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MINI REVIEW

Hypothesis: Iron chelation plays a vital role in neutrophilic inflammation

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Neutrophil influx into tissues occurs in many diverse diseases and can be associated with both beneficial and injurious effects. We hypothesize that the stimulus for certain neutrophilic inflammatory responses can be reduced to a series of competing reactions for iron, with either a labile or reactive coordination site available, between host chelators and chelators not indigenous to that specific living system. The iron focuses the transport of host phagocytic cells through a metal catalyzed generation of oxidant sensitive mediators including cytokines and eicosanoids. Many of these products are chemotactic for neutrophils. We also postulate that the iron increases the activity of the phagocyte associated NADPH oxidoreductase in the neutrophil. The function of this enzyme is likely to be the generation of superoxide in the host's attempt to chemically reduce and dislodge the iron from its chelate complex. After the reoxidation of Fe^{2+} in an aerobic environment, Fe^{3+} will be coordinated by host lactoferrin released by the neutrophil. When complexed by this glycoprotein, the metal does not readily undergo oxidation/reduction and is safely transported to the macrophages of the reticuloendothelial system where it is stored in ferritin. Finally, we propose that the neutrophil will attempt to destroy the chelator not indigenous to the host by releasing granular contents other than lactoferrin. Inability to eliminate the chelator allows this sequence to repeat itself, which can lead to tissue injury. Such persistence of a metal chelate in the host may be associated with biomineralization, fibrosis, and cancer.

Keywords: free radicals, inflammation, iron, iron chelates, neutrophils

Introduction

Iron is an essential micronutrient utilized in almost every aspect of normal cell function and is particularly crucial for the conservation of energy (Crichton & Ward 1995). All higher forms of life derive energy through reactions of organic molecules and, in animals, through the oxidation of hydrocarbons to carbon dioxide. This process is dependent on oxygen (O_2) as a final electron acceptor. Ground state O_2 exists as a triplet which presents a kinetic block to reactions with singlet molecules. Transfer of electrons to O_2 is often catalyzed by transition metals such as iron since they can provide a pathway to overcome this singlet–triplet reaction barrier. Iron also has the characteristic that it forms stable six coordinate complexes with octahedral or near-octahedral symmetry. As a result of its interactions with O_2, its tendency towards donor–acceptor complex formation (coordination), and its abundance in nature, iron was selected in molecular evolution to carry out a wide range of biological functions.

Those same chemical properties which allow iron, with either a labile or reactive coordination site available, to function as a catalyst in the reactions

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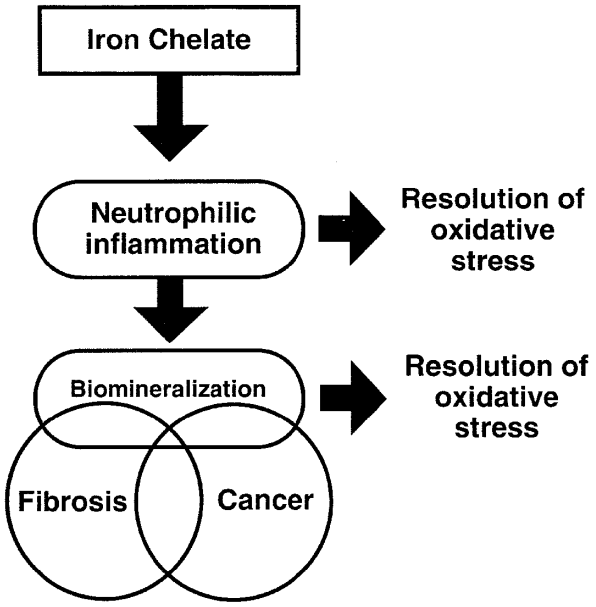

Figure 1. Schematic representation of the relationships of iron chelates, neutrophilic inflammation, biomineralization, fibrosis, and cancer. It is also possible to have a neutrophilic inflammatory response present with biomineralization, fibrosis, and cancer.

