Iron Loading and Disease Surveillance

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Iron is an oxidant as well as a nutrient for invading microbial and neoplastic cells. Excessive iron in specific tissues and cells (iron loading) promotes development of infection, neoplasia, cardiomyopathy, arthropathy, and various endocrine and possibly neurodegenerative disorders. To contain and detoxify the metal, hosts have evolved an iron withholding defense system, but the system can be compromised by numerous factors. An array of behavioral, medical, and immunologic methods are in place or in development to strengthen iron withholding. Routine screening for iron loading could provide valuable information in epidemiologic, diagnostic, prophylactic, and therapeutic studies of emerging infectious diseases.

Excessive iron in specific tissues (iron loading) promotes infection, neoplasia, cardiomyopathy, arthropathy, and a profusion of endocrine and possibly neurodegenerative disorders (1-5). An array of behavioral, medical, and immunologic methods are being developed to decrease iron loading or its detrimental effects. Routine screening for iron loading in populations exposed to certain diseases can provide valuable epidemiologic, diagnostic, prophylactic, and therapeutic information.

Hazards of Iron Loading

Iron can contribute to disease development in several ways. Excessive amounts of the metal in specific tissues and cells can hinder the ability of proteins, such as transferrin and ferritin, to prevent accretion of free iron. Moreover, in infectious diseases, inflammatory diseases, and illnesses that involve ischemia and reperfusion, iron causes reactions that produce superoxide radicals (6). Nonprotein bound ferric ions are reduced by superoxide, and the ferrous product is reoxidized by peroxide to regenerate ferric ions and yield hydroxyl radicals, which attack all classes of biologic macromolecules. Hydroxyl radicals can depolymerize polysaccharides, cause DNA strand breaks, inactivate enzymes, and initiate lipid peroxidation (6).

Iron can also increase disease risk by functioning as a readily available essential nutrient for invading microbial and neoplastic cells. To survive and replicate in hosts, microbial pathogens must acquire host iron. Highly virulent strains possess exceptionally powerful mechanisms for obtaining host iron from healthy hosts (7). In persons whose tissues and cells contain excessive iron, pathogens can much more readily procure iron from molecules of transferrin that are elevated in iron saturation. In such cases, even microbial strains that are not ordinarily dangerous can cause illness. Markedly invasive neoplastic cell strains can glean host iron more easily than less malignant strains or normal host cells (3). Moreover, iron-loaded tissues are especially susceptible to growth of malignant cells (Table 1).

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Table 1. Iron loading in specific tissues and increased risk for disease

Tissue type	Disease
Alveolar macrophages	Pulmonary neoplasia and infection
Anterior pituitary	Gonadal and growth dysfunction
Aorta; carotid and coronary arteries	Atherosclerosis
Colorectal mucosa	Adenoma, carcinoma
Heart	Arrhythmia, cardiomyopathy
Infant intestine	Botulism, salmonellosis, sudden death
Joints	Arthropathy
Liver	Viral hepatitis, cirrhosis, carcinoma
Macrophages	Intracellular infections
Pancreas	Acinar and beta cell necrosis, carcinoma
Plasma and lymph	Extracellular infections
Skeletal system	Osteoporosis
Skin	Leprosy, melanoma
Soft tissue	Sarcoma
Substantia nigra	Parkinson's disease

How Microbes Acquire Iron: A Determinant of Host Range and of Tissue Localization

The number of infectious disease agents whose virulence is enhanced by iron continues to increase (Table 2). To obtain host iron, successful pathogens use one or more of four strategies: binding of ferrated siderophilins with extraction of iron at the cell surface; erythrocyte lysis, digestion of hemoglobin, and heme assimilation; use of siderophores that withdraw iron from transferrin; and procurement of host intracellular iron.

