Iron overload and immunity

Graça Porto, Maria De Sousa

Abstract

Progress in the characterization of genes involved in the control of iron homeostasis in humans and in mice has improved the definition of iron overload and of the cells affected by it. The cell involved in iron overload with the greatest effect on immunity is the macrophage. Intriguing evidence has emerged, however, in the last 12 years indicating that parenchymal iron overload is linked to genes classically associated with the immune system. This review offers an update of the genes and proteins relevant to iron metabolism expressed in cells of the innate immune system, and addresses the question of how this system is affected in clinical situations of iron overload. The relationship between iron and the major cells of adaptive immunity, the T lymphocytes, will also be reviewed. Most studies addressing this last question in humans were performed in the clinical model of Hereditary Hemochromatosis. Data will also be reviewed demonstrating how the disruption of molecules essentially involved in adaptive immune responses result in the spontaneous development of iron overload and how they act as modifiers of iron overload.

© 2007 WJG. All rights reserved.

Key words: Iron; Iron overload; Innate immunity; Adaptive immunity

Porto G, De Sousa M. Iron overload and immunity, World J Gastroenterol 2007; 13(35): 4707-4715

http://www.wjgnet.com/1007-9327/13/4707.asp

THE TWO IMMUNITIES

The last decade has seen a growing understanding of the numerous functions of the immune system beyond the two classical attributes of adaptive immunity: antigen specificity and memory. The key effector cell of adaptive immunity is the lymphocyte, a cell no longer known from its two principal origins in mammals, the thymus (T) and bone marrow (B), but with several subtypes characterized by different function and cytokine production profiles[1]. “Behind” adaptive immunity lays a complex world of cells and molecules involved in less antigen-specific tasks collectively dedicated to the function of innate immunity. Two competing models have sought to explain innate immunity. One where evolutionary conserved features of pathogens are recognized by pattern recognizing receptors[2] known generally as toll-like-receptors (TLRs). A second model, known as the danger model, is based on the assumption that products from damaged or stressed cells provide a danger signal to the host, thus evoking an innate immunity response[3]. The effector cells of innate immunity are mostly myeloid cells and lymphocytes whose interaction with target cells does not depend on the recognition of the Major Histocompatibility Complex (MHC). A third cell, the dendritic cell (DC), was first described by Steinman and Cohn in 1973[4]. DCs have been described most elegantly as “the nexus for translating signals from innate recognition into cells guiding adaptive immune function”[5].

One hallmark of immune system cells, whether involved in innate or adaptive immunity, is their capacity to circulate and migrate from the blood compartment into lymphoid and non-lymphoid tissues (innate immunity cells) from non-lymphoid into lymphoid tissues (DCs) and from the blood into the lymph through the peripheral lymphoid organs (lymphocytes). The other is their role in protection from pathogen infections. Since early descriptions of immune cell functions in iron overload[6] knowledge of the reciprocal interactions between immune cell responses, intracellular iron load and response to micro-environmental changes in iron levels has increased considerably. A decisive contribution to this understanding was the clarification of the genes involved in iron homeostasis. Reviews of the functions of such genes and their link to the reticuloendothelial system and immunity have been published recently[7,8]. The present review will focus on the evidence that the cells of the immune system are equipped to modulate iron homeostasis through the expression of several iron related genes and proteins, and how do innate and adaptive immune cells respond in conditions of iron overload. In addition, the reader will be reminded of the evidence indicating that defective immune system models, particularly experimental models,
are associated with the spontaneous development of iron overload[9]. The mechanisms underlying what could be called the “reverse” side of this same coin, i.e. immunity and iron overload are less well understood. However, taking into account that practically all cells of the immune system express iron related genes their contribution to systemic iron homeostasis should no longer be ignored.