of molecular oxygen make it a threat to life via the generation of O_2 based free radicals:

$$Fe^{2+} + O_2 \leftrightarrows Fe^{3+} + O_2^-$$

$$Fe^{2+} + O_2^- + 2H^+ \leftrightarrows Fe^{3+} + H_2O_2$$

$$Fe^{2+} + H_2O_2^- \leftrightarrows Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$$

While an organism must obtain iron to catalyze homeostatic and synthetic functions, O_2^-, H_2O_2, and $^{\bullet}OH$ generated by the metal have a capacity to damage biological molecules. Consequently, living systems transport and store iron with all coordination sites of the metal fully complexed (Crichton & Charloteaux-Wauters 1987). However, environments can expose the host to iron chelates that have either a labile or reactive coordination site available (e.g. siderophores, substances in microbial membranes, and functional groups at the surfaces of mineral oxide dusts). Oxidant generation due to the presence of such iron chelates might be expected to damage host tissues (Torti & Torti 1994). For the survival of the host, mechanisms must have evolved to respond to these agents. We hypothesize that neutrophilic inflammation opposes the injurious effects which extracellular iron, with either a labile or reactive coordination site available, presents (Figure 1).

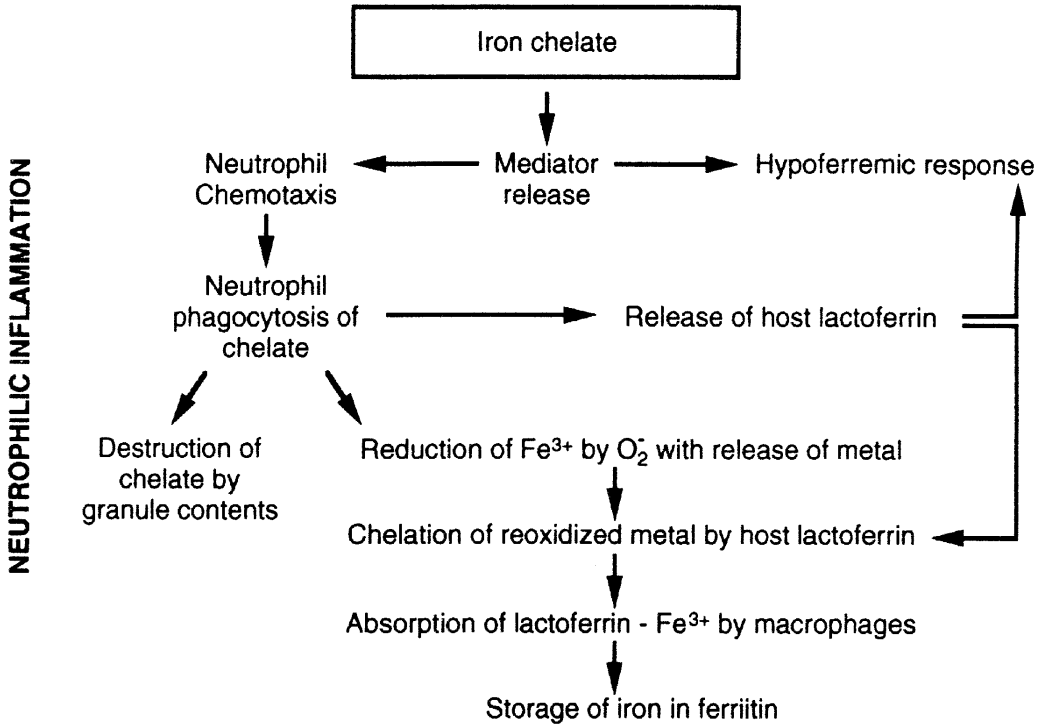

Figure 2. Neutrophilic inflammation can be viewed as a series of reactions accounting for the reduction and displacement of iron from a chelate not indigenous to a specific living system. The iron is later complexed by host lactoferrin and sequestered in ferritin.

