Review: Effects of Iron Overload on the Immune System

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Abstract. Iron and its binding proteins have immunoregulatory properties, and shifting of immunoregulatory balances by iron excess or deficiency may produce severe, deleterious physiological effects. Effects of iron overload include decreased antibody-mediated and mitogen-stimulated phagocytosis by monocytes and macrophages, alterations in T-lymphocyte subsets, and modification of lymphocyte distribution in different compartments of the immune system. The importance of iron in regulating the expression of T-lymphocyte cell surface markers, influencing the expansion of different T-cell subsets, and affecting immune cell functions can be demonstrated in vitro and in vivo. The poor ability of lymphocytes to sequester excess iron in ferritin may help to explain the immune system abnormalities in iron-overloaded patients. Iron overload as seen in hereditary hemochromatosis patients enhances suppressor T-cell (CD8) numbers and activity, decreases the proliferative capacity, numbers, and activity of helper T cells (CD4) with increases in CD8/CD4 ratios, impairs the generation of cytotoxic T cells, and alters immunoglobulin secretion when compared to treated hereditary hemochromatosis patients or controls. A correlation has recently been found between low CD8+ lymphocyte numbers, liver damage associated with HCV positivity, and severity of iron overload in beta-thalassemia major patients. Iron overload, with its associated increases of serum iron levels and transferrin saturation, may cause a poor response to interferon therapy. Iron overload with hyperferremia is associated with suppressed functions of the complement system (classic or alternative types). High plasma ferritin content in patients with chronic, diffuse diseases of the liver (cirrhosis, chronic hepatitis), beta-thalassemia major, dyserythropoiesis, and hereditary hemochromatosis may induce the development of anti-ferritin antibodies with the production of circulating immune complexes. Increased body stores of iron in various clinical situations may tip the immunoregulatory balance unfavorably to allow increased growth rates of cancer cells and infectious organisms, and complicate the clinical management of preexisting acute and chronic diseases. (received 10 April 2000; accepted 8 June 2000)

Keywords: iron overload, immune system, hemochromatosis, macrophages, phagocytosis, T-cells, B-cells, delayed hypersensitivity, infectious diseases, interferon, tumor growth

Introduction

There is a progressive, generalized, age-related decline in human immune function, which exhibits variability in extent and characteristics among individuals. Environmental, genetic, and nutritional factors, including tissue and cellular damage by oxidants, appear to be involved. Some degree of immune decline can be found in every aged human, and may become serious by the seventh decade. Findings in elderly persons that reflect immune alterations include susceptibility to various infectious organisms and malignancies, decreased response to foreign antigenic stimuli and delayed hypersensitivity, increased response to self-antigens, and immune alterations characteristic for specific components of immune function [1]. Mounting evidence suggests that specific age-related immune functional deficits are related to iron concentrations and that iron can induce molecular damage by the generation of reactive oxygen free radicals at extracellular and intracellular loci [1].
The quantity of free radicals generated, the extent and degree of immune damage, and the concentrations of iron in tissue or cells all appear to be positively correlated. Sustained high levels of iron may lead to the production of sufficient oxygen free radicals to overwhelm protective antioxidants and other body defenses, and allow a vigorous attack on immune components and cells. Increased body iron stores may accelerate, enhance, and broaden the scope of short- and long-term immune damage, beyond that naturally produced by aging.

Control of Iron Uptake in Cells and Tissues

Hereditary hemochromatosis is generally associated with homozygosity for a point mutation of the gene that encodes the HFE protein, which is closely related to class I proteins of the major histocompatibility complex (MHC) [2]. HFE protein interacts with beta2-microglobulin and is incorporated into plasma membranes of cells that are regulated for iron content, including macrophages and other cells of the immune system [2]. HFE expression in cell membranes appears to initiate steps to regulate cellular iron absorption by affecting the activity of iron regulatory proteins (IRPs).

IRPs (chiefly IRP1 and IRP2) are cytoplasmic RNA-binding proteins that control iron metabolism in mammalian cells [3]. The IRPs interact with iron-response elements in the mRNAs (3' or 5' untranslated regions) of genes associated with iron metabolism [3,4]. In iron-deficient cells, increased membrane HFE expression causes increased IRP (IRP1 and IRP2) binding to iron-response elements that regulate gene expression. This produces down-regulation of the iron storage protein (ferritin) and up-regulation of transferrin receptor levels [2,4], leading to decreases in intracellular iron concentrations.

In hereditary hemochromatosis, the lack of functional HFE protein is equivalent to low HFE membrane expression and results in iron overload in multiple tissues. IRP activity is down-regulated in these tissues according to the degree of iron overload. Therefore, the ability of the IRP regulatory system to sense intracellular iron concentrations appears to be intact in patients with hereditary hemochromatosis [2].

