has a high affinity for ferric iron and a greater iron-binding efficiency than transferrin. Desferrioxamine provides iron to bacteria through mobilization from tissue deposits (the first step in the elimination of the excess iron burden and an incidental occurrence when desferrioxamine is being used for aluminum chelation). Iron is thus made available to microorganisms, which having the capacity through their siderophore receptors to internalize and utilize it, can increase their growth and virulence [85, 86].

There are many reports on the increased incidence of certain types of infections, such as yersinia [82, 87–89] and zygomycete [90–92] infections, related to the therapeutic use of desferrioxamine. The frequent use of desferrioxamine for treatment of aluminum toxic effects in patients with chronic renal failure potentially increases the risk of infection with iron-dependent organisms. In the setting of renal failure, the half-lives of desferrioxamine and its chelates (ferroxamine and aluminoxamine) are markedly prolonged, by as much as 10-fold. This alteration in desferrioxamine pharmacokinetics will allow microorganisms longer periods to utilize the chelated iron. There have been multiple reports of invasive zygomycosis in patients with chronic renal failure who are treated with desferrioxamine [93–96]. On the basis of these reports, the use of this agent for patients with renal failure should be approached with great caution.

Although the main indications for the use of desferrioxamine are iron and aluminum overload, it is likely that its therapeutic indications will extend to such conditions as malignancy and autoimmune disorders [97]. Thus, the number of patients at risk for desferrioxamine-associated invasive yersinia infection and mucormycosis may increase in the near future.

**Blood Transfusion**

Repeated blood transfusions can lead to iron overload. In addition to iron overload, transfused blood per se has been associated with certain bacterial infections in which free iron in the stored blood (as a result of in vitro hemolysis) may play a role. Increased iron availability in the stored blood facilitates the proliferation of certain microorganisms. In most cases of transfusion-associated yersinia bacteremia reported in the literature, the involved blood had been stored for >3 weeks (there is a linear correlation between duration of storage and extent of hemolysis) [98]. Besides iron availability, the temperature at which blood is stored plays a role in the specific organisms involved. *Yersinia* and other psychrophilic organisms (such as *Enterobacter agglomerans*) grow well at 4°C, the temperature at which blood is stored. *Yersinia* can multiply to large numbers in the stored blood without showing visible evidence of contamination, such as microagglutination, turbidity, or overt hemolysis. *Yersinia* is not commonly found in the environment, and most cases of transfusion-related yersinia bacteremia appear to be related to asymptomatic or only mildly symptomatic donors (as evidenced by clinical or serological data) [98, 99].

**Diabetic Ketoacidosis**

The classical association of mucormycosis (zygomycete) with diabetic ketoacidosis and other disorders characterized by acidosis (i.e., renal tubular acidosis) is most likely multifactorial. There is evidence that one of the pathogenetic factors is increased iron availability secondary to acidosis [12, 13].

**Therapeutic Implications of the Role of Iron in Infections**

Understanding the molecular basis of iron acquisition by pathogenic microorganisms has provided new insights that are potentially useful for the development of innovative therapeutic measures and alternatives to antibiotic use. One such approach is the production of antibacterial agents that interfere with the siderophore–siderophore receptor system. The siderophore enterochelin is produced by a number of pathogenic enterobacteria and could be complexed with ions other than iron (such as scandium [Sc³⁺] or indium [In³⁺]) to yield antimetabolites. Experimental data suggest this may be a viable approach [100–102]. The use of ferrisiderophore receptors as antigenic sites for the development of vaccines has also been explored [103]. Eaton et al. [70] have proposed the use of haptoglobin in the treatment of potentially fatal infections in which available hemoglobin may have a role.

**Summary**

Iron plays a role both in bacterial pathogenicity and in host defense mechanisms, which has frequently been underestimated. Bacterial pathogenicity is multifactorial, and iron is but one of its components. Besides the complex role iron plays directly in microbial biology (as described in this review), excess iron also leads to decreased phagocytosis and deficient oxidative metabolism. Furthering our knowledge in this field
may provide us with a better understanding of microbial pathogenicity and lead to some innovative approaches to antimicrobial therapy.

References


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