Microbial strains that use siderophilin binding often have a very narrow host range (7). Bacterial receptors recognize siderophilins generally from a single or closely related host species. Strains of *Haemophilus somnus*, for example, form receptors for bovine but not for human transferrin; these bacteria are virulent for cattle but not for humans (9). The human pathogen, *Neisseria meningitidis*, can bind ferrated transferrins from humans and such hominids as chimpanzees, gorillas, and orangutans, but not from monkeys or nonprimate mammals (10,11). *Actinobacillus pleuropneumoniae* synthesizes a swine-specific transferrin receptor and causes pneumonia only in hogs (12).

Each of the above three pathogens, as well as other organisms that use siderophilin binding, can often obtain iron from heme. *Helicobacter pylori*, for instance, first obtains iron from human ferrated lactoferrin in the gastric lumen. Then, as it migrates into intercellular junctions of epithelial cells in the gastric wall, its sole source of iron is heme. This pathogen binds neither bovine ferrated lactoferrin nor human, bovine, or equine ferrated transferrin (13).

However, not every pathogen that uses siderophilin binding has a narrow host range. For example, *Staphylococcus aureus* can be virulent for a variety of mammalian species. Strains of this organism can bind human, rat, and rabbit transferrins and, much less efficiently, bovine, porcine, and avian transferrins (14). Moreover, isolates of *S. aureus* also may produce siderophores (15,16). These small molecules can withdraw iron from transferrins synthesized by a variety of host species. The siderophore, staphyloferrin A, removes iron from both human and porcine transferrin; thus, the metal can be available to invading cells in humans and in hogs. Erythrocyte lysis, digestion of hemoglobin, and heme assimilation are available to strains of *S. aureus*. Bacterial hemolysins generally are active against erythrocytes from several, although not from all, potential host species.

Virulent streptococci are examples of bacteria that neither bind siderophilins nor produce siderophores yet proficiently invade and replicate in many tissues in diverse host species. The cellulytic activities of these pathogens enable them to access such intracellular sources of host iron as hemoglobin, myoglobin, catalase, and ferritin (17).

The remarkable versatility for host species shown by *Listeria monocytogenes* illustrates the adeptness of this organism in procuring iron. Although mainly a saprophyte that lives in the plant-soil environment, *L. monocytogenes* can be acquired by humans and other mammals through ingestion of undercooked tissue of other mammals, birds, fish, and Crustacea, as well as from raw vegetables. Unable to bind siderophilins or form siderophores, *L. monocytogenes* obtains

Table 2. Microbial genera with strains whose growth in body fluids, cells, tissues, and intact vertebrate hosts is stimulated by excess iron (8)

Fungi	Protozoa	Gram-positive and acid-fast bacteria	Gram-negative bacteria	
Candida	*Entamoeba*	*Bacillus*	*Acinetobacter*	*Klebsiella*
Cryptococcus	*Leishmania*	*Clostridium*	*Aeromonas*	*Legionella*
Histoplasma	*Naegleria*	*Corynebacterium*	*Alcaligenes*	*Moraxella*
Paracoccidioides	*Plasmodium*	*Erysipelothrix*	*Campylobacter*	*Neisseria*
Pneumocystis	*Toxoplasma*	*Listeria*	*Capnocytophaga*	*Pasteurella*
Pythium	*Trypanosoma*	*Mycobacterium*	*Chlamydia*	*Proteus*
Rhizopus		*Staphylococcus*	*Ehrlichia*	*Pseudomonas*
Trichosporon		*Streptococcus*	*Enterobacter*	*Salmonella*
			Escherichia	*Shigella*

iron by using either exogenous siderophores of other microorganisms or natural catechols, such as dopamine and norepinephrine, in host tissues. The pathogen expresses a cell surface ferric reductase that recognizes the siderophoric chelated iron site; the metal is then reduced and assimilated (18). Furthermore, in contrast to saprophytic strains, systemic pathogenic strains of *L. monocytogenes* are hemolytic.

To grow within host cells, pathogens apparently are not required to synthesize siderophilin binding sites or form siderophores. For instance, unlike the wild type, siderophore-minus mutants of *Salmonella* Typhimurium cannot grow in extracellular compartments of the host. However, both the wild and mutant strains replicate within host cells (19). Possible sources of intracellular iron are heme, iron released from transferrin at pH 5.5-6, and ferritin.