The various mechanisms of cellular iron control that have been discussed can be modulated by changes in the redox state or oxidative stress of cells, especially in loci of inflammation [4].

Iron and the Monocyte/Macrophage System

**Phagocytosis.** Phagocytic functions of monocytes (MN) and polymorphonuclear (PMN) leukocytes have been studied in patients with iron overload and compared to those of healthy donors [6]. Abnormal phagocytic functions in the iron-overloaded patients included significant decreases in phagocytosis of opsonized *Staphylococcus albicans* by MM and PMN, decreased bactericidal activity of MN, and decreased mean chemotactic responsiveness of PMN leukocytes. Iron-overloaded patients may have associated functional impairment of monocytes and granulocytes, and the excess of available iron may stimulate bacterial growth [6].

Iron overloading can increase susceptibility to *L. monocytogenes* in patients by decreasing the phagocytic capacity of monocytes and by increasing the virulence of the organism [7].

Biopsies of the terminal duodenum from 11 patients with hereditary hemochromatosis revealed iron localization in basal parts of the villi, between the crypts of Lieberkuhn, stored in plasma cells but not in macrophages. Impaired iron-storing of macrophages in hereditary hemochromatosis may be related to increased intestinal iron absorption [8].

Monocytes from hereditary hemochromatosis patients exhibited decreased antibody-mediated phagocytosis of rabbit erythrocytes and *Staphylococcus aureus*, compared to monocytes from normal persons. The altered phagocytosis by hereditary hemochromatosis monocytes did not reflect the levels of expression of Fc gamma receptors (Fc gamma R) involved in phagocytosis or Fc gamma RIIa polymorphism [9].
Monocytes (MN) and monocyte-derived macrophages (MDM) from hereditary hemochromatosis patients demonstrated significantly decreased ability to phagocytose rabbit red blood cells. Hereditary hemochromatosis MNs also showed decreased capacity to phagocytose *Staphylococcus aureus*, but with *S. aureus* the decrease may be kinetic in nature, explaining why the prevalence of bacterial infections is not increased among hereditary hemochromatosis patients. Phagocytosis defects were consistently found in hereditary hemochromatosis patients and were independent of the magnitude of iron overload, age, or severity of liver damage, and affected the antibody-mediated uptake of bacteria and rabbit RBCs [10].

Macrophages have been shown to possess a great ability to take up exogenous iron, producing lysosomal iron accumulation that may be cytotoxic [11]. They can also autophagocytose endogenous ferritin/apoferritin, which may chelate lysosomal iron and combat iron-mediated intralysosomal oxidative reactions. It appears that human monocyte-derived macrophages accumulate iron in response to exposure to iron, that iron can destabilize secondary lysosomes of macrophages when cells are exposed to H₂O₂, and that endocytosed apoferritin stabilizes the acidic vacuolar compartment of iron-loaded macrophages. However, induction of ferritin synthesis may not be sufficient to protect completely against iron-mediated cytotoxic effects (e.g., H₂O₂ effects) under conditions of severe macrophage lysosomal iron overload [11].

Macrophages acquire iron from transferrin via receptor-mediated endocytosis and via uptake of extracellular low molecular weight iron chelates, especially under conditions of iron overload. Iron acquisition depends upon the nature of iron chelates; Fe-ascorbate > Fe-citrate > Fe-nitrilotriacetate (NTA) = Fe-ADP > Fe-glycyl-L-histidyl-L-lysine > Fe-diethylenetriamine pentaacetic acid >= Fe-EDTA = Fe-deferoxamine. Evidence suggests that myeloid cells are also able to salvage low molecular weight iron chelates. Acquisition of low molecular weight chelates may allow macrophages and other leukocytes to clear local states of iron overload in vivo [12].

**Chemotaxis.** As noted in the previous section, hereditary hemochromatosis is associated with defective function of the monocyte-macrophage system. However, a careful study did not show any differences in mean chemotactic responsiveness between monocytes of control patients and hereditary hemochromatosis patients, despite the abnormal migration and location of gut wall macrophages in hereditary hemochromatosis patients [13]. Increased transferrin receptor expression was seen in 12 hereditary hemochromatosis patients, but the increase was not correlated with the degree of iron overload. It appears that increases in transferrin receptors are not secondary to systemic iron overload but may signify a primary defect of iron metabolism in patients with hereditary hemochromatosis [14].