**IRON AND INNATE IMMUNITY**

As a critical element of cellular activity, iron plays a pivotal role in the fight for survival between mammalian hosts and their pathogens, each displaying a wide range of mechanisms for controlling iron acquisition and utilization. Micro-organisms have developed a large number of strategies to acquire iron from the environment and to transport the element to sites of incorporation into biologically important molecules[10,11]. On the other side, the host has developed the capacity to modulate cellular iron metabolism, not only for its optimal utilization as a catalyst for the generation of reactive oxygen species acting as strong antimicrobial molecules, but also in order to make iron less available for the micro-organisms[11,12]. A number of genes and proteins primary involved in iron homeostasis, namely in iron binding, transport and storage are now recognised to display related immunological functions. These are summarized in Table 1[13-24]. In addition, it is becoming clear that the cells of the innate immune system, as part of a non-specific defense against infection, are equipped to express genes and proteins that can modulate iron homeostasis both at the cellular and the systemic levels (see also Table 1)[25-42]. One central player in this modulation is hepcidin, first described as a liver derived antimicrobial peptide[43], and now well recognised as a key regulator of iron homeostasis and the anaemia of inflammation, at the interface of innate immunity and iron metabolism[43]. Liver-derived hepcidin is strongly induced during infection and inflammation, causing intracellular iron sequestration and decreased plasma iron levels, a process mediated by the inflammatory cytokine cascade, namely macrophage derived IL-6 and IL-1[32,33]. The mechanism underlying intracellular iron sequestration is mediated by the hepcidin-induced internalization and degradation of ferroportin, the only known iron exporter[16]. The reduction in extracellular iron concentrations is believed to limit iron availability to invading microorganisms, thus contributing to host defense. Recently, hepcidin has been shown to be endogenously expressed by innate immune cells, i.e. macrophages and neutrophils, capable of migrating from the blood to a site of infection, constituting a newly recognised component of the local innate immune response to bacterial pathogens[35]. Myeloid endogenous hepcidin mRNA expression in response to bacterial pathogens was shown to be dependent on the specific activation by the toll-like receptor 4 (TLR-4), the key pattern recognition receptor for LPS[43]. This activation also produces down-regulation of the iron exporter

---

**Table 1 Relevant genes/proteins involved in iron homeostasis and iron overload, and their related immunological functions**

| Genes/Proteins | Main role in iron homeostasis | Related immunological functions |
|----------------|-------------------------------|---------------------------------|
| Lactoferrin    | Iron binding, transport and storage | Bactericidal and antiviral[13,14]. Immuno-regulatory effects on Th1/Th2 cell activities[40]. |
| Transferrin    | Iron chelator                  | Present in monocyte/macrophages and T lymphocytes[44]. |
| Transferrin receptor 1 | Cellular iron transporter | Required for early T cell differentiation[73]. |
| Ferritin       | Iron storage                   | Synthesised by macrophages and T lymphocytes[92,93]. |
| Nramp1 (SCLA11) | Iron transfer in late phagolysosome | Resistance to infection with intracellular pathogens[20,22]. |
| Nramp2/DMT-1   | Iron uptake and transfer in late phagolysosome | Resistance to infection with intracellular pathogens[20,22]. |
| Ferroportin 1 (IREG1) | Cellular iron exporter | TLR4 mediated downmodulation in infection[20]. |
| Lipocalin      | Unknown                        | Limits bacterial growth by sequestering the siderophore[94]. |
| Heme-oxygenase 1 | Required for mammalian iron reutilization. Targeted Hmx1 mutations induce spontaneous iron overload[69]. | Derived from macrophages during infection and inflammation[22,24]. |
| HFE           | Hereditary Hemochromatosis associated gene[25]. | Unknown |
| β2-Microglobulin | hemochromatosis[90]. β2m deficiency induces spontaneous iron overload | High CD4:CD8 ratios with activated CD4+ cells in Hmx1 deficient mice[95]. |
| MHC-class I    | MHC-class I deficient mice develop hepatic iron overload[40]. | Critical to the folding of MHC-class I molecules and selection of MHC class I molecule-associated peptides[41]. |

www.wjgnet.com
ferroportin. Endogenous myeloid hepcidin production is not stimulated by iron, pointing to different pathways of hepcidin activation in response to infection or iron overload[23]. The mechanisms underlying the differential stimulatory effects of infection and iron overload on hepcidin are still not understood. Interestingly, it was recently shown that hepcidin levels in fish also respond to iron overload and infection, demonstrating the evolutionary conservation of hepcidin's dual function[26].

In addition to the demonstrated role of neutrophils and macrophages on hepcidin gene activation and ferroportin down-modulation, other iron genes and proteins involved in iron transport from the human phagosome into the cytosol also play critical roles in TLR-4 mediated innate immunity. The natural resistance-associated macrophage protein 1 (Nramp1) is expressed in circulating phagocytes, and is recruited from the lysosomal compartment to the phagosome membrane where it functions as an efficient antimicrobial through a mechanism of iron deprivation[25]. Nramp1 mutations in mice are shown to cause susceptibility to infection with various intracellular pathogens including Salmonella, Mycobacterium and Leishmania[21]. Mutations in the human homologue of the gene, NRAMP1, were also shown to strongly affect the susceptibility to tuberculosis[7]. Nramp2, also known as the divalent metal transporter 1 (DMT1) is another important iron transporter in mammals[48,49] and, as Nramp1, it is also induced by infection with intracellular pathogens, namely Mycobacterium[50]. In addition, it is suggested to play an important role in recycling iron from RBC-containing phagosomes to the cytoplasm[51]. The amount of available iron in the phagosome is also decreased by the entry in macrophages of neutrophil derived lactoferrin (Lf), another well known potent iron chelator found recently to have also a bridging role between innate and adaptive immunity. Both in vitro and in vivo studies showed that Lf is able to stimulate the proliferation and differentiation of T lymphocytes from their immature precursors into the Th1 or the Th2 phenotypes thus having an immunoregulatory effect on Th1/Th2 activities[18,52].