Iron and neutrophilic inflammation

(Figure 2)

Teleologically, microbial utilization of host iron would be predicted to be an extremely important example of the formation of a chelate not indigenous to a living, multicellular system. Pathogenic bacteria must acquire iron from the host to multiply (Bullen *et al.* 1974). In support of this, the capacity of bacteria to procure host iron can correlate both with the ability of the infectious agent to cause disease, and with the severity of the resultant injury (Neilands 1981, Cross 1984). In further support of this dependence of microbials on available metal, iron chelators can inhibit their replication (Bullen & Armstrong 1979, Arnold *et al.* 1980, Wright *et al.* 1981, Bortner *et al.* 1986). During the acquisition of host metal by a bacterium, a coordination site on the iron can become available and the metal then presents an oxidative burden (Britigan *et al.* 1992). An influx of neutrophils to the site of infection is integral in the host response to microbes. Other metal chelates (e.g. mineral oxide dusts and bleomycin) can also introduce an oxidative stress to a living system and an incursion of host neutrophils follows comparable to the influx of these cells with infection (Burger *et al.* 1981, Lugano *et al.* 1982, White *et al.* 1987, Schapira *et al.* 1995). We postulate that iron, with either a labile or reactive coordination site available, focuses such an influx through a generation of oxidant sensitive mediators. The oxidative stress resulting from exposures to such chelates is associated with a release of cytokines and arachidonate products which are chemotactic for neutrophils (Lewis *et al.* 1988, Barradas *et al.* 1989, Affres *et al.* 1991, Vogt *et al.* 1991, Simeonova & Luster 1995).

It would be advantageous to the host to: (1) sequester the metal; and (2) destroy the chelator which is not indigenous to that specific living system. Metal exchange mechanisms require a host ligand that can compete for Fe^{3+} with bacterial siderophores, substances in the microbial membrane, and surface functional groups of inorganic particles. We hypothesize that, in animals, this function is accomplished by lactoferrin. In support of such a role, this glycoprotein has a stability constant (K_{stab}) approximating 10^{36} and radiolabelled iron associated with a microbe can be complexed by lactoferrin (Molloy & Winterbourn 1990). However, it is unlikely that lactoferrin alone can sequester iron away from these chelates. All ligands, including siderophores, have different affinities for Fe^{2+} and Fe^{3+}, with K_{stab} values for ferrous ion being considerably less than those for ferric ion in many cases. These dissimilarities offer a possible mechanism to remove ferric iron from a chelate such as a siderophore, a substance in a microbial membrane, and the surface of a mineral oxide dust. Parallel evolutionary development may account for similarities in the methods employed by all living organisms in the acquisition of iron. Bacteria, phytoplankton, and plants most frequently procure requisite Fe^{3+} by reduction of the metal using superoxide (Bienfait *et al.* 1983, Jones *et al.* 1987, Dancis *et al.* 1992). The enzyme responsible for this reduction of iron is an NADPH oxidase which catalyzes the reaction:

$$2NADPH + 2O_2 \leftrightarrows 2NADP + 2O_2^- + 2H^+$$

Neutrophils perform the same enzymatic function with an NADPH oxidoreductase (Bellavite 1988). This enzyme is normally inactive in the resting cell and appears to be assembled on a membrane after exposure to specific stimuli (Rossi 1986, Jesaitis *et al.* 1990). We propose that available iron will increase the activity of this enzyme and that, once at the membrane, the superoxide produced by this NADPH oxidoreductase reduces the metal, dislodging it from the ferric ligand:

$$Fe^{3+}L + O_2^- \leftrightarrows Fe^{2+}L + O_2$$

$$Fe^{2+}L \leftrightarrows Fe^{2+}\ (aq.) + L$$

Evidence supports this postulate. First, the NADPH to $NADP^+$ ratio can be increased with iron depletion and, with iron supplementation, the ratio corrects itself (Sijmons *et al.* 1984). Second, exposure of phagocytes to microbes grown in iron depleted conditions is associated with less chemiluminescence, reflecting a diminished activity of NADPH oxidoreductase (Domingue *et al.* 1989). Third, O_2^- production by resting neutrophils can correlate with serum ferritin (Lipschitz *et al.* 1974, de Martino *et al.* 1984). Finally, there is a significant homology between the gene for a subunit of the phagocyte associated NADPH oxidoreductase and the ferric reductase gene *frpi*$^+$ (Romas *et al.* 1993). Comparable structure suggests a similar purpose which is likely the reduction and transport of iron.

Iron is then displaced from sites of inflammation after its reoxidation to Fe^{3+} in an aerobic environment and its coordination by lactoferrin released from the secondary granules of neutrophils:

$$Fe^{2+} + L_1 \leftrightarrows Fe^{2+}L_1$$

(where L_1 is a foreign chelator)

$$Fe^{2+}L_1 + O_2 \leftrightarrows Fe^{3+}L_1 + O_2^-$$

$$Fe^{3+}L_1 + L_2 \leftrightarrows Fe^{3+}L_2 + L_1$$

(where L_2 is lactoferrin)

This is the back reaction of the reduction of iron by superoxide generated by NADPH oxidoreductase. Both reactions can occur because of the differences in stability constants of chelates for Fe^{3+} and Fe^{2+}, strong host chelates with Fe^{3+}, and variation in the availability of superoxide and oxygen in the immediate environment. The resultant lactoferrin–Fe^{3+} is taken up by specific receptors on macrophages (van Snick *et al.* 1975, Markowetz *et al.* 1979). The transport of the iron, implicated as the target of neutrophilic inflammation, concludes as the metal is stored in ferritin (Birgens *et al.* 1988, Birgens 1991). This deposition in the macrophage is histologically detected as a sideromacrophage and can be a reliable marker of neutrophilic inflammation (van Snick *et al.* 1975, Konijn & Hershko 1977, Birgegard & Caro 1984).

In addition to the events that occur in the phagosome, lactoferrin in secondary granules is released extracellularly (Leffell & Sptiznagel 1975). Extracellular lactoferrin can react with small pools of available iron causing a fall in plasma iron (van Snick *et al.* 1974) followed by the movement of the metal to stores of ferritin and hemosiderin in monocytes and the macrophages of the reticuloendothelial system (Cartwright & Lee 1971). Extracellular lactoferrin will also inhibit iron absorption at the small intestine (de Vet & ten Hoopen 1978) and suppress hematopoiesis (Bullen 1987). The 'anemia of chronic disease' which results is likely to be a host mediated attempt to limit the availability of iron to a chelator not indigenous to that living system (Bharadwaj *et al.* 1991).

Finally, after the release of secondary granules containing lactoferrin and the burst of O_2^- generation, contents of the primary granule are delivered to the phagosome in an attempt by the host to destroy the foreign chelate (e.g. the microbe).

Rather than being a maladaptive response, neutrophilic inflammation is absolutely essential for survival. The iron was initially present with a labile or reactive coordination site available and was consequently capable of oxidant generation and injury to host tissues. After reduction by superoxide produced by NADPH oxidoreductase in the neutrophil, reaction with lactoferrin released by the neutrophil (Britigan *et al.* 1989), and deposition in ferritin of the reticuloendothelial system (Olakanmi *et al.* 1993), the iron is rendered catalytically less active.

The risk a foreign iron chelate poses to the host is not always successfully resolved by the neutrophilic inflammatory response. In these instances, rather than terminating any threat and therefore being of benefit, inflammation can become detrimental to the host. For example, iron chelates of mineral oxide dusts may perpetuate a chronic neutrophilic inflammatory response in the lung. Neutrophils converge to the iron complexed by the surface of the dust and can both successfully reduce, chelate, and store the Fe^{3+} using the phagocyte associated NADPH oxidoreductase, lactoferrin, and ferritin, respectively. However, the phagocytes are unable to destroy the particle with proteases and endogenous oxidants such as hypochlorous acid. Rather than the elimination of the chelate, the released granular contents contain the particle which can take up more Fe^{3+} from the host and reinitiate the cycle. As the cycle repeats itself, the neutrophils ultimately produce tissue injury. The host response to the metal chelate can then include biomineralization, fibrosis, and cancer.