**Release of cytokines.** In a study by Gordeuk et al [15], monocytes from healthy controls and hereditary hemochromatosis patients were incubated in a medium with or without added lipopolysaccharide. The mean concentrations of immunoreactive tumor necrosis factor-alpha (TNF-alpha) in the supernatants were lower in the hereditary hemochromatosis patients compared to healthy controls, suggesting that monocyte production of TNF-alpha may be selectively impaired in hereditary hemochromatosis patients [15].

**Antigen processing.** Carrasco-Martin et al [16] reported that iron salts produced a time- and dose-dependent blockage of antigen presentation. Antigen processing interference correlated with the production of lipid peroxide. The authors suggested that iron porphyrins and free iron could cause peroxidation of key lipids involved in specific protein interactions in antigen processing [16].

**Overview.** Based on discussions in the four previous sections, iron overload has adverse effects on four major facets of macrophage/monocyte functions: (a) immune and nonimmune phagocytosis, (b) chemotaxis, (c) cytokine production and release, and (d) antigen processing for subsequent presentation to B- and T-lymphocytes. Interference with such macrophage/monocyte functions can alter the recognition of, and the response to, foreign antigens, cancer cells, and infected cells. Iron overload interferes with macrophage/monocyte attraction to and participation in acute and chronic inflammatory responses, affecting the immune response in general [17].
Iron Effects on T and B Lymphocytes.

T suppressor cells (CD8) and T helper cells (CD4). Effector T cells play a role in cellular immunity, in regulating B-lymphocyte function/activity, and in delayed hypersensitivity [17]. B lymphocytes are transformed into plasma cells that produce circulating antibodies (humoral immunity). Helper T cells (CD4) stimulate cellular and humoral activity of the immune system, while suppressor T cells (CD8) inhibit cellular and humoral immune activity. CD4/CD8 ratios set the relative activity or inactivity of the immune system. CD4/CD8 ratios in human peripheral blood range from 0.0 to 2.7 [17].

Iron-induced decreases or increases in CD4 and/or CD8 numbers or distribution (with resulting changes in CD4/CD8 ratios) may drastically influence the relative activity or inactivity of the immune system. Arosa et al [18] compared the phenotypic and functional characteristics of peripheral blood T lymphocytes from 21 patients with hereditary hemochromatosis to those from 30 healthy controls, and determined the HLA phenotypes of both groups. The greatest changes were observed in HLA-A3 antigen positive patients with hereditary hemochromatosis, and included increased percentages of CD8+CD28- T cells and decreased percentages of CD8+CD28+ T cells. No CD28 expression anomalies were seen in the CD4+ subset. CD8+ cytotoxic T lymphocytes (CTL) from hereditary hemochromatosis patients exhibited diminished cytotoxic activity as compared to CTL from healthy controls, suggesting an association between hereditary hemochromatosis and functional anomalies of the peripheral CD8+ T-cell pool [18].

Arosa et al [19] reported that some hereditary hemochromatosis patients have abnormally low numbers of peripheral CD8+T cells. CD8-p561ck kinase activity was decreased in 16 of 18 hereditary hemochromatosis patients, but levels of CD4-p561ck activity were not decreased when compared to healthy controls. Decreased CD8-p561ck activity was seen in hereditary hemochromatosis patients undergoing intensive phlebotomy treatment or maintenance therapy, so the anomaly was not corrected by iron removal. This was the initial evidence of an abnormality in a src-receptor-associated kinase in a human disease that is linked to MHC class-I antigens [19].

Decreased CD8 numbers and increased CD4/CD8 ratios are seen in many patients with hereditary hemochromatosis. Notably, Porto et al [20] observed in 21 hereditary hemochromatosis patients a positive correlation between their CD4/CD8 ratios and the amount of iron removed by phlebotomies.

A study by Bryan et al [21] of 7 patients with hereditary hemochromatosis demonstrated increases in the absolute numbers of CD8+ T cells in untreated patients, and reduced numbers in treated patients, compared to controls. Untreated patients with hereditary hemochromatosis showed suboptimal proliferative responses of peripheral blood mononuclear (PBM) cells to mitogens, although normal responses were found in treated patients. Immunoglobulin secretion by PBM cells in hereditary hemochromatosis patients was altered from controls. A subset of mature non-activated T lymphocytes caused formation of thermostable erythrocyte rosettes, and this response was unrelated to treatment status. The authors concluded that cellular immunity may be influenced by the high level of storage iron in patients with hereditary hemochromatosis [21].

Numerous studies have shown the importance of iron in regulating the expression of T-lymphoid cell surface markers, influencing the expansion of different T-cell subsets, and affecting different immune cells functions in vitro. The majority of such observations made in vitro have an in vivo counterpart, thus providing compelling evidence for the importance of iron as an immunoregulator [22].