Finally, lipocalin 2 has been recently described as a pivotal component of the innate immune system and the acute phase response[53]. Upon infection the toll-like receptors on immune cells stimulate the transcription, translation and secretion of lipocalin 2 which then limits bacterial growth by sequestering the iron-loaded siderophore[54].

INNATE IMMUNITY AND IRON OVERLOAD

If one or all of the above described iron related immune response genes either fail or the cells are overwhelmed by continuous iron loading such as seen in transfusional iron overload, one consequence is the development of infection. Infections including rare microorganisms[55] are among the major complications in patients with thalassemia, a group of common genetic disorders of hemoglobin synthesis clinically characterized by severe anemia and blood transfusion-dependent iron overload. Besides the well-known risks of blood borne viral infections associated with multiple transfusions, the increased susceptibility of these patients to infection is known to be associated with a wide spectrum of immune abnormalities which are, at least in part, due to the effect of iron overload, including defective chemotaxis and phagocytosis by neutrophils and macrophages and decreased natural killer cell activity[54].

In contrast with the findings in transfusional iron loading, patients with severe iron overload due to Hereditary Hemochromatosis (HH) do not show evidence of increased susceptibility to infections or iron loading of macrophages. HH is a common genetic disorder of iron overload, the majority of HH patients being homozygous for the C282Y mutation in HFE, a gene encoding a protein of the Major Histocompatibility Complex class I (MHC class I). The C282Y mutation disrupts the correct folding of the α3 domain of the protein, interfering with its interaction with β2-microglobulin (β2m) and consequently abolishing the cell surface expression of the molecule[59]. The surface expression of HFE was shown to have a prominent role in the regulation of iron export from macrophages[50,54].

Monocyte/macrophage abnormalities in HH

Anomalies in monocyte/macrophage cells have been consistently described in HH patients, including low TNF-α production by peripheral blood macrophages upon stimulation with lipopolysaccharide[57] and a significant increase in iron regulatory protein (IRP) activity in monocytes[58], an anomaly that was corrected after phlebotomy treatment. Interestingly, subjects with a tissue iron burden similar to HH patients, but due to secondary iron overload have an IRP activity significantly decreased suggesting that the increased IRP activity in iron overload is specific to the HFE related hereditary form of hemochromatosis[59]. In addition, a study that investigated the release of erythrocyte-derived iron from purified human monocytes obtained from controls and HH patients showed that although HH monocytes phagocytosed less than half the number of erythrocytes taken up by control monocytes, they released twice as much iron in the form of LMW-Fe complex than controls[59]. More recently, increased iron content was described in macrophages from HH patients transfected with wt HFE when compared with HH macrophages transfected with an empty vector[56]. These observations are consistent with previously reported observations in macrophage cell lines derived from C282Y mutated HH patients, where HFE was shown to loose its ability to inhibit iron release leading to a relative macrophage iron deficiency[60]. Interestingly, Mycobacterium tuberculosis (M.tb) residing within phagosomes of macrophages from HH patients exhibit a profound defect in their ability to acquire iron from exogenous transferrin and lactoferrin relative to M.tb-infected macrophages from normal controls[89]. Moreover, macrophages from HH patients failed to induce Nef-mediated iron and ferritin accumulation upon HIV-1 infection in contrast to macrophages expressing wild-type HFE[81], thus suggesting that HFE mutated macrophages may be better equipped to protect from HIV-1 infection, compatible with the description of a long survival in a patient with AIDS.
and hereditary hemochromatosis\(^{[60]}\). One must therefore wonder whether the failure of macrophages from C282Y HFE\(^{-}\) HH patients to hold on to the iron is the expression of a putative selective advantage protecting from infection brought about by the appearance and the establishment of such a mutation.

**Iron and the dendritic cells**

Very little work has addressed the interaction between iron loading and dendritic cell (DC) function. There is, however, recent evidence indicating that, upon endotoxin induced maturation, DCs increase significantly the expression of Tlr1 and down regulate expression of the export molecule ferroportin\(^{[65]}\), an observation compatible with an earlier finding of Kramer \textit{et al}\(^{[64]}\) who reported that DCs generated under iron deprivation conditions were phenotypically undifferentiated and could not stimulate T cells.

