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Hemochromatosis and the enigma of misplaced iron: Implications for infectious disease and survival

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Abstract

The mystery surrounding the apparent lack of iron within the macrophages of individuals with hereditary hemochromatosis, a condition of excessive uptake of dietary iron, has yet to be fully explained. We have suggested that iron deficiency of macrophages in people with hereditary hemochromatosis mutations is associated with increased resistance to infection by Yersinia and other intracellular pathogens, a selection pressure resulting in unusually high current population frequencies of hereditary hemochromatosis mutations. Such selection pressure has been called Epidemic Pathogenic Selection (EPS). In support of the theory of EPS, a considerable number of virulent species of bacteria multiply mainly in iron-rich macrophages of their mammalian hosts. Among these fastidious pathogens are strains of Chlamydia, Coxiella, Francisella, Legionella, Mycobacterium, Salmonella and Yersinia. Iron deficiency of macrophages of persons with hereditary hemochromatosis gene mutations may result in increased resistance to members of these bacterial pathogens. People with genes that result in hereditary hemochromatosis may be protected against coronary artery disease associated with Chlamydia and Coxiella infection in the absence of iron overload. In the clinical setting, when a patient appears to be iron deficient, the reason for this should be carefully evaluated. Iron supplementation may adversely affect the health of individuals who have mounted an acute phase response to infection, injury or stress, or who carry genes predisposing them to iron overload disorders.

Introduction

Hereditary hemochromatosis is a genetic condition whereby too much iron is absorbed through the diet (Jazwinska 1998). In people with hereditary hemochromatosis, iron overload of parenchymal cells may lead to destruction of the liver, heart and pancreas. Two mutations (C282Y and H63D) in a 'non-classical' HLA class-I gene named HFE have been found to be associated with hereditary hemochromatosis (Feder *et al.* 1996). The wild-type HFE protein is thought to associate with the transferrin receptor on the cell surface thus preventing the binding and internalization of iron loaded transferrin (Montosi *et al.* 2000). The C282Y mutation is the more penetrant of the two mutations and is found in the majority of patients with iron overload of western and northern European descent. The C282Y mutation prevents the association of the HFE protein's α-3 domain with β-2 microglobulin and the C282Y protein is thus not properly anchored to the cell surface (Jazwinska 1998). Since the C282Y mutation is found almost exclusively in those of western and northern European descent, it is thought that a series of specific evolutionary pressures might have resulted in the selection of these mutations. We have previously outlined an hypothesis regarding the origins of the mutations involved in hereditary hemochromatosis as being the result of Epidemic Pathogenic Selection (EPS). In this hypothesis, we suggested that mutations in HFE that lead to hereditary hemochromatosis might have conferred a selection advantage to those individuals as the result of having, among other things, iron deficient macrophages (Moalem *et al.* 2002). As opposed to other conditions of iron loading

such as transfusional iron overload, or African siderosis, the iron loading in hereditary hemochromatosis is not equally distributed throughout the body (Montosi *et al.* 2000). The enigmatic sequestering of iron found in hereditary hemochromatosis, whereby there is a preferential loading in parenchymal cells as opposed to cells of the reticuloendothelial system, has yet to be fully explained. In an animal model of iron overload that mimics transfusional iron overload, tissue loading by iron-dextran supplementation included broncho-alveolar macrophages (Legssyer *et al.* 2003). Thus the iron concentration of broncho-alveolar macrophages as well as circulating macrophages and other tissue macrophages may be iron-deficient in hereditary hemochromatosis. Since many intracellular pathogens require iron within reticuloendothelial cells such as macrophages to become virulent, people with hereditary hemochromatosis mutations may be protected against infection by a number of iron-seeking organisms that require macrophage iron for their replication (Moalem *et al.* 2002).

The purpose of this paper is to review the importance of macrophage iron in infection by virulent pathogens, and some of the consequences that mutations resulting in altered iron loading in the body can have on the susceptibility to infectious disease and on the manifestation of certain human diseases.