Iron and biomineralization

(Figure 3)

We hypothesize that, in addition to the neutrophilic inflammatory response, biomineralization (e.g. dystrophic calcification) also functions to inactivate the oxidative stress which iron chelates pose to a living system. Dystrophic calcification can be observed in tissue injury associated with neutrophilic inflammation (e.g. asbestos related pleural plaques, histoplasmosis, and atherosclerosis). Such biomineralization has previously been postulated to be a method of detoxification of metal cations by cells (Simkiss 1977, Mason & Nott 1981). The anionic component of the secreted product (e.g. phosphate and carbonate) may occupy either labile or reactive coordination sites on the metal. In support of a role of iron chelates in biomineralization, subcutaneous injection of iron salts induces a calciphylaxis (Boivin 1975, Raica 1990, Anghileri 1992) and iron chelators have a capacity to inhibit ectopic calcification (Williams *et al.* 1984, Putcha *et al.* 1985, Soriano *et al.* 1987). In further support of this relationship, the metal can be detected in the outer rim and core of Blue bodies which are inclusion bodies of calcium carbonate (Gardiner & Uff 1978, Koss *et al.* 1981). Finally, microlithiasis could be the presentation of extensive biomineralization at sites of inflammation after exposure of the host to different chelates (e.g. M. tuberculosis) (Koss *et al.* 1981).

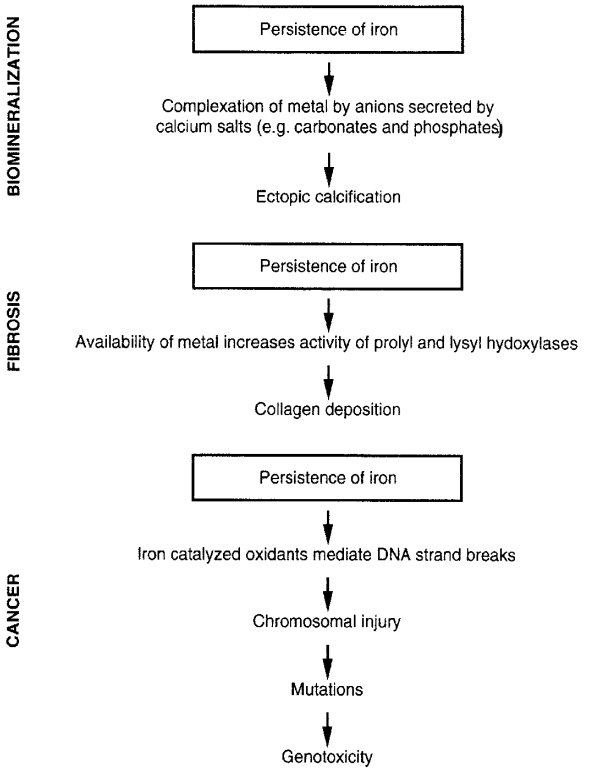

Figure 3. Sequelae of neutrophilic inflammation possibly result from the persistence of chemically reactive iron complexed to a chelate not indigenous to a specific living system.