**IRON OVERLOAD AND ADAPTIVE IMMUNITY**

The postulate that the immunological system could have a role in monitoring tissue iron toxicity, as part of its surveillance function, was first advanced in 1978, based on studies on lymphocyte traffic and positioning\(^{[65-67]}\). It was implicit in that postulate that the lymphomyeloid system, and its circulating components participate in the recognition and binding of metals as a protective device against metal toxicity, and the preferential use of indispensable metals, such as iron, by bacteria or transformed cells. While a vast number of studies have clarified the reciprocal interactions between myeloid innate immunity cells and iron metabolism, fewer studies addressed this question in lymphocyte populations. Lymphocyte activation and expansion depend on the expression of transferrin receptors, required for DNA synthesis and cell division\(^{[68]}\) and both activated and non-activated T lymphocytes synthesize ferritin\(^{[18,20]}\). Lymphocytes could, therefore, act as a "mobile" and easily "mobilizable" iron-storage compartment protecting from iron-mediated toxicity\(^{[69]}\). This hypothesis motivated the study of lymphocyte function in iron overload\(^{[69]}\). The influence of iron on the expansion of different T-cell subsets was demonstrated both \textit{in vitro}\(^{[70]}\) and \textit{in vivo}, namely in patients with thalassemia\(^{[70]}\). Results in this clinical model, however, are difficult to interpret due to the inseparable effects of blood transfusion, splenectomy, iron chelation therapy and infection. Imbalances of the relative proportions of CD4+ and CD8+ T lymphocytes, with abnormally high CD4/CD8 ratios were later reported in HH patients\(^{[5,22]}\), a clinical model where iron overload is not complicated by the effects of transfusion, splenectomy or desferrioxamine. Curiously, in one experimental model of iron overload seen in mice generated with targeted Hmx1 mutations, high CD4:CD8 ratios have been seen in the splenic cell populations of older mice aged 50 wk\(^{[66]}\). These mice also exhibited numerous activated CD4+ cells. Hepatic inflammatory cell infiltrates were seen in the mice, a finding similar to that reported by Rodrigues \textit{et al}\(^{[73]}\) in aging Hfe deficient mice.

**T lymphocyte abnormalities in HH**

Abnormalities in the relative proportions of the two major T lymphocyte subpopulations have been consistently described in HH patients. Reimão and co-workers first described that patients with abnormally high CD4/CD8 ratios displayed a faster re-entry of iron into the serum transferrin pool after intensive phlebotomy treatment than patients without those abnormalities\(^{[71]}\). It was shown later that the amount of iron mobilized by phlebotomy correlated significantly with the number of CD8+ T cells, but not with CD4+ T cells\(^{[72,74]}\). An independent study examining patients homozygous for the C282Y mutation showed that the low percentages of CD8+ T cells seen in the peripheral blood of HH patients were associated with low numbers of the same cells in the liver and with higher levels of hepatic tissue iron\(^{[73]}\). HH patients had been shown earlier to have reduced percentages of CD8+ CD28+ T cells in peripheral blood\(^{[76]}\). No anomalies of CD8 expression were found in the CD4+ subset. The apparent failure of the CD8+ CD28+ T cell population to expand coincided with an expansion of CD8+ CD28- T cells in peripheral blood of HLA-A3+ but not HLA-A3-HH patients\(^{[77]}\). Although the described abnormalities in lymphocyte populations were systematically found in the sub-population of CD8+ T lymphocytes, the association with total body iron stores is also reflected in the total lymphocyte counts. Low total lymphocyte counts were found associated significantly with a higher degree of iron overload in HFE-linked HH, but not in African iron overload\(^{[77]}\). More recently, Barton \textit{et al}\(^{[78]}\) also described a significant inverse relationship of total blood lymphocyte counts and severity of iron overload in hemochromatosis probands with HFE C282Y homozygosity. Fabio \textit{et al}\(^{[79]}\) confirmed that the presence of low numbers of total lymphocyte counts and CD8+CD28+ T cells in C282Y homozygous patients was inversely related to the transferrin saturation. In addition, they found low numbers of CD4+ T and NK cells, and a major increase in IL-4 and IL-10 production in the CD3+ CD8- T cell subset\(^{[79]}\). A study of the Vα/Vβ T cell receptor (TCR) repertoire in a population of C282Y homozygous HH patients showed that the frequency of Vα/Vβ TCR expansions within the CD8+ pool in the group of HH patients was significantly higher in those with iron overload related pathology (9/16) than in patients (1/16) without pathology\(^{[80]}\). In the same study it was found that control subjects heterozygous for the C282Y mutation had an absence of expansions of the Vβ5.2 and Vβ12 chains in the CD8+ pool, suggesting that the HFE could have an effect in the shaping of T cell receptor repertoire.