The importance of macrophage iron for certain virulent infections

Many reports have outlined the protective nature of lack of iron within the macrophages that encounter pathogens whose virulence is dependent on the availability of iron (Weinberg 1999; Olakanmi *et al.* 2002). Our inherent iron-withholding defence is crucial to our survival; without it we would most definitely succumb quite quickly to one of a dozen common bacterial organisms. Most people are familiar with the acute phase response found in many mammals, which involves the imminent sequestration of iron and is incumbent upon the down-regulation of transferrin and the upregulation of ferritin and a decrease in absorption of iron through the diet (Weiss 2002). There have been many theories postulated for the biological changes that occur during the acute phase response. There seems to be a general consensus that the acute phase response arose as a defensive adaptation against pathogenic organisms, although this occurs in response to different types of stresses. Since many pathogens require iron to be pathogenic to humans, it is not surprising that the body employs many defensive measures to withhold the availability of iron.

Examples of bacterial organisms whose virulence and/or replication depend upon macrophage iron include *Mycobacterium tuberculosis*, *Salmonella typhi*, and Chlamydia *pneumoniae* (Weinberg 1999) (Table 1). Of the eight genera capitalized in the table, two (Chlamydia, Coxiella) are characterized as being *obligate* intracellular bacteria; i.e., they must grow within cells, such as macrophages. The other six are characterized as being *facultative* intracellular bacteria; i.e., they can grow inside or outside of host cells. Generally, the highly virulent strains of facultative organisms are most dangerous when they are growing within the host's iron-rich macrophages.

In cases of transfusional iron overload, where the excessive iron is loaded due to multiple blood transfusions, or African siderosis, an iron loading condition in those of African descent, of unknown aetiology, the iron loading seems to be quite uniform throughout the body (Iancu *et al.* 1978; Witte *et al.* 1996; Walker *et al.* 1999; Legssyer *et al.* 2003). Yet in hemochromatosis the iron is not uniformly loaded and even appears lacking in certain types of tissues and cells including macrophages (Montosi *et al.* 2000). Since, as mentioned, many pathogens become increasingly virulent with the increased availability of iron, especially within the macrophage, it is most interesting that in hereditary hemochromatosis there seems to be a lack of iron within macrophages in these individuals (Cairo *et al.* 1997; Recalcati *et al.* 1998; Montosi *et al.*, 2000).

The withholding of iron has often led to confusion in clinical care of patients since many caregivers are only familiar with the problems associated with the lack of iron. The result is that many patients have received and still are receiving iron supplementation when their body is working hard to remove the iron away from invading pathogens (Oppenheimer 1998).

African siderosis (AS)

Formerly known as Bantu siderosis and first described by Strachan in 1929, African siderosis is an inherited iron loading disorder, affecting individuals of African descent, with some limited similarity to hereditary hemochromatosis but of unknown aetiology (Andrews 1999, Walker *et al.* 1999). It is also thought that dietary intake of excess iron, especially from traditional

Table 1. Bacterial genera with strains whose growth in body fluids, cells tissues or intact vertebrate hosts is stimulated by excess iron.

Gram positive and acid-fast bacteria:
Bacillus, Clostridium, Corynebacterium, Erysipelothrix, Listeria, MYCOBACTERIUM, Staphylococcus, Streptococcus, Tropheryma
Gram negative bacteria:
Acinetobacter, Aeromonas, Alcaligenes, Campylobacter, Capnocytophaga, CHLAMYDIA, COXIELLA, EHRLICHIA, Enterobacter, Escherichia, FRANCISELLA, Helicobacter, Klebsiella, LEGIONELLA, Moraxella, Neisseria, PASTEURELLA, Proteus, Pseudomonas, SALMONELLA, Shigella, Vibrio, YERSINIA

Capitalization of genera indicates complete dependence of these virulent strains upon iron-rich macrophages in order to grow within the host.