Delayed hypersensitivity. Bryan [23] reported a patient with hereditary hemochromatosis who showed specific immune alterations including delayed cutaneous-type hypersensitivity anergy. The patient developed a poorly differentiated stomach adenocarcinoma four years after the diagnosis of hereditary hemochromatosis. One may speculate that, in certain clinical situations of elevated body iron stores, the immunoregulatory balance or metabolic environment may be tipped in favor of growth and development of cancer cells [23].

of the F344 strain. Stimulated lymphocyte proliferation was significantly lower in the old rats with iron overload, compared to the young iron-overloaded or control rats. Iron overloading increased LP and PG metabolism in rats. Mitogen-stimulated PGE2 synthesis and increased T-cell proliferation were observed in young rats after only 4 wk of iron overloading [24].

In iron-overloaded rats, Pietrangelo et al [25] demonstrated a marked reduction in lymphocyte proliferative capability after a mitogenic stimulus and a dramatic decrease in the ability to repair DNA damage. The close association of iron overload with cancer may be related to the deleterious effects of iron overload on cellular DNA structure and function.

According to Smith et al [26], liver nonheme iron concentrations were 67-, 42-, and 21-fold higher than controls at 4, 24, and 78 wk, respectively, after iron overloading with a single sc injection of iron dextran (600 mg/kg) to C57BL/10ScSn mice. Iron was evident in macrophages and in hepatocytes. Iron-positive nuclear inclusions were found in 37% of hepatocytes at 24 wk after iron overloading. The inclusions were not membrane-limited and they increased in size with time after iron overloading to reach diameters of 3 μm and to occupy >25% of the nuclear volume. Immunocytochemical and energy dispersive x-ray microanalysis studies showed iron to be present as ferritin aggregates. The study revealed slow but ongoing passage of ferritin through nuclear pores to form aggregates that progressively increased in size. Intranuclear ferritin may constitute an iron source for catalyzing intranuclear formation of hydroxyl radicals during some toxic, carcinogenic, and aging processes [26].

Changes in lymphocyte subsets were evaluated by Cardier et al [27] in male iron-overloaded Sprague-Dawley rats. A total dose of 1.5 mg/kg iron dextran was divided into equal aliquots and injected sc daily for 20 consecutive days. Blood, splenic, and mesenteric lymph node subsets (ie, T cells (W3.13), helper T cells (W3.25), and cytotoxic T cells (OX-8)) were estimated by indirect immunofluorescence using monoclonal antibodies at 20 and 50 da after the initiation of iron injections. Compared to control rats, the number of W3.25+ T cells in blood was unchanged, but the number of OX.8+ T cells was significantly increased at 20 and 50 da. In the spleen, W3.25+ T cells were decreased and OX-8+ T cells were increased at 20 da but not at 50 da. No changes in mesenteric lymph node W3.25+/OX-8 ratios were seen. These results demonstrate that iron overload alters the distribution of T lymphocytes in various compartments of the immune system in male Sprague-Dawley rats [27].

Cardier et al [28] observed progressively increased lipid peroxidation in spleen homogenates during a 20-day period of iron dextran overloading of male Sprague-Dawley rats. The lipid peroxidation was attended by the development of immunological abnormalities. Additions of superoxide dismutase or catalase reversed iron-induced decreases of a splenic lymphocyte proliferative response to concanavalin-A, suggesting that reactive oxygen species were responsible for the alterations. Damage of cell membranes and DNA by intracellular and extracellular peroxidation probably plays a role in the immunological abnormalities associated with iron overload [28].

Different forms of iron and iron-binding proteins were tested by Djeha et al [29] for effects on the proliferative response of human lymphocytes to phytohaemagglutinin (PHA). Transferrin enhanced lymphocyte proliferation proportionally to the degree of iron saturation up to 100%, but the presence of excess iron caused decreased proliferation. A lipophilic complex, ferric pyridoxal isonicotinoyl hydrazone (FePIH), increased lymphocyte proliferation, whereas a hydrophilic complex, ferric nitritotriacetate (FeNTA), decreased lymphocyte proliferation. Lymphocyte ferritin levels increased 4-fold as transferrin iron saturation rose from 0% to 90%, but no further increase occurred at higher iron levels. This suggests that lymphocytes are poorly equipped to detoxify excess iron through stimulation of ferritin synthesis. Ferritin (0% to 75% iron saturation) produced CD4/CD8 ratios of 2.0:1.0 in cells previously incubated with PHA for 72 hr, while FeNTA produced ratios of 1.1:1.0. Iron complexes apparently can affect lymphocyte proliferation and subset ratios, depending on the form and amount of iron present [29].