Functional abnormalities in CD8+ T lymphocytes were also described in HH patients. The level of autophosphorylation of the CD8-associated p56lck as well as its phosphotransferase activity, as determined by phosphorylation of an exogenous substrate, was significantly reduced by two- to three-fold in HH patients relative to a control population of healthy donors\(^{[81]}\). By contrast, the level of CD4-p56lck activity did not show an
overall decrease relative to controls. The decreased CD8- p56lk activity seen in patients was not corrected by iron depletion. A significantly higher percentage of HLA- DR+$^+$, but not CD45RO+$^+$ cells was also found within the peripheral CD8+$^+$ T cell subset in HH patients relative to controls.$^{[56]}$ Moreover, functional studies showed that CD8+$^+$ cytotoxic T lymphocytes (CTL) from HH patients exhibited a diminished cytotoxic activity when compared with CD8+$^+$ CTL from healthy controls.$^{[56]}$

The finding of a significant association of abnormally low CD8+$^+$ T lymphocytes with a more severe clinical expression of hemochromatosis in HH patients$^{[72,83]}$ raises the obvious question: are these abnormalities the follow or precede the development of iron overload. The fact that those abnormalities are remarkably stable in each individual patient, that they are not corrected by phlebotomy treatment, and that they are observed in asymptomatic patients at young ages, favors the hypothesis that they are intrinsic to the genetic defect and not a consequence of the progressive iron overload. More recently it was shown that the numbers of CD8+$^+$ T lymphocytes are genetically determined, in association with other genetic determinants at the MHC class I region close to HLA and $HFE^{[87,88]}$. It is, therefore, conceivable that the inherited abnormalities in CD8+$^+$ T lymphocytes in HH are modifiers of the clinical expression of the disease as proposed by Cruz et al.$^{[85]}$ or genetically located close to a yet unidentified modifier gene of iron metabolism.

THE REVERSE TOPIC: ADAPTIVE IMMUNITY AND IRON OVERLOAD

Genetically manipulated animal models have, therefore, become wonderful and decisive tools to address the questions of the effect on iron overload of a specific gene or protein at the systemic level. Several animal models of spontaneous iron overload were described that illustrate the influence of proteins of the adaptive immunological system on iron homeostasis, all pointing to the putative importance of the MHC class I region.

The β2-microglobulin deficient (β$^2m$−/−) mice constitute the first described model of spontaneous iron overload$^{[38,85,86]}$. These mice develop a hepatic iron overload with a distribution similar to that seen in HH liver pathology, i.e., mainly in the liver parenchyma with no evidence of iron loading in the Kupffer cells$^{[38,86]}$. These mice present severe decreased cell surface expression of the MHC-class I molecules and consequently almost no CD8+$^+$ T lymphocytes$^{[72,86]}$. Intestinal uptake of ferric iron and the subsequent transfer into the plasma is inappropriately increased in β$^2m$−/− mice.$^{[86]}$ Upon treatment with hematopoietic cells derived from normal mice fetal liver iron overload is attenuated and shifted from the parenchymal to the Kupffer cells$^{[80,89]}$. However, TISat and intestinal iron absorption remain high, suggesting that the primary defect of iron overload is not corrected. With the discovery of the $HFE$ gene and the demonstration that the C282Y mutated form failed to bind to β$^2m$,$^{[35]}$ it was assumed that the earlier findings in β$^2m$−/− were due to an impaired $HFE$ function. However, several subsequent studies showed that this was not sufficient to explain the pathology of β$^2m$ deficient mice. In contrast to Hfe$^{−/−}$, the β$^2m$−/− mice display increased expression of the duodenal iron transporters DMT1 and ferroportin1, implicating a broader role of β$^2m$ in mammalian iron overload$^{[80]}$. More recently, Rodrigues et al.$^{[92]}$ described results of a comparative study of these two models in older mice. The results confirmed that the β$^2m$−/− old mice present a more severe hepatic iron overload than the Hfe−/− counterparts. β$^2m$−/− old mice which also showed liver steatosis, probably as a reflection of the higher hepatic iron content causing lipid peroxidation. Earlier Levy et al.$^{[91]}$ had reported the finding that in mice lacking both the Hfe and the β$^2m$ molecules, liver iron deposition is observed in greater levels than in mice lacking Hfe alone.

The β$^2m$−/− Rag1$^{−/−}$ double knock out mice lack mature T and B lymphocytes as well as MHC-class I and Hfe expression. Rag1 is required for normal T and B lymphocyte development. β$^2m$ is required for correct folding and cell surface expression of MHC-class I like proteins. These mice present a more severe body iron overload than each of the single knock out models$^{[90]}$. Besides liver iron deposition in the parenchymal cells, the β$^2m$−/− Rag1$^{−/−}$ also showed iron deposition in pancreas and heart. Older mice under an iron-enriched diet develop heart fibrosis, which could be prevented by treatment with normal fetal liver hematopoietic cells. To determine whether the effect of the β$^2m$ deficiency in the β$^2m$−/− Rag1$^{−/−}$ double knock out mouse was only due to lack of Hfe expression, double knock out mice for Hfe and Rag1 were generated$^{[92]}$. Hfe$^{−/−}$Rag1$^{−/−}$ double knock out mice showed increased liver iron overload compared to each of the single knock out, or the β$^2m$−/− Rag1$^{−/−}$. The distribution of the iron loading in Hfe$^{−/−}$Rag1$^{−/−}$ mice did not recapitulate the iron loading of the β$^2m$−/−Rag1$^{−/−}$, since they did not present heart or pancreas iron loading.