For at least two pathogens, *Francisella tularensis* and *Legionella pneumophila*, the host intracellular niche is obligatory. Like the mutant strain of *S.* Typhimurium, these organisms are unable to access iron in extracellular fluids and tissues. Culturing these bacteria in laboratory media requires markedly elevated concentrations of iron (20,21).

In host intracellular niches, growth of microbial pathogens is stimulated by elevation and depressed by decrease of iron. Indeed, at least one bacterial pathogen, *Ehrlichia chaffeensis*, induces elevation of iron in its host cells; intracellular inclusions of the organism cause the host cell to upregulate expression of the transferrin receptor mRNA (22).

Iron Withholding Defense System

Hosts use several mechanisms (Table 3) to withhold iron from invading microbial and neoplastic cells: stationing of potent iron binding proteins at sites of impending microbial invasion; lowering iron levels in body fluids, diseased tissues, and invaded cells during invasion; and synthesizing immunoglobulins to the iron acquisition antigens of microbes.

High concentrations of iron not only benefit invading cells, they may also mediate antimicrobial activities of defense cells. In in vitro studies, 150 µM iron augmented macrophage killing of *Brucella abortus* (24) and, without altering phagocytosis, 250 µM iron enhanced anti-Candida activity of microglia (25). In the latter system, the metal suppressed synthesis of nitric oxide but not of tumor necrosis factor A. By generating oxidant-sensitive mediators, iron may focus influx of neutrophils to sites of infection (26). Iron loading of staphylococci increased their killing by peroxide, macrophages,

Table 3. The iron withholding defense system (1,8)

Constitutive components
- Siderophilins
  - Transferrin in plasma, lymph, cerebrospinal fluid
  - Lactoferrin in secretions of lachrymal and mammary glands and of respiratory, gastrointestinal, and genital tracts
- Ferritin within host cells

Processes induced at time of invasion
- Suppression of assimilation of 80% of dietary iron[a]
- Suppression of iron efflux from macrophages that have digested effete erythrocytes to result in 70% reduction in plasma iron[a]
- Increased synthesis of ferritin to sequester withheld iron[a]
- Release of neutrophils from bone marrow into circulation and then into site of infection[a]
- Release of apolactoferrin from neutrophil granules followed by binding of iron in septic sites
- Macrophage scavenging of ferrated lactoferrin in areas of sepsis and of tumor cell clusters
- Hepatic release of haptoglobin and hemopexin (to bind extravasated hemoglobin and hemin, respectively)
- Synthesis of nitric oxide (from L-arginine) by macrophages to disrupt iron metabolism of invaders[b]
- Suppression of growth of microbial cells within macrophages via downshift of expression of transferrin receptors and enhanced synthesis of Nrampl (23) by the host cells[b]
- Induction in B lymphocytes of synthesis of immunoglobulins to iron-repressible cell surface proteins that bind either heme, ferrated siderophilins, or ferrated siderophores

[a]Activated by interleukin-1 or -6 or by tumor necrosis factor-α.
[b]Activated by interferon-γ.

and neutrophil-derived cytoplasts but not by neutrophils (27). Certain conditions can impair iron withholding (Table 4); numerous studies have presented evidence that risk for infection or neoplasia is increased significantly in persons with these conditions.

Table 4. Conditions that can compromise iron withholding (1,3)