In a study by Good et al [30], cultures of splenic cells from carbonyl iron-loaded C57 mice generated
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reduced amounts of interleukin 2 (IL-2) following stimulation with concanavalin-A, probably due to decreased numbers of splenic IL-2 secreting cells. These splenic cells showed reduced capacity to generate an allo-specific cytotoxic response, but this abnormality was reversed by the addition of exogenous IL-2. These results suggest that iron overload is associated with defective immunoregulatory control [30].

In another study, Good et al [31] investigated the effects of ferric iron and normal human liver ferritin on the proliferative response of human memory T4+ lymphocytes to tetanus toxoid. Ferric citrate significantly reduced the cloning efficiency of precursor T cells. Fe3+ levels comparable to iron concentrations in the serum of iron-overloaded patients interfered with antigen-specific lymphocyte responses, which may help to explain the development of infections and neoplasia in patients with iron overload [31].

Grady et al [32] found that the percentage of OKT8-positive (T suppressor) cells in chronically transfused beta-thalassemia major patients increased linearly with the number of units transfused. The results indicated that transfusion of blood products may lead to decreased T4/T8 cell ratios (increased numbers of T8 suppressor cells) and increased numbers of B-cell (SIg-positive) populations. Splenectomized beta-thalassemia major patients showed marked, persistent lymphocytosis with increases in numbers of T cells but even greater increases in B-cell numbers, unrelated to the number of transfusions. None of the patients with beta-thalassemia major and with decreased T4/T8 cell ratios exhibited the clinical symptoms of acquired immune deficiency syndrome (AIDS) [32].

Natural killer cells. Natural killer (NK) cells have an innate ability to lyse foreign cells, including virus infected cells, cancer cells, and in some cases, normal cells. Prior exposure of the organism to the inciting antigen is not required [17]. Beta-thalassemia major patients exhibit depressed NK-cell activity toward K562 cells and decreased cytotoxicity against human fibroblasts infected with herpes simplex virus type-1 (HSV-1). Akbar et al [33] found that desferrioxamine and alpha-interferon enhanced or restored NK activity by different mechanisms, both of which appear to be reversibly impaired in patients with beta-thalassemia major [33]. There may be a positive correlation between low numbers of CD8+ lymphocytes, HCV-induced liver damage, and the severity of iron overload in patients with beta-thalassemia major [34].

Other immune effects. Cultured lymphocytes from 11 patients with hereditary hemochromatosis showed significant increases in spontaneous and radiation-induced DNA strand-breaks, compared to lymphocytes from matched controls, suggesting that the iron overload of hemochromatosis causes chromosomal damage [35]. Monocytes from iron-loaded patients with hereditary hemochromatosis also express increased surface ferritin levels [36].

Iron Effects on the Complement System

Complement activation, an essential component of the acute inflammatory response, is associated with an anaphylatoxic effect (vasodilation, increased vascular permeability, and fluid exudation), chemotaxis for neutrophils, and induction of immune phagocytosis of antigens and membrane lysis of target cells [17]. Complement may be activated by either the classic or alternative (properdin) pathways [17].

Settarova et al [37] evaluated the complement system in 114 hereditary hemochromatosis homozygotes and 48 hereditary hemochromatosis heterozygotes, by assays for complement protein function, circulating immune complex level, neutrophil phagocytic activity, completeness of the phagocytic reaction, and C3c levels. The results showed suppression of complement system function, according to the classic or alternative types, associated with hyperferremia and reflected damage to the immunocompetant organs and cells synthesizing individual complement proteins [37].

Overview of Immune Effects of Iron.

In an extensive review of the effects of iron and iron-binding proteins on the immune system, De Sousa et al [38] emphasized the following points: (a) iron and iron-binding proteins act as regulators of immune function, (b) immune system cell subsets respond differently to in vitro and in vivo increases in iron concentrations, (c) macrophages and lymphocytes differ in the H- and L-subunit content of the ferritins synthesized in response to in vitro increases in iron
concentrations, (d) NK activity of adherent and nonadherent cells differ in susceptibility to in vitro enhancing effects of lactoferrin, (e) iron causes diminished responses to mitogen stimulation by PHA and concanavalin-A, (f) iron pretreatment of effector cells (but not target cells) causes diminished responses in the MLR, which appear related to the HLA-A locus, (g) transferrin is synthesized by a specific subset of T lymphocytes, and (h) transient increases of serum iron concentrations above the full saturation of transferrin (reproducing a common situation in patients with hereditary hemochromatosis) induce cellular changes in the synovium that mimic changes during the course of an experimental rat arthritis model [38].