The δ TCR$^{−/−}$ mice lack the γδ intraepithelial lymphocytes. Following the administration of an iron supplemented diet, these mice showed an increased liver iron accumulation in relation to control mice$^{[89]}$. In addition, δ TCR$^{−/−}$ mice had a marked reduction of tumor necrosis factor alpha (TNF-α) production by intraepithelial lymphocytes when compared with controls suggesting a role for this cytokine in intestinal iron regulation.

Finally, mice deficient in the MHC class I molecules H2K$^b$ and D$^b$ have a strong reduction in CD8+$^+$ T-lymphocyte numbers$^{[94]}$. These mice present a spontaneous increase of iron content in the liver preferentially in hepatocytes with occasionally Kupffer cells iron staining$^{[94]}$. The liver iron content in this model was shown to correlate directly with the number of residual CD8+$^+$ T lymphocytes (Cardoso E and M de Sousa, unpublished observations).

The above described double knockout mouse models provide a good illustration of the modifier effect of the components of the adaptive immune system, namely a MHC class I dependent effect, on the iron overload phenotype, a conclusion also reached separately by Muckenthaler et al.$^{[90]}$ in a study of differential gene expression in β$^2m$ deficient mice. Altogether the results described in animal models of hemochromatosis may help to explain why immunological anomalies
modify the severity of iron overload in Hereditary Hemochromatosis.}

**CLOSING REMARKS**

The growing knowledge and availability of genetic techniques dissecting the fine components of the host response to the challenge of infection have strengthened the opportunity of revising the topic “iron and immunity”. When the main concern for this topic resided in the effects of iron deficiency on the immune response, Weinberg pioneered the opposite concern for iron overload and infection. Today it is evident that in response to infection numerous innate immunity components display metal chelating properties, including synthesis and release of lactoferrin, lipocalin and hepcidin, as discussed above. The cells involved in that response are neutrophils and macrophages. But, during evolution the macrophage, particularly the splenic macrophage, assumed the key physiological role of recognizing senescent red blood cells and recycling the iron in hemoglobin. Exhaustion of that pool in vivo leads to “phagocytic” iron overload, such as seen in transfusional iron overload, with the expected consequences in the development of infection. If on the other hand the macrophage fails, it is to be expected that “parenchymal” iron overload will develop. This is the case in HFE-Hereditary Hemochromatosis. Therefore, iron overload must not be seen as one entity, but two separate entities with different relationships to immunity (Figure 1). Evidence was provided in this review that innate immunity is affected and affects “phagocytic” iron overload. On the reverse side, defective immune system models, namely natural and experimental defects in MHC-class I related genes (HFE, β2-microglobulin and HLA) are associated with the spontaneous development of “non-phagocytic” or parenchymal iron overload. The mechanisms underlying the spontaneous development of iron overload in these models are not yet understood. Three possible (not mutually exclusive) mechanisms are represented in this figure: (1) the fact that both Hfe and β2m knockout mouse models lack the liver hepcidin response to iron overload may support the notion of a liver-derived, systemically induced iron overload. The involvement of liver hepcidin in the development of iron overload in mice deficient in the MHC-class I molecules H2Kβ and Db (HLA) was never tested; (2) the iron overload seen in the β2m−/− Rag1−/− and Hfe−/− Rag1−/− mouse models support the involvement of lymphocytes themselves as modifiers of the severe parenchymal iron overload phenotype seen in those mice. Finally, the recent demonstration of a strong link between the MHC-class I region and the genetic transmission of low CDB+ T lymphocyte numbers in humans and the association of total lymphocyte numbers with a more severe iron overload phenotype in patients with Hereditary Hemochromatosis opens a new possibility that another, still unidentified MHC-class I associated gene(s) may simultaneously regulate CD8+ T lymphocyte numbers and influence modification of iron overload.
the MHC class I region harbor, in addition to HFE, other gene or genes that besides regulating lymphocyte numbers also influence the development of “non-phagocytic” or parenchymal iron overload? The search for the answer to this question will probably guide many research interests for years to come.

