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Mini-Review

Microbial pathogens with impaired ability to acquire host iron

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Abstract

Successful microbial pathogens must be adept in obtaining growth-essential iron from healthy hosts. Some potential pathogens, however, are sufficiently impaired in iron acquisition ability so as to be dangerous mainly in hosts with such iron loading conditions as alcoholism, asplenia, hemochromatosis, β-thalassemia major, or tobacco smoking. The association of six impaired pathogens (*Capnocytophaga canimorsis*, *Yersinia enterocolitica* and *Y. pseudotuberculosis*, *Vibrio vulnificus*, *Tropheryma whippelii*, and *Legionella pneumophila)* with iron loaded humans is described.

Abbreviations: AIDS – acquired immunodeficiency disease syndrome, AM – alveolar macrophages; HH – hemochromatosis.

Introduction

> "Study of the rare and curious . . . often brings to light general phenomena which may be exaggerated in the rare but overlooked in the commonplace."
>
> (Henrici 1940)

For successful proliferation in animal tissues, microbial pathogens must possess ability to acquire growth-essential iron from their hosts. The latter, however, have evolved an elaborate iron withholding defense system that permits host cells to maintain access to iron while inhibiting potential invaders from acquiring the metal (Weinberg 1999a). This powerful feature of innate immunity protects healthy hosts from all but the most skillful of iron-acquiring aggressors.

Nevertheless, there exist potential invaders that have very little iron acquisition capability. Such microorganisms cause disease primarily in iron loaded hosts. Because of the relatively low percentage of iron loaded humans in any given population, patients with these pathogens usually are not contagious. These impaired pathogens generally are confined to specific host cells or tissues that provide readily available iron. A few examples of these singular pathogens are described below.

Etiologies of iron loading

Numerous behavioral, physiologic, and genetic factors can contribute to iron loading (Weinberg 1999a). This paper will focus mainly on five conditions: alcoholism, asplenia, hemochromatosis, β-thalassemia major, and tobacco smoking. Chronic alcoholics can absorb up to twice the amount of alimentary iron as do normal persons (Duane *et al.* 1992). About 20–30% of alcohol misusers have pronounced hepatic iron loading as well as elevated values for serum iron and ferritin (Chapman *et al.* 1983). Excessive absorption of inorganic iron by alcoholics often has been proposed to be due to augmented gastric acid secretion leading to enhanced reduction of ferric iron. However, Fe(II)-ascorbate absorption likewise is increased in alcoholics (Duane *et al.* 1992).

Patients with spherocytosis (Parkin *et al.*. 1974) or β-thalassemia major (Erlandson *et al.*. 1962; Pootrakul *et al.*. 1980) tend to absorb twice the amount of iron and to have a two-fold elevation of serum iron values after splenectomy as compared with persons with these diseases who have intact spleens. Conceivably, the spleen produces a cytokine that participates in the normal regulation of intestinal iron absorption.

Hereditary hemochromatosis (HH) is endemic in populations of European descent at a rate of 0.1–0.5%; most subjects, when they present clinically, are between 40 and 60 yr of age (Bothwell & MacPhail 1998). The gene disorder is not sex related; nevertheless, because of greater alimentary heme iron intake as well as absence of menstruation in earlier years, disease manifestations occur more frequently in males than in females (Bothwell & MacPhail 1998; Witte *et al.* 1996). Uptake of ferric ions by freshly isolated duodenal mucosal biopsy specimens is elevated two fold in HH as compared with normal controls and, in untreated cases, serum iron likewise is increased two fold (Raja *et al.* 1996). In longstanding HH, iron loading is present not only in parenchymal cells but also begins to appear in macrophages (Witte *et al.* 1996).

The β-thalassemias are widespread throughout the Mediterranean region, Africa, the Middle East, the Indian subcontinent, and the countries of southeast Asia including Indonesia (Olivieri 1999). In some areas, the altered gene frequencies range from 3 to 10%. Because of ineffective erythropoiesis, the homozygotic patients have enhanced iron absorption; additionally, they frequently require blood transfusions. Accordingly, iron overload is the most important complication of the disease and is a major focus of management (Olivieri 1999). Many of the clinical manifestations of iron loading appear by the second decade of life. The spleen can become excessively swollen due to accumulation of erythrocytes with non-functional hemoglobin; thus splenectomy often is performed.