**Excess Iron in Relation to Ferritin Levels**

Levina et al [39] measured parameters of iron metabolism and humoral immunity in groups of patients including those with beta-thalassemia major and hereditary hemochromatosis. High plasma ferritin levels led to the formation of anti-ferritin antibodies, followed by development of circulating immune complexes that caused clinical complications. Plasma ferritin and circulating immune complex levels were reduced by plasmapheresis and deferoxamine therapy with favorable clinical outcomes [39].

In patients with hereditary hemochromatosis, iron overload affects mainly parenchymal cells, and little iron accumulates in the reticuloendothelial cells. Reticuloendothelial cells from hereditary hemochromatosis patients have high activities of iron regulatory protein (IRP), a key regulator of intracellular iron homeostasis. Elevated IRP should cause a reduction in the intracellular iron pool, reflecting failure of cellular retention of iron. Recalcati et al [40] found that monocytes and macrophages from control subjects treated with lipopolysaccharide and interferon-gamma showed a transient increase in IRP activity, followed by a greater decrease. In patients with hereditary hemochromatosis, monocytes and macrophages also showed increased IRP activity but no subsequent decrease. Ferritin content increased 24-hr post-treatment in monocytes from control patients, but not in those from hemochromatosis patients. Thus, the control of iron release from reticuloendothelial cells is defective in hereditary hemochromatosis patients [40].

**Effects of Excess Iron on Interferon**

The interferons (INF) are a family of agents (alpha, beta, gamma) with antiviral and antitumor effects [17]. INF-gamma is an important mediator of delayed-type hypersensitivity and a potent macrophage activator, enhancing phagocytosis of foreign or offending antigens, macrophage-induced cell lysis, and the ability to kill microorganisms and tumor cells [41].

Clemente et al [42] showed that the response of patients with transfusion-dependent thalassemia and chronic active hepatitis C to IFN-alpha therapy is inversely related to liver iron burden. They concluded that INF-alpha therapy represents a useful option for children with transfusion-dependent thalassemia and chronic active hepatitis C with a mild to moderate iron burden [42].

Iron excess and iron deficiency can both lead to disease states. Iron overload facilitates infection, as in the increased risk of hereditary hemochromatosis patients for certain infections and in the fact that patients with chronic viral hepatitis and lower hepatic iron levels respond better to interferon therapy than patients with larger amounts of hepatic iron [43]. These studies suggest that excess iron antagonizes interferon activity [17,41-43].

**Excess Iron and Neoplasia**

Iron inhibits the nonspecific tumoricidal activity of macrophages, which may contribute to the increased incidence and growth of neoplasia in patients with hemochromatosis [44]. Iron overload conditions are associated with increased susceptibility to both infection and neoplasia. Effects include impairment of antigen-specific immune responses, reduction in numbers of functional helper precursor cells, impaired generation of cytotoxic T cells, enhancement of suppressor T-cell activity, reduction of the proliferative capacity of helper T cells, and increased incidence of hepatocellular carcinoma. Iron deficiency is also associated with altered response to infection, possibly involving impaired T- and B-cell function [45].

Beckman et al [46] analyzed the interactions between the hemochromatosis and transferrin receptor genes in various malignancies. The genes for the transferrin receptor (TFR) and for hereditary
hemochromatosis (HFE) are both involved in iron metabolism. The wild-type HFE gene product complexes with the transferrin receptor and two different mutations of the HFE gene product (Cys282Tyr and His63Asp) increase the affinity of HFE for TFR and enhance cellular iron uptake. Individuals with the HFE Tyr282 allele (homozygous and heterozygous) in combination with the TFR Ser142 allele have increased risk for cancer. Interaction between HFE and TFR genotypes has been noted in patients with multiple myeloma, breast cancer, and colorectal cancer. Thus, an interaction between HFE Tyr282 homozygotes or heterozygotes in combination with TFR Ser142 homozygosity may enhance the risk for various neoplastic disorders [46].

Heterozygous carriers of hereditary hemocho­matosis usually have increased iron stores compared to control noncarriers, and heterozygous carriers of hereditary hemochromatosis may comprise up to 15% of the American population. Increased body iron stores give increased risk of heart disease in men and cancer and colonic adenoma in women. Nelson et al [47] reported an evaluation of family history questionnaires and other data for 1950 hereditary hemochromatosis heterozygotes and 1656 normal controls. The results indicated that heterozygosity was attended by increased risk for colorectal neoplasia, diabetes, hematologic malignancy, and gastric cancer, but not for heart disease, cancer death, or cancers of the lung, breast, or cervix [47].