REFERENCES

1. O’Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004; 10: 801-805
2. Janeway CA. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 1989; 54 Pt 1: 1-13
3. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994; 12: 991-1045
4. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 1973; 137: 1142-1162
5. Germain RN. An innately interesting decade of research in immunology. Nat Med 2004; 10: 1307-1320
6. de Sousa M. Immune cell functions in iron overload. Clin Exp Immunol 1989; 75: 1-6
7. Knutson M, Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. Crit Rev Biochem Mol Biol 2003; 38: 61-88
8. Vyoral D, Petrák J. Hepcidin: a direct link between iron metabolism and immunity. Int J Biochem Cell Biol 2005; 37: 1768-1773
9. Cardoso CS, Weiss G and de Sousa M. Iron, genes and the Immune System. Hershko R, Gordeuk V and Weiss G eds. Anemia of Chronic Disease. Taylor and Francis Group, Boca Raton 2005: 281-334
10. Marx J. Iron and infection: competition between host and microbes for a precious element. Best Pract Res Clin Haematol 2002; 15: 411-426
11. Radtke AL, O’Riordan MX. Intracellular innate resistance to bacterial pathogens. Cell Microbiol 2006; 8: 1720-1729
12. Weinberg ED. Iron withholding as a defense strategy. In: Hershko R, Gordeuk V and Weiss G eds. Anemia of Chronic Disease. Taylor and Francis Group, Boca Raton 2005: 255-280
13. Weinberg ED. Iron withholding: a defense against infection and neoplasia. Physiol Rev 1984; 64: 65-102
14. van der Steade BW, Beljaars L, Molema G, Harmsen MC, Neckers LM. Antiviral activities of lactoferrin. Proc Natl Acad Sci USA 1998; 95: 7806-7810
15. Nishiyama Y, Chiao JW, de Sousa M. Iron binding proteins in selected human peripheral blood cell sets: immunofluorescence. Br J Haematol 1980; 42: 255-245
16. Macedo MF, de Sousa M, Ned RM, Mascarenhas C, Andrews NC, Correia-Neves M. Transferrin is required for early T-cell immunofluorescence. Br J Haematol 1980; 42: 225-239
17. Vezzoni P, Levi S, Gabri E, Pozzi MR, Spinazzè S, Arosio P. Ferritin in malignant and non-malignant lymphoid cells. Br J Haematol 1986; 62: 105-110
18. Vidal S, Gros P, Skamene E. Natural resistance to infection with intracellular parasites: molecular genetics identifies Nramp1 as the Bcg/Ity/Lsh locus. J Leukoc Biol 1995; 58: 382-390
19. Jabbado N, Cuellar-Mata P, Grinstein S, Gros P. Iron chelators modulate the fusogenic properties of Salmonella-containing phagosomes. Proc Natl Acad Sci USA 2003; 100: 6127-6132
20. Peyssonnaux C, Zinkernagel AS, Datta V, Lauth X, Johnson RS, Nizet V. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood 2006; 107: 3272-3273
21. Flo TH, Smith KD, Sato S, Rodrigue DJ, Holmes MA, Strong RK, Akira S, Aderem A. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature 2004; 432: 917-921
22. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003; 102: 783-788
23. Lesbos-Brión JC, Vlante L, Bennoun M, Lou DQ, Ramey G, Houbour C, Hamard G, Kahn A, Vaulent S. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. Blood 2006; 108: 1402-1405
24. Roetto A, Papanikolau G, Politou M, Alberti F, Girelli D, Christakis J, Loukopoulos D, Camaschella C. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet 2003; 33: 21-22
25. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001; 276: 7806-7810
26. Lin L, Goldberg YP, Ganz T. Competitive regulation of hepcidin mRNA by soluble and cell-associated hemoglovin. Blood 2005; 106: 2884-2889
27. Truksa J, Peng H, Lee P, Beutler E. Bone morphogenetic proteins 2, 4, and 9 stimulate murine hepcidin 1 expression independently of Hfe, transferrin receptor 2 (Tfr2), and IL-6. Proc Natl Acad Sci USA 2006; 103: 10289-10293
28. Papanikolau G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dubé MP, Andres L, MacFarlane J, Sakellaropoulos N, Politou M, Nemeth E, Thompson J, Risler JK, Zaborowska C, Babakafif R, Radomski CC, Pape TD, Davidas O, Christakis J, Brissot P, Lockitch G, Ganz T, Hayden MR, Goldberg YP. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 2004; 36: 77-82
29. Nemeth E, Rivera S, Gabayn V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004; 113: 1271-1276
30. Lee P, Peng H, Gelbart T, Wang L, Beutler E. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc Natl Acad Sci USA 2005; 102: 1906-1910
31. Poss KD, Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. Proc Natl Acad Sci USA 1997; 94: 10919-10924
32. Feder JN, Gnikre A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McCluggage E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Sanders SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolf R. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13: 399-408
33. Zhou XY, Tomatsu S, Fleming RE, Parkkila S, Waheed A, Jiang J, Fei Y, Brunt EM, Ruddy DA, Prass CE, Schatzman RC, O’Neill R, Britton RS, Bacon BR, Sly WS. HFE gene knockout produces mouse model of hereditary hemochromatosis. Proc Natl Acad Sci USA 1998; 95: 2492-2497
34. Cardoso CS, de Sousa M, HFE, the MHC and hemochromatosis: paradigm for an extended function for MHC class I. Tissue Antigens 2003; 61: 263-275
35. de Sousa M, Reimão R, Racerda R, Hugo P, Kaufmann SH, Porto G. Iron overload in beta 2-microglobulin-deficient mice. Immunol Lett 1994; 39: 105-111
36. Santos MM, de Sousa M, Rademakers LH, Clevers H, Marx JJ, Schilham MW. Iron overload and heart fibrosis in mice deficient for both beta2-microglobulin and Rag1. Am J Pathol 2000; 157: 1885-1892
37. Péramba B, Siegrist CA, Gillet A, Vincent C, Kimura S, Lemonnier FA. Beta 2-microglobulin restriction of antigen
Cardoso MG, Macedo MC, Rohrich P, Ribeiro E, Silva MT, Lemmonier FA, de Sousa M. Increased hepatic iron in mice lacking classical MHC class I molecules. Blood 2002; 100: 4239-4244.