beer brewed in non-galvanized steel drums, might exacerbate African siderosis (Andrews 1999). Some studies have indicated that there may be a genetic component to African siderosis, which unlike hereditary hemochromatosis is not HLA linked (Gorduek *et al.* 1992; Moyo *et al.* 1998). In a cohort of 808 African Americans, three subpopulations based on transferrin iron saturation were apparent (Gordeuk *et al.* 1998). One population, comprised of 0.9% of the total, had a mean transferrin iron saturation of 63.4%±5.7%; a second group comprised of 13.6%, had a mean transferrin iron saturation of 38%±5.7%; and the remaining 85.6% of the population had a mean transferrin iron saturation of 24.6%±5.7% (Gordeuk *et al.* 1998). These proportions are consistent with a yet to be discovered genetic mutation. The first group, postulated to be homozygous, could lead to an iron overload state with even a normal diet. The second group, postulated to be carriers or heterozygotes, might load a significant amount of iron only with a high iron diet. The third group, postulated to be wild-type, might have normal iron metabolism.

An important difference between hemochromatosis and African siderosis is that in hereditary hemochromatosis the macrophages are iron-poor and in African siderosis the macrophages are iron-rich (Gangaidzo *et al.* 1999). Thus in contrast to hereditary hemochromatosis, persons with African siderosis are quite susceptible to intracellular pathogens such as *M. tuberculosis* (Gangaidzo *et al.* 2001). Accordingly, *M. tuberculosis* or yersinia spp. infections cannot be invoked to explain the prevalence of the postulated mutation responsible for African siderosis.

Researchers have also found that the human monocyte-derived macrophages from hemochromatosis and healthy donors both acquired similar amounts of iron following incubation with labelled iron. Yet when the pathogen *M. tuberculosis* was exposed to iron loaded macrophages, this organism acquired markedly less iron from the cells of individuals with hereditary hemochromatosis than from healthy donors (Olakanmi *et al.*, 2002). Since *M. tuberculosis* requires iron for replication, growth and pathogenicity, the genetic mutations responsible for hereditary hemochromatosis could limit this pathogen's impact by preventing its acquisition of iron. The study of Olakanmi *et al.* is in keeping with our hypothesis that EPS may have selected for hereditary hemochromatosis mutations, and raises the possibility that *M. tuberculosis* might have been one of the selection pressures responsible for the high prevalence of hereditary hemochromatosis mutations found in certain populations (Moalem *et al.* 2002). Thus evidence continues to increase that certain alterations in iron distribution and metabolism could be protective against certain pathogens and may have resulted in hemochromatosis being one of the most prevalent genetic conditions in those of western and northern European descent. Reports of increased prevalence of bacterial infections in individuals with hemochromatosis are minimal, unlike reports for those with transfusional overload or African siderosis (Weinberg *et al.* 2000; Vikram *et al.* 2002). An exception in hemochromatosis is susceptibility to *Vibrio vulnificus*, a facultative organism that can propagate outside of macrophages. Increased susceptibility to *V. vulnificus* occurs since the level of iron is increased in serum thus permitting propagation extracellularly. People with hemochromatosis may possibly also be unusually susceptible to infection with other facultative organisms.

Iron overload and infection in coronary heart disease

Iron overload in hereditary hemochromatosis can lead to destruction of heart tissue. An important question that is receiving much attention is whether hereditary hemochromatosis also predisposes to coronary artery disease.

