The importance of iron in the pathogenesis of infection and neoplasia

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Iron is essential for all living organisms, with the exception of some Lactobacilli species, because of its key role in electron transport, DNA synthesis and various essential redox reactions[1,2]. Consequently, iron deprivation in bacteria results in cessation of growth and eventual death of the population. This essential requirement of living cells for iron has important implications in the pathogenesis of infection and neoplasia. Thus, when microorganisms invade the vertebrate host, or when neoplastic cells disseminate, iron availability affects the successful establishment of the disease.

Iron is not freely available to microbes in the vertebrate host because of its association with the high-affinity iron-binding proteins lactoferrin and transferrin[3]. Lactoferrin (Lf) is positioned on mucosal surfaces and is also present in exocrine secretions such as tears and mucus[3]. By virtue of its high affinity for iron, Lf can defeat microbial growth at strategic sites of entry. The unavailability of iron is also a problem for pathogens which invade the blood stream. The blood of vertebrate hosts contains two major pools of iron. First, there is iron associated with hemoglobin, the oxygen-carrying protein of blood. However, because hemoglobin is sequestered within erythrocytes it is not directly available to an extracellular pathogen. The second pool of iron is that associated with the glycoprotein transferrin (Tf) in the plasma. Transferrin is the major iron transport protein of plasma and, as such, acts as a shuttle between sites of absorption of iron and sites of utilization[3]. It is present in concentrations of 2–4 mg ml^{-1} of plasma and each molecule of the protein bears two binding sites, each of which can bind one atom of ferric iron with very high affinity[3]. Under normal conditions, only 30% of the binding sites of Tf are occupied by iron[3]. The high affinity of Tf for iron limits the availability of the metal to microbial invaders, and thus forms part of the bacteriostatic activity of plasma[4–6]. Extensive evidence indicates that the antimicrobial activity of plasma is inversely proportional to the percentage saturation of circulating Tf[5,6]. Hence, microbial growth *in vitro* is severely reduced in plasma samples with abnormally low concentrations of iron bound to Tf; this bacteriostatic effect is readily reversed by adding sufficient iron to saturate the Tf pool[5,6]. The evidence obtained from studies *in vivo* is also particularly convincing. In naturally occurring hyperferremic states, such as hemachromatosis and siderosis, patients show a greatly increased susceptibility to various infectious diseases and to neoplasia[7–11]. Likewise, administering iron compounds to experimental animals before exposure to infectious organisms greatly increases the magnitude and the severity of the infection[12–15]. This phenomenon has particularly significant implications for therapeutic iron supplementation, since such therapy has been shown to be associated with a recrudescence of tuberculosis, brucellosis and malaria[5]. The ability to enhance infections and neoplasia is unique to iron and has not been observed with other metals[5]. Because of its *partial* saturation in the normal state, the plasma Tf pool can be regarded as a biochemical buffer which can bind excess iron. This ability to withhold iron from pathogenic organisms in hyperferremic states, acts as a protective mechanism.

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Aquisition of iron by microbes and neoplastic cells

To overcome the difficulty of obtaining iron in the normal host, a few pathogenic species of bacteria produce iron-binding molecules of low molecular weight, called siderophores[16]. Siderophore production is induced by low iron concentrations and the molecules are generally secreted extracellularly to chelate iron in the environment. By virtue of their high affinity for iron, siderophores can successfully compete with Tf for the metal, thereby satisfying the iron requirements of the producing organism[16]. Siderophore production has been shown to be an important virulence factor in a variety of studies *in vitro* and *in vivo*[5,6]. The remarkable efforts that microbial species make to obtain iron from their host underline the importance of iron in disease pathogenesis. More recently, research on iron requirements of biological systems has been greatly stimulated by extending the concept to neoplastic cells. Growth of tumor cells within the host depends upon sufficient iron acting as a co-factor for the enzyme ribotide reductase, an essential enzyme in DNA synthesis. Still higher concentrations of iron are required by the enzymes involved in energy metabolism. Accumulating evidence indicates that neoplastic cells can also acquire iron from Tf, either directly via membrane associated Tf receptors[17–20] or indirectly by using a siderophore-like growth factor[21].

Regulation of iron metabolism during infection and neoplasia

From the above discussion, it would appear that microorganisms and neoplastic cells equipped with efficient means of acquiring iron from Tf would circumvent the problem of iron unavailability in the host. Fortunately, the picture is more complex as a result of metabolic alterations induced by disease states. A distinctive response to infection, inflammation and neoplasia is a reduction in the concentration of iron associated with the plasma Tf pool[5,6]. This response has been regarded as a host's attempt to withhold essential iron from the bacterial pathogens or neoplastic cells[5,6], but its exact role in other disease states has not yet been defined. The frequent occurrence of hypoferremia in inflammatory states suggests that this is a systemic and non-specific response. Hypoferremia can be induced experimentally by injecting turpentine or administering sham infectious agents such as endotoxin, bacterial cell walls or live attenuated bacteria. Induction of the response has been shown to protect an organism subsequently challenged with infectious agents or neoplastic cells[5]. Conversely, when the host is overloaded with iron, thus impairing its ability to mount an efficient hypoferremic response, the incidence and severity of infection greatly increases[5,6]. Despite the widespread occurrence of the hypoferremic response and its underlying importance as a defence mechanism, the exact nature of the processes mediating

the reduction of Tf iron has remained obscure for many years.