Tobacco has been reported to contain 84 μg iron/g. A cigarette contains approximately 0.7 g tobacco. About 0.1% of the cigarette iron is present in mainstream smoke. Thus, a one pack/d smoker might inhale 1.2 μg iron/d (Weinberg 1999b). Alveolar macrophages (AM) attempt to scavenge the inhaled iron. In one study, the ratio of iron content of AM of smokers to non-smokers was 3.6:1 (McGowan *et al.* 1986). In another report, smokers with chronic airway obstruction had a four-fold increase in iron content of AM over the amount in AM of non-smokers (Wesselius *et al.* 1992).

Capnocytophaga canimorsus

Capnocytophaga canimorsus, formerly DF-2, is a fastidious gram negative bacillus associated with septicemia and other severe infections in iron loaded persons following exposure to saliva of carrier cats and dogs (Weinberg 1987; Brenner *et al.* 1989; Blanche *et al.* 1998). The pathogen first was isolated from a febrile patient in 1973; since then, only a few hundred cases have been described in the medical literature (Blanche *et al.* 1998). Prominent risk factors include alcoholism, asplenia, and males of European descent in middle age or older. Although about 30% of persons bitten by dogs are children, this age group in Europe and the United States is resistant to *C. canimorsus* (Pers *et al.* 1996). With the exception of thalassemia, iron values in children generally are not elevated. Reasons for earlier splenectomy in patients with *C. canimorsus* infection include trauma, Hodgkin's disease, events incidental to abdominal surgery, and thrombocytopenic purpura (Hicklin *et al.* 1987). Only 15–25% of cats and dogs carry detectable amounts of *C. canimorus* in their saliva (Blanche *et al.* 1998). Possibly, the positive animals have inadequate quantities of lactoferrin or other iron-withholding factors in their oral cavities.

Yersinia enterocolitica and *Yersinia pseudotuberculosis*

Systemic infections caused by *Yersinia enterocolitica* and *Y. pseudotuberculosis* are uncommon. They occur almost exclusively in persons of any age who have an underlying iron loading condition. Among the large spectrum of such conditions that have been cited for risk of these pathogens are African siderosis, alcoholism, asplenia, excessive parenteral or oral iron therapy, hemochromatosis, sideroblastic anemia, thalassemia, and transfusional siderosis (Capron *et al.* 1984; Abbott *et al.* 1986; Adams & Gregor 1990; Vadillo *et al.* 1994; Adamkiewicz *et al.* 1998). These gram negative bacillary pathogens can grow both in iron-enriched host fluids as well as in macrophages that contain elevated iron. The pathogens lack siderophores but contain siderophore receptors. Thus iron loaded patients who are being de-ironed with the siderophore, deferoxamine, are especially at risk (Adams & Gregor 1990).

Vibrio vulnificus

Vibrio vulnificus, an estuarine gram negative bacterial species, causes (i) wound infections associated with sea water exposure, and (ii) septicemias ensuing from consumption of contaminated raw oysters. An iron loading condition such as alcoholism, chronic hepatitis, hemochromatosis, or thalassemia is present in a very high percentage of patients (Linkous & Oliver 1999). In the United States, patients tend to be predominantly male and middle aged or older (Linkous & Oliver 1999).

Virulence of isolates of *V. vulnificus* is significantly associated with ability to obtain iron from transferrin of iron loaded patients. In a study of eight isolates from patients and eight from the marine environment, none could grow in the presence of transferrin that had a normal saturation value of 30%. At 100% iron saturation, six of the patient isolates and two of the environmental isolates grew ($p = 0.04$) (Morris *et al.* 1987). Intraperitoneal injection of a virulent strain into normal mice required, for a 50% lethal dose, one million bacterial cells. In contrast, in mice injected with a non-toxic dose of ferric salts, the 50% lethal dose was only 1.1 bacterial cell (Wright *et al.* 1981).

In a confirming study, the pathogen was observed to die in normal plasma but to rapidly replicate in plasma of hemochromatotic patients whose transferrin iron saturation values were 84–95% (Bullen *et al.* 1991). The bacteria grew also in normal blood fortified with iron salts or with hematin, but not with hemoglobin (Bullen *et al.* 1991). As with *Y. enterocolitica*, provision of deferoxamine to *V. vulnificus* assisted the pathogen to obtain iron from transferrin (Wright *et al.* 1981). Results of these and more recent studies (Shapiro *et al.* 1998; Hor *et al.* 1999) have consistently demonstrated that excessive iron plays a major role in the pathogenesis of *V. vulnificus.*

Tropheryma whippelii

Tropheryma whippelii, an actinomycete, causes a rare systemic disease (Whipple's disease) predominately in middle aged males of European descent (Dobbins 1995; Singer 1998). As in hereditary hemochromatosis, patients often will have manifested polyarthralgia, fatigue, and impotence for years prior to diagnosis. Unlike prototypical hemochromatosis, many of the patients proceed to develop steatorrhea with accompanying weight loss and diarrhea due to malabsorption of fat.