Tiniakos and Williams [48] analyzed a series of needle liver biopsies performed on 71 patients with hereditary hemochromatosis who were treated by venesection therapy for 1 to 12 yr (mean 7). The tissue samples included initial biopsies, biopsies following venesection therapy, and follow-up or autopsy biopsies (30 cases). The final histological evaluations of liver pathology remained unchanged in 44 (62%) cases, became worse in 23 (32%) cases, and improved in 4 (5%) cases. Long-term venesection therapy did not prevent a high incidence of hepatocellular cancer (18%) or the development of a malignant neoplasm in another organ (8%). This study supports the conclusion that the longer survival time of hereditary hemochromatosis patients treated by venesection increases the possibility of developing a hepatocellular carcinoma or a malignancy in another organ [48].

Excess Iron and Infectious Diseases

Two monoclonal antibodies, ED1 and ED2, recognize epitopes on spleen macrophages related to antigen presenting activity. In a study by Wu and Hayashi [49], red and white pulp macrophages contained hemosiderin deposits and demonstrated reduced expression of ED1 and ED2 and reduced macrophage phagocytic activity, which, according to the authors, may influence macrophage antigen presentation and anti-infection functions [49].

Even normal or slightly elevated iron concentrations in liver can be damaging when combined with other hepatotoxic agents (alcohol, drugs, viruses). Iron enhances the pathogenicity of microorganisms, adversely affects macrophage and lymphocyte function, enhances fibrogenesis, and may be a co-carcinogen or promote neoplasia (eg, hepatocellular carcinoma) [50].

Human proteins such as transferrin and lactoferrin, used for iron acquisition and transport, can sometimes withhold iron from the siderophores of invading bacteria and fungi and from protozoa and neoplastic cells. Some microorganisms have adapted by developing methods to take iron away from host proteins and cells, such as red blood cells, and such microorganisms are particularly dangerous in hosts with iron overload in specific body fluids, tissues, or cells. The excesses of available iron may stimulate invading organisms to grow and help them to overwhelm host immune defenses [51].

Pulmonary tuberculosis and dietary iron overload are both common in sub-Saharan Africa. The spread of AIDS from the human immunodeficiency virus (HIV) has allowed increased incidence of tuberculosis. Dietary iron overload affects up to 10% of adults in the rural population and is characterized by heavy iron deposition in parenchymal cells and macrophages. Mycobacterium tuberculosis grows in macrophages and macrophage function is important in the body's defense against the infection. Increased iron concentration in macrophages reduces their response to activation by INF-gamma and diminishes their antimicrobial toxicity [52].

According to Moyo et al [52], dietary iron overload may increase the risk of death from tuberculosis even in the absence of AIDS. The combination of dietary iron overload and HIV infection, with impaired
function of both macrophages and T cells, renders patients especially vulnerable to tuberculosis. Thus, prevention and treatment of iron overload might contribute to the control of tuberculosis in the African population [52].

Minn et al [53] reported that human serum, transferrin, and apotransferrin can profoundly inhibit the growth of *Candida albicans* by iron deprivation. They showed that iron overload (iron saturated transferrin) is a serious risk factor for candidiasis in newborns and leukemic patients. Fluconazole and effector cells exhibit synergy against *Candida albicans*. Minn et al [53] observed that exogenous iron inhibited the fungistatic activity of monocyte-derived macrophages, but did not prevent the synergistic antifungal activity conferred by fluconazole and monocyte-derived macrophages. In fact, fluconazole activity was often increased in the presence of extra iron, suggesting that the effectiveness of fluconazole therapy is not compromised in vivo by iron overload conditions [53].

According to Mencacci et al [54], murine iron overload greatly increases the host's susceptibility to disseminated infection with low-virulence *Candida albicans* cells of exogenous origin. The candidacidal activity and the ability to release nitric oxide and bioactive interleukin (IL)-12 were greatly impaired in neutrophils and macrophages from infected mice. CD4 T cells from spleens of iron-overloaded mice produced high levels of IL-4 and IL-10 and low levels of INF-gamma. Treatment of iron-overloaded mice with the iron chelator, deferoxamine, resulted in cure of mice from candidial infections, restored the antifungal effector and immunomodulatory functions of phagocytic cells, and allowed the occurrence of CD4 Th1 protective antifungal responses. Thus, iron overload may negatively affect CD4 Th1 development in mice with candidiasis, a function that is efficiently restored by therapy with deferoxamine [54].

Iron overload may play an important role in the progression of human immunodeficiency virus (HIV) toward its more advanced stages. Iron accumulates in macrophages, microglia, endothelial cells and monocytes. The iron burden is especially heavy in bone marrow, brain white matter, muscle, and liver. Excess iron potentially enhances oxidative stress, impairs the already compromised immune defense mechanisms, and directly promotes the growth of microbial cells.