- Excessive intake of iron through intestinal absorption
  - Behavioral and nutritional factors
    - Accidental ingestion of iron tablets
    - Adulteration of processed foods with inorganic iron or blood
    - Excessive consumption of red meats (heme iron)
    - Excessive intake of alcohol (HCl secretion enhanced)
    - Folic acid deficiency
    - Ingestion of ascorbic acid with inorganic iron
    - Use of iron cookware
  - Genetic and physiological factors
    - African siderosis
    - Asplenia (mechanism unknown)
    - Pancreatic deficiency of bicarbonate ions
    - Porphyria cutanea tarda
    - Regulatory defect in mucosal cells in hemochromatosis
    - Thalassemia, sicklemia, other hemoglobinopathies
- Parenteral iron
  - Intramuscular and intravenous iron saccharate injections in excess
  - Multiple transfusions of whole blood or erythrocytes in excess
- Inhaled iron
  - Exposure to amosite, crocidolite, or tremolite asbestos
  - Exposure to urban air particulates
  - Mining iron ore, welding, grinding steel
  - Painting with iron oxide powder
  - Tobacco smoking (1-2 µg iron inhaled per cigarette pack)
- Release of body iron from compartments into plasma
  - Efflux of erythrocyte iron in hemolytic diseases
  - Efflux of hepatocyte iron in hepatitis
- Deficit in iron withholding
  - Transferrin
  - Decreased synthesis
    - Congenital defect
    - Lack of dietary amino acids in kwashiorkor or in jejunoileal bypass
    - Decreased activity in acidosis
  - Lactoferrin
    - Neutropenia
    - Substitution of bovine milk or milk formula for human milk in nursling nutrition
  - Haptoglobin
    - Decreased synthesis in persons with haplotype 2-2 (28)

Detection of Iron Loading

Screening of large populations for iron loading can be accomplished with inexpensive, noninvasive methods. A useful indicator of iron loading is marked elevation of serum ferritin (sFt). However, sole reliance on this measurement can be misleading because sFt increases moderately during inflammatory episodes. Accordingly, concurrent determination of the percentage of iron saturation of serum transferrin (%TS) provides useful information (29). In iron loaded persons, hyperferritinemia generally is accompanied by an elevation in %TS. In contrast, in patients with an inflammatory process, hyperferritinemia generally is accompanied by a reduction in %TS.

Iron loading is associated also with moderate depression of a third variable, serum transferrin receptor (sTfR). The ratio of sTfR/sFt, apparently independent of inflammation, is significantly reduced in persons with high levels of iron (5).

Strengthening the Iron Withholding Defense

A considerable array of behavioral, medical, and immunologic methods are in place or in development for strengthening iron withholding (Table 5) (3). Additional precautions are indicated for persons who are known to be (or have a tendency to become) iron loaded. For example, persons with elevated iron due to either hemochromatosis or alcoholism are cautioned to avoid eating raw oysters, which may contain *Vibrio vulnificus* (30). Another pathogen that likewise causes severe systemic infection in hosts with elevated iron is *Capnocytophaga canimorsis*. Accordingly, persons who have hemochromatosis, alcoholism, or asplenia are advised to receive prompt antibiotic therapy if they are exposed to a dog bite (31).

De-ironing by phlebotomy is effective in lowering risk for cardiovascular diseases (32,33) and various neoplasms (34), as well as in therapy for hepatitis C (35). Interfering with iron metabolism by administering gallium can be useful in suppressing growth of lymphoma and bladder cancer cells (36). The antineoplastic action of monoclonal antibodies against ferrated transferrin receptors has been examined (37).

Table 5. Methods of strengthening the iron withholding defense system

- Reduction of excessive intake of ingested iron
  - Decreased consumption of red meats (heme iron)
  - Avoidance of processed foods that have been adulterated with inorganic iron or with blood
  - Decreased consumption of alcohol and ascorbic acid
  - Elimination of iron supplements unless an iron deficiency has been correctly diagnosed
- Reduction of excessive intake of parenteral iron
  - Inject iron saccharates only if unequivocally justified
  - Transfuse blood or erythrocytes only if unequivocally justified
  - Substitute erythropoietin (+ minimal amount of iron) for whole blood transfusions when possible
- Reduction of excessive inhalation of iron
  - Eliminate use of tobacco
  - Use iron-free chrysotile in place of iron-loaded amosite, crocidolite, tremolite varieties of asbestos
  - Use mask to avoid inhalation of urban air particulates
  - Use mask and protective clothing when mining or cutting ferriferous substances
- Reduction of iron burden by regular depletion of whole blood or erythrocytes
  - Avoidance of premature hysterectomy
  - Routine ingestion of aspirin
  - Regular donations of whole blood or erythrocytes
  - Vigorous exercise
- Increased use of iron chelators
  - Use human milk (high in lactoferrin, low in iron) rather than milk formula (lacking in lactoferrin, high in iron) in nursling nutrition
  - Use tea (iron-binding tannins) and bran (iron-binding phytic acid)
  - Continue research and development (R&D) of potential iron chelator drugs (e.g., recombinant human lactoferrin; hydroxpyridones; pyridoxal isonicotinoyl hydrazones)
- Initiation of prompt therapy of chronic infections and neoplastic diseases to forestall saturation of iron withholding defense system
  - Continued R&D of cytokines such as interferon γ that induce cellular iron withholding
  - Continued R&D of passive and active methods of immunization against surface receptor proteins used by microbial and neoplastic cells to obtain iron