The bacterium is believed to be acquired orally; it is carried in the gastointestinal tracts of healthy humans (Street *et al.* 1999). The principal sites of multiplication in patients include macrophages in such systems as gastrointestinal, cardiovascular, endocrine, and central nervous, as well as intestinal epithelial cells, lymphatic and capillary endothelial cells, myocytes, and neutrophiles. In some patients, cardiac tissue is invaded without evidence of overt gastrointestinal disease (Gubler *et al.* 1999).

The pathogen has not yet been cultured either in laboratory media, animal cell cultures, or animals. However, it has been grown successfully in human macrophages deactivated with interleukin-4 (Schoeden *et al.* 1997). Activated macrophages suppress intracellular growth of pathogens in part by down regulating transferrin receptor expression (Weinberg 2000). In contrast, interleukin-4 augments transferrin receptor mRNA expression thus enhancing iron uptake while permitting phagocytosis of the invader (Weiss *et al.* 1997). Note that such obligate intracellular pathogens as *Ehrlichia chaffeensis* (Barnewall *et al.* 1999) and *Coxiella burnetii* (Howe & Mallavia 1999) induce their host cells to upregulate transferrin receptor mRNA. It is not yet known if *T. whippelii* possesses this ability.

Legionella pneumophila

Legionella pneumophila is a pleomorphic gram negative bacterium that survives in nature by growing within a variety of fresh water amoeba and ciliated protozoa (Yu 1995). It enters humans upon inhalation of contaminated water droplets. *L. pneumophila* can evade host defenses only by parasitizing and multiplying within alveolar macrophages. The ensuing pneumonia is not contagious. Evidence that the pathogen requires unusually iron-rich macrophages is derived from both clinical and laboratory observations.

Risk factors for development of pneumonia due to *L. pneumophila* include cigarette smoking, alcoholism, male gender (male:female ratio is 3:1), and median age of 55 yr (England & Fraser 1981). Additional risk factors include transfusion siderosis in renal dialysis patients, cancer chemotherapy and organ transplantation, and acquired immunodeficiency disease syndrome (AIDS) (Yu 1995). Intensive chemotherapy for neoplasias or in preparation

for organ transplantation can result in high levels of transferrin iron saturation as well as accumulation of non-transferrin bound iron (Harrison *et al.* 1994; Bradley *et al.* 1997). In AIDS patients, iron loading of macrophages is well documented (Boelaert *et al.* 1996; Al-Khafaji *et al.* 1997).

Laboratory investigations have observed that, for extracellular growth in vitro, *L. pneumophila* must be cultured in iron-enriched media (Quinn & Weinberg 1988). The pathogen produces neither siderophores nor siderophilin binding proteins; it fails to multiply in normal extracellular host tissues or fluids. Moreover, *L. pneumophila* cannot proliferate within human phagocytic cells whose iron level remains low because of activation by interferon-γ (Horwitz 1992). This cytokine suppresses expression of transferrin receptors. Similarly, chloroquine prevents pathogen growth by raising the pH value of the host cell endosome thereby preventing iron-transferrin from releasing the metal to the bacteria (Byrd & Horwitz 1991). Mutants of *L. pneumophila*, whose ability to acquire iron is even more impaired than is the wild type, lose pathogenicity as well as ability to grow in saprophytic protozoa (Pope *et al.* 1996). As with *T. whippelii*, it is not yet known if virulent strains of *L. pneumophila* might be able to upregulate expression of transferrin receptor mRNA.

Perspectives

Methods for prevention of diseases due to the pathogens cited above, as well as to numerous other microorganisms that may be more efficient in acquiring host iron, are well established. Tests for body iron values are reliable and inexpensive; also genetic testing is becoming available for several of the iron loading disorders. Many iron loaded patients can be de-ironed by appropriate phlebotomies; others require an iron chelating drug such as deferoxamine.

Inhalation of excessive iron can be prevented by cessation of smoking and by wearing masks while working with such ferriferous minerals as asbestos, iron ore, and steel. Injection of excessive iron can be prevented by avoiding use of parenteral iron saccharates or of whole blood unless unequivocal medical justification is obtained.

Intestinal absorption of excessive iron can be prevented by lowering intake of alcohol, red meats, and iron-adulterated processed foods, and by cessation of use of iron supplements in the absence of medical justification. Also helpful is the use of recombinant erythropoietin (with limited amounts of iron) in place of blood transfusions in renal dialysis patients. In victims of trauma who have splenic damage, restoration of the organ in place of routine splenectomy should be practiced.

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