It is possible that prevention or reduction of iron loading might slow the progression of the infectious complications of HIV infection, and perhaps indirectly, the HIV infection itself. Boelaert et al [55] proposed a twofold strategy consisting of limitation of iron intake through the alimentary, parenteral, and respiratory routes, and possible use of iron chelating agents to decrease the iron burden, redistribute the metal to erythroblasts, and suppress growth of microorganisms. Such an approach must be considered hypothetical. However, there is urgent need for studies on the effect of iron status on the course of HIV infection [55].

Abe et al [56] induced experimental candidiasis in iron- and non-iron-overloaded mice by iv injections of 1 X 10⁷ *Candida albicans* spores. The infected mice were observed for 28 da post-inoculation. The iron-overloaded mice received iv injections of 60 mg/kg colloidal iron on 3 consecutive days prior to the fungal inoculation. The mortality rate in iron-overloaded mice was 80% compared to 40% in controls. Increased serum iron and iron saturation enhanced candidial growth, loaded phagocytic cells with iron, decreasing their phagocytic activity against the inoculated *Candida* organisms. Proliferation of the candidial infection involved the iron-rich kidneys. In this experiment, iron overloading clearly promoted the proliferation of iv-inoculated *Candida* organisms [56].

**Excess Iron and Marrow Transplants**

Strasser et al [57] studied the degree of hepatic iron overload and marrow iron content in 10 patients given marrow transplants for hematologic malignancies who died between 50 and 100 days after the transplant. The patients received 30 ± 17 units of red cells during the transplant period and 48 ± 26 units of red cells from diagnosis to death. The mean time from disease onset until death was 2.2 yr (range 0.5–8.7) and the mean age was 35 yr (range 10-59). The authors found that these marrow transplant recipients had high liver iron content, comparable to the range seen in patients with hereditary hemochromatosis [57].

**Excess Iron and the Thymus**

Presence of iron within cells of the thymus has rarely been reported. However, Vigorita and Hutchins [58]
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evaluated four cases of hereditary hemochromatosis at autopsy. Iron-containing moieties were found within lymphocytes and epithelial cells of the involuted thymic glands, suggesting either iron uptake from the vascular pool or synthesis of iron-containing moieties within the cells themselves [58].

Conclusions and Research Needs

Iron overload adversely affects macrophage/monocyte function directly and indirectly. Interferences with antigen processing, phagocytosis, and release of cytokines appear to be direct, while the indirect effects include reduced chemotaxis, reduced complement activation by macrophages, and impaired macrophage activation by interferon. Modification of macrophage/monocyte function appears to be the most consistent iron-induced abnormality of the immune system, with far-reaching effects on acute and chronic inflammatory responses, delayed hypersensitivity, and other immune activities that involve macrophages/monocytes.

Iron excess appears to cause alterations of T-cell (cellular immunity) and B-cell (humoral immunity) function. Iron-induced modifications of helper T-cell and suppressor T-cell numbers, their distribution, and CD4/CD8 ratios also influence the lymphocyte component of the immune system [17].

The ability of excess iron to cause a spectrum of immune dysfunctions is beginning to be understood and appreciated. Further research must elucidate the effects of acute and chronic exposure to excess iron on the immune system. What are the short- and long-term effects of iron overload on the physiological functions of diverse organ systems, the aging process, and many other facets of human health and well-being? Which effects of iron overload result from intracellular and extracellular oxidative damage to cell membranes and macromolecules? What role does chronic exposure to excess iron have in the pathogenesis of atherosclerosis and associated cardiovascular disease, in diabetes, and in hepatic failure? Do iron-induced abnormalities of the immune system play a role in these diseases? Studies are needed in animals or humans of the effects of acute and chronic iron exposures on the central nervous system and their possible associations with neurodegenerative diseases. Does iron overload cause additive or synergistic deleterious effects on individuals who suffer from AIDS, autoimmune diseases, and other conditions affecting the immune system? Does iron overload influence the incidence, progression, aggressiveness, susceptibility to therapeutic modalities (chemotherapy, radiotherapy, immunotherapy, and surgery) and survival outcomes of human cancers? Does excess iron, via oxidative damage to DNA and other macromolecules, initiate tumor induction or cause benign malignancies to become aggressive and lethal? What are the effects of iron overload on parasitic infestations such as malaria and schistosomiasis?

Although considerable data have been derived from animal studies regarding iron-induced alterations of immune functions, such data from clinical research are sparse. Valuable insights on the immune effects of iron overload have been obtained in patients with hereditary hemochromatosis and hemolytic blood diseases, such as beta thalassemia major. However, more research is needed to understand, prevent, and treat the adverse effects of excess iron on human health.

References