Combinations of the iron chelator, deferoxamine, with gallium or with antibodies against ferrated transferrin receptors increase effectiveness against tumor cells.

The natural iron scavenger, lactoferrin, has been shown to remove free iron from synovial fluid aspirated from joints of rheumatoid arthritic patients (38). Recombinant human lactoferrin, which is indistinguishable from native breast milk lactoferrin with respect to its iron binding properties, is now available (39) and could become a very useful addition to our array of de-ironing pharmaceutical products.

A recently discovered integral membrane phosphoglycoprotein, Nrampl, is expressed exclusively in macrophages and is localized to phagolysosomes. The protein suppresses replication of intramacrophage microbial invaders apparently by altering iron availability (23). A second protein, Nramp2, is involved in enhancement of intestinal iron absorption (40). Future research might develop useful medical procedures for modulation of the actions of these proteins.

Potential vaccines that incorporate iron acquisition antigens of pathogens in the families *Neisseriaceae* and *Pasteurellaceae* are being developed by several research groups. For example, in *Moraxella catarrhalis*, the recombinant transferrin binding protein B (TbpB) has been shown to elicit bactericidal antibodies (41) In *N. meningitidis*, antisera to TbpA and TbpB were bactericidal for both homologous and heterologous strains (42,43). Because the antigenic proteins function at the cell surfaces of the pathogens, the receptors are potentially ideal vaccine candidates. For synthesis of the receptors, the organisms must be cultured in iron-restricted media.

Perspectives and Conclusions

There is growing awareness that transmissible agents are involved in diseases not earlier suspected of being infectious (44-46). A recent review contains a list of 34 degenerative, inflammatory, and neoplastic diseases associated in various ways with specific infectious agents (44). Other chronic inflammatory diseases, such as sarcoidosis, inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, Wegener granulomatosis, diabetes mellitus, primary biliary cirrhosis, tropical sprue, and Kawasaki disease may also have infectious etiologies (45). Excessive iron is correlated with synovial damage in rheumatoid arthritis (47) and with impaired glucose metabolism in diabetes (48). The association of *Chlamydia pneumoniae* (49) and excessive iron (5) with cardiovascular disease is well established. Growth of this pathogen is strongly suppressed by iron restriction (50).

Proving the role of infection in chronic inflammatory diseases and cancer presents challenges (46). The means by which pathogens suppress, subvert, or evade host defenses to establish chronic or latent infection have received little attention. However, the association and causal role of infectious agents in chronic inflammatory diseases and cancer have major implications for public health, treatment, and prevention (44-46).

Iron loading is a risk factor in these illnesses, as well as in classic infectious diseases. Because the prevalence of iron loading in various populations can be remarkably high, routine screening of iron values in host populations could provide valuable information in epidemiologic, diagnostic, prophylactic, and therapeutic studies of emerging infectious diseases.

Acknowledgment

Dedicated to Jerome L. Sullivan, pioneer and leader in our awareness of the role of iron in cardiovascular disease.

Support for this review was provided by the Office of Research and the University Graduate School, Indiana University, Bloomington, IN, USA.

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