Proposed mechanism for the hypoferremic response

Under normal conditions, iron metabolism proceeds as a closed cycle characterized by a very efficient reutilization of endogenous sources of iron (Fig. 1). In the plasma compartment, Tf is the major iron transport protein distributing the metal to sites of utilization (predominantly the erythropoietic tissues of the bone marrow). In this compartment, iron is incorporated into heme and returns to the vascular compartment as hemoglobin in erythrocytes. Eventually, erythrocytes are phagocytosed by macrophages of the reticuloendothelial (RE) system, hemoglobin is catabolized and heme-derived iron is released into the cytosol of RE cells. Some of this iron enters the intracellular pool of the iron-binding protein, ferritin, and the rest associates with soluble intermediates of low-molecular-weight in the cytosol. This latter pool supplies plasma Tf with iron, completing the cycle.

In view of the cyclic nature of iron metabolism, we can postulate two mechanisms by which iron concentrations in the plasma Tf pool are reduced. Thus, hypoferremia can result from accelerated removal of iron from the plasma Tf pool, and/or impaired return of RE-associated iron to the plasma compartment. Van Snick *et al*[22] have reported evidence for accelerated removal of iron from the plasma Tf pool during inflammation. In their model, lactoferrin (Lf) released at sites of inflammation by neutrophils, removes iron from Tf or competes with Tf for iron released into the plasma pool. Subsequently, Lf is rapidly taken up by macrophages of the RE system where iron is stored. The short-coming of this proposal stems from the chemistry of iron-binding of Lf and Tf. Transferrin has a much higher affinity for iron at physiological pH than does Lf, while the opposite is true as pH decreases[3]. Assuming that the relative affinities of Tf and Lf for iron on the basis of studies *in vitro* are the same *in vivo*, it seems reasonable that Lf could obtain iron from Tf under conditions of low pH at localized sites of inflammation. However, this would be far less likely in the plasma compartment where physiological pH prevails.

The possibility of accelerated removal of iron from the plasma Tf pool during pathogenesis has been investigated during meningococcal infection in mice[23].

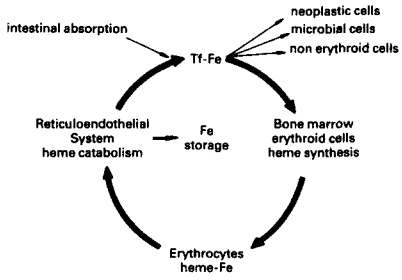

Fig. 1. Cycle of iron metabolism in vertebrates. (Tf = transferrin.) Reproduced from *Infection and Immunity* by permission of the American Society of Microbiology.

Iron supplied specifically as Tf-iron left the plasma compartment at normal rates in infected animals[23]. This is direct evidence that there is no accelerated removal of iron from the plasma Tf pool during infection and that hypoferremia results from an impaired release of RE-associated iron.

The kinetics of iron release by RE cells can be studied using ^{59}Fe-labeled heat-denatured erythrocytes. Heme iron acquired by the RE system apparently enters a labile iron pool, part of which is immediately returned to circulating Tf while the rest becomes associated with a ferritin pool with a slower turnover (Fig. 2)[24]. This has been shown in normal mice where some heme-derived iron is processed and promptly released to the extracellular plasma Tf pool[25]. The rest of the heme-derived iron stays within the ferritin pool of RE cells for later release. However, during infection all heme-derived iron remains within RE cells of the liver. Thus, hypoferremia results from impaired release of heme-derived iron from the RE system to the plasma Tf pool. This mechanism has now been reported during infection in mice[25] and after injection of endotoxin and turpentine in rats[26,27].

The impaired iron release could result from three main mechanisms, as outlined in Fig. 2. Firstly, heme catabolism might be reduced; secondly, the intracellular movement of heme-derived iron might be altered; and thirdly, the transfer of iron across the RE cell-plasma interface could be blocked. Fractionation of RE cell iron compartments in liver homogenates of infected and normal mice indicates that heme catabolism proceeds at a normal rate during infection[25], ruling out the first mechanism. If the transfer of iron across the RE cell-plasma interface had in any way been hampered, one would expect a build-up of intracellular soluble iron intermediates but this was not seen. The impairment of iron release appears to result from a difference in the relative distribution of iron between the intracellular soluble and ferritin pools. In normal mice, heme-derived iron is directed to both the soluble and ferritin pool simultaneously. However, during meningococcal infection in mice, iron is preferentially incorporated into ferritin and only low concentrations enter the soluble pool. Consequently, little iron is available for release to the circulating Tf pool[25]. This preferential incorporation of heme-derived iron to the ferritin pool within the cytosol of RE cells appears to be the underlying mechanism for impaired RE-iron release during the hypoferremic phase of infection.