Iron and fibrosis

(Figure 3)

We also propose that an increased availability of iron, with either a labile or reactive coordination site, not eliminated by inflammatory cells and not incapacitated by biomineralization, can result in a fibrotic injury to a tissue. Prolyl and lysyl hydroxylases are enzymes which play a central role in collagen synthesis (Prockop 1971). These dioxygenases couple the oxidative decarboxylation of 2-oxoglutarate to the hydroxylation of prolyl and lysyl residues, respectively. Both enzymes use molecular oxygen as a substrate and require ascorbate and iron as cofactors (Hutton *et al.* 1967). Prolyl hydroxylase does not contain stoichiometric amounts of iron (Pankalainen & Kivirikko 1971) but rather the metal is loosely bound in a non-heme form. Elevations in the concentration of available iron could increase the activity of prolyl and lysyl hydroxylases, resulting in collagen deposition and fibrosis. Another dioxygenase, tyrosine hydroxylase, which catalyzes the conversion of tyrosine to L-DOPA, is stimulated by the addition of iron *in vitro* (Rausch *et al.* 1988). In support of this relationship of metal availability to collagen synthesis, exposure to iron chelates with a labile or reactive coordination site, such as the ferrous–bleomycin coordination complex, can directly increase prolyl hydroxylase activity (Giri *et al.* 1983). In further support, iron chelators, including 2,2′-dipyridyl and deferoxamine (Hunt *et al.* 1979, Franklin *et al.* 1991, Geesin *et al.* 1991), can inhibit prolyl hydroxylase. Finally, dietary depletion of iron (Chandler *et al.* 1988) and metal chelation (Kennedy *et al.* 1986) can inhibit lung fibrosis after exposure to such chelates.

Iron and cancer

(Figure 3)

Finally, we hypothesize that carcinogenesis can be a consequence of an exposure of host tissues to increased concentrations of available iron with a labile or reactive coordination site. Cancers are associated with both inflammation and fibrosis (e.g. scar carcinoma and hepatocarcinoma after cirrhosis) (Cruickshank *et al.* 1963, Ames 1983, Cerutti 1985). Accumulation of iron, not eliminated by inflammation and not deactivated by biomineralization, can mediate damage to DNA by its catalysis of hydroxyl and ferryl radicals (Hutchinson 1985, Imlay & Linn 1988). DNA strand nicks and mutations resulting from oxidant exposure are assumed to be events in tumor induction. There are several indications that iron can participate in the pathogenesis of cancer. A role for available iron in cancer induction is suggested by an increased risk of developing primary liver cancer (14%) and other extrahepatic malignancies in patients with idiopathic hemochromatosis (Hann *et al.* 1991). Second, iron dextran given intramuscularly can induce cancers (Weinbren *et al.* 1978). In addition, ferritin, an indicator of iron stores, can reflect the biological activity and potential aggressiveness of neoplasms (Parry *et al.* 1975, Jacobs *et al.* 1976a,b, Wahren *et al.* 1977, Matzner *et al.* 1980, Patel *et al.* 1980, Zandman-Goddard *et al.* 1986, Zhou *et al.* 1987, Iancu 1989, Penneys & Zlatkiss 1990). Fourth, depletion of iron in the diet will diminish the incidence of certain malignancies (Hann *et al.* 1988). Similarly, complexation of available iron by phytate may suppress colon cancer (Graf & Eaton 1985). Finally, hepatocellular carcinoma cell growth can be inhibited using deferoxamine (Tabor & Kim 1991).

Inhibition of neutrophilic inflammation

We hypothesize that this series of biochemical reactions to iron chelates with a labile or reactive coordination site, recognized as neutrophilic inflammation, is of benefit when the host can successfully destroy the foreign chelator (e.g. the microbe). In other circumstances, inflammation and its sequelae including biomineralization, fibrosis, and cancer can be detrimental to the host and result in tissue injury and mortality. In these specific situations, it might be advantageous to suppress the host response to the increased availability of iron. This can be done by depletion of host concentrations of the available metal. In iron overload syndromes, this has been used therapeutically by employing low iron diets, phlebotomy, iron chelators, and metals with a single stable valence state to displace iron from its reaction sites (Denny *et al*. 1937, Foster *et al*. 1986, Kennedy *et al.* 1986, Chandler *et al.* 1988, Dubois *et al.* 1988).

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