Participation of an inflammatory process in the development of coronary artery disease is becoming increasingly apparent. Among the proposed risk factors for the disease are chronic infections with such pathogens as *Chlamydia pneumoniae* (Sullivan *et al.* 1999) and *Coxiella burnetti* (Weinberg 2001). These obligate intracellular bacteria are inhaled and multiply in iron rich alveolar macrophages to result in 'atypical pneumonia'. Some of the infected host cells migrate through the vascular system and, over time, contribute to the development of arterial wall lesions. For multiplication in host cells, a requirement for elevated intracellular iron has been demonstrated for each of these pathogens. Indeed, in murine macrophages, *C. burnetti* can, within 3–6 h post-infection, induce upregulation of transferrin receptor (Howe *et al.* 1999). By 12 h post-infection, total macrophage iron can increase 2 1/2-fold and by 72 h, the number of intracellular bacteria can multiply 7-fold. Accordingly, since individuals with hereditary hemochromatosis have below normal iron containing macrophages, they might be at no increased risk for coronary heart disease associated with Chlamydia or Cloxiella.

This prediction may, in fact, be supported by recent clinical studies. For instance, in an Australian cohort of 1185 men and 1141 women aged 20 and 79 years of age, neither the C282Y nor the H63D mutations the two most common HFE mutations, nor elevated serum iron parameters were associated with increased risk for coronary heart disease (Fox *et al.* 2002). In an American study of 15,362 men and 15,554 women aged between 43 and 72 years of age, similar observations were made except that compound heterozygote men for the HFE mutations (C282Y/H63D) had a slightly higher prevalence of coronary heart disease as compared with men lacking the HFE mutations (Waalen *et al.* 2002). As in the Australian study, there was no significant association between elevated serum iron values and the prevalence of coronary heart disease.

Table 2. Iron values for Caucasian females, 43–72 years of age.

Genotype	Number (%)	Transferrin Saturation% mean ± (SD)	Serum ferritin ng/ml; mean (95% CI)
wt/wt	9707 (62.4)	23±(9)	53 (52-54)
C282Y/C282Y	69 (0.4)	52±(22)	175 (126-245)
C282Y/wt	1558 (10.0)	27±(10)	57 (54-57)
C282Y/H63D	286 (1.8)	33±(13)	71 (63-79)
H63D/H63D	372 (2.4)	29±(11)	61 (55-67)
H63D/wt	3562 (22.9)	25±(9)	56 (54-57)

wt = wild-type; CI = confidence interval.
Adapted from a portion of Table 1 of Waalen *et al.* (2002).

Nutritional theory of survival of mutations leading to iron overload

In contrast to the EPS theory for the origin of hereditary hemochromatosis mutations, it has been previously reported that the selective value of carriage of HFE gene mutations is associated with prevention of iron deficiency primarily in women during childbearing years (Simon *et al.* 1980; Jazwinska 1998). The frequency of iron deficiency has been suggested to be less in female heterozygotes (C282Y) than in the general population (Crawford *et al.* 1998). As well, others have proposed that C282Y homozygosity in women would be beneficial during their reproductive years (Crawford *et al.* 1996; Jazwinska 1998).

Table 2 contains mean iron values for the American female population cited earlier (Waalen *et al.* 2002). Unfortunately, data are not presently available for younger women. Thus it is not yet known if C282Y homozygotes might have an iron nutritional advantage during their reproductive decades.

It would not be surprising if nutritional iron deficiency as well as EPS promoted high frequencies of genetic variants resulting in body iron overload. As well, it will be of interest in subsequent studies to explore effects that other genetic variants affecting iron metabolism have on emerging relationships between nutrition, the topography of body iron load, infection and human diseases resulting from interactions between such factors. Knowledge gleaned from studies of infectious diseases, physiology and cellular biology must go hand in hand with studies of genetics and other risk factors in order for us to learn how to prevent and to effectively treat serious human diseases that include not only heart disease, but also diabetes, Alzheimer disease, Parkinson disease and other disorders in which iron overload has been implicated

(Moalem *et al.* 2000; Moczulski *et al.* 2001; Moalem *et al.* 2002). Iron is essential for life and deficiency may affect survival. However, iron supplementation is not without risks. It may adversely affect the health of individuals who have mounted an acute phase response to infection, injury or stress, or who carry genes predisposing them to iron overload disorders. In particular, clinicians must pay careful attention to distinguish between states of true iron deficiency and the acute phase response that accompanies inflammation.

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