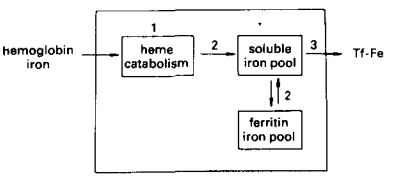

Fig. 2. Possible mechanisms for impaired processing of iron by the RE system during infection. Impaired release of iron during infection could result from reduced heme catabolism[1], impaired intracellular movement of heme-derived iron[2], or blockage of the transfer of iron across the RE cell–plasma interface. (Tf = transferrin.) Reproduced from *Infection and Immunity* by permission of the American Society of Microbiology.

The nature of the mechanism mediating the altered intracellular movement of iron during hypoferremia remains undetermined. Two studies have reported a *de novo* synthesis of intracellular ferritin during infection[25] and inflammation[27]. An enlarged ferritin pool creates a driving force for the preferential incorporation of heme-derived iron into that pool. However, if this is the case, what triggers the synthesis of ferritin? A potential candidate for the signal which induces the alterations in iron metabolism is the leukocytic endogenous mediator (LEM) released by macrophages at sites of inflammation. This small molecule induced hypoferremia in several experimental systems[28–30]. Furthermore, LEM also triggers the acute-phase response of infection, which is characterized by the *de novo* synthesis of some plasma proteins[30]. The molecular mechanism of LEM action and its role in mediating hypoferremia remain to be determined.

Conclusions and perspectives

The extensive evidence supporting the importance of iron during infection and neoplasia raises some interesting questions. What triggers the mechanism

leading to hypoferremia? Could this be used to induce hypoferremia for prophylaxis or therapy on a short-term basis? Can infections and neoplasms be controlled by interfering with the iron acquisition systems of microorganisms and tumor cells? More research on the regulation of iron metabolism during pathogenesis of disease should ultimately reveal ways of using it to help control infections and neoplasia.

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Phosphoinositide turnover provides a link in stimulus–response coupling

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Much excitement has resulted from the discovery that phosphatidylinositol and its phosphorylated derivatives are storage forms of messenger molecules that can be released in response to specific extracellular signals. Signal molecules bind to cell surface receptors and the messenger molecules derived from the phosphoinositides couple the signal to a variety of responses within and between cells.

Phosphoinositides are minor phospholipids (~5% of phospholipid) that turnover much more rapidly than other membrane lipids. Hokin and Hokin were the first to observe that acetylcholine accelerates this turnover rate[1]. Their results with pancreatic tissue have since been observed repeatedly in a myriad of tissues from yeast and insects to man[2]. Although many theories have been proposed, no clear function for the process was demonstrated until recently.

The production of icosanoid mediators (oxygenated derivatives of arachidonic acid and related polyunsaturated fatty acids such as prostaglandins, thromboxanes, leukotrienes) is limited by the release of arachidonate from phospholipids. In 1979, it was discovered that hydrolysis of phosphatidylinositol (PI) by phospholipase C resulted in transient accumulation of 1,2-diglyceride in platelets[3]. Diglyceride[4]- and monoglyceride-lipases[5] were found to be involved in sequential degradation of diglyceride in platelets to release arachidonate. This was the first evidence for phosphoinositide turnover as a signal-generating system. The mechanism for arachidonate release operates in many cells and tissues, although other mechanisms do exist. In platelets, it appears that the initial release of arachidonate after stimulation is from inositol phospholipids, while that released later is from phosphatidylcholine[6].

A second function for diglyceride was discovered by Nishizuka *et al.* who described a diglyceride-dependent protein kinase, designated protein kinase C, that phosphorylates serine and threonine residues of various cellular proteins[7]. This enzyme has a hydrophilic catalytic domain and a hydrophobic membrane-binding domain and is located in the cytosol of unstimulated cells where it is presumably inactive. According to a current hypothesis, when 1,2-diglyceride is produced, protein kinase C binds to the cell membrane (presumably to phosphatidylserine) and shifts the Ca^{2+}-dependence of the enzyme from mM to μM concentrations, thereby activating the enzyme. Protein kinase C activation has been studied most extensively in blood platelets where phosphorylation of a 40 kDa protein follows stimulation by physiological agonists. The functions of this and other protein substrates phosphorylated by protein kinase C are not yet known.

The third and most recently discovered signal molecule generated by phosphoinositide turnover is inositol 1,4,5-trisphosphate (IP_3), one of the products of phospholipase C hydrolysis of phosphatidylinositol 4,5-diphosphate (PI 4,5-P_2, see Fig. 1). Berridge *et al.*[8] proposed that IP_3 is the messenger that triggers intracellular calcium fluxes in cells on the basis (initially) of two observations: phosphatidylinositol turnover takes place in all tissues in which calcium fluxes occur upon cell activation[9]; and polyphosphoinositide turnover precedes

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