Doherty DG, O'Farrelly C. Innate and adaptive lymphoid cells in the human liver. Immunol Rev 2000; 174: 5-20.

Ganz T. Heparin: a peptide hormone at the interface of innate immunity and iron metabolism. Curt Top Microbiol Immunol 2006; 306: 183-198.

Nemeth E, Tuttle MS, Powelson J, Vaughan MB, Donovan A, Ward DM, Ganz T, Kaplan J. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004; 306: 2090-2093.

Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003; 21: 335-376.

Rodrigues PN, Vázquez-Dorado S, Neves JV, Wilson JM. Dual function of fish hepcidin: response to experimental iron overload and bacterial infection in sea bass (Dicentrarchus labrax). Dev Comp Immunol 2006; 30: 1156-1167.

Bellamy R, Ruvende C, Corrh T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. N Engl J Med 1998; 338: 640-644.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Collan JL, Ediger MA. Cloning and characterization of a mammalian copper-proton-coupled metal-ion transporter. Nature 1997; 388: 482-488.

Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci USA 1998; 95: 1148-1153.

Zhong W, Lafuse WP, Zwilling BS. Infection with Mycobacterium avium differentially regulates the expression of iron transport protein mRNA in murine peritoneal macrophages. Infect Immun 2001; 69: 6616-6624.

Jabado N, Canonne-Hergaux F, Gruenheid S, Picard V, Gros P. Iron transporter Nramp2/DMT-1 is associated with the membrane of phagosomes in macrophages and Sertoli cells. Blood 2002; 100: 2617-2622.

Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 2002; 10: 1033-1043.

Ozkayal N, Anil M, Agus N, Helvaci M, Sirti S. Community-acquired meningitis and sepsis caused by Chryseobacterium meningosepticum in a patient diagnosed with thalassemia major. Clin Microbiol 2006; 44: 3037-3039.

De Sousa M. The Immunology of Iron Overload. In: De Sousa M and Brock J eds. Iron in Immunity, Cancer and Inflammation. John Wiley & Sons Ltd 1999: 247-258.

Drakesmith H, Sweetland E, Schimanski L, Edwards J, Cowley D, Ashraf M, Bastin J, Townsend AR. The haemochromatosis protein HFE inhibits iron export from macrophages. Proc Natl Acad Sci USA 2002; 99: 15602-15607.

Montosi G, Paglia P, Garuti C, Guzman CA, Bastin JM, Colombo MP, Pietrangelo A. Wild-type HFE protein normalizes transferrin iron accumulation in macrophages from subjects with hereditary haemochromatosis. Blood 2000; 96: 1125-1129.

Gordeuk VR, Ballou S, Lozanski G, Brittenham GM. Decreased concentrations of tumor necrosis factor-alpha in supernatants of monocytes from haemochromatotic individuals. Blood 1992; 79: 1855-1860.

Cairo G, Recalcati S, Montosi G, Castrusini E, Conte D, Pietrangelo A. Inappropriately high iron regulatory protein activity in monocytes of patients with genetic haemochromatosis. Blood 1997; 89: 2546-2553.

Moura E, Noordermeer MA, Verhoeven N, Verheul AF, Marx JJ. Iron release from human monocytes after erythropagocytosis in vitro: an investigation in normal subjects and hereditary haemochromatosis patients. Blood 1998; 92: 2511-2519.

Olakanoi O, Schlesinger LS, Britigan BE. Hereditary haemochromatosis results in decreased iron acquisition and growth by Mycobacterium tuberculosis within human macrophages. J Leukoc Biol 2007; 81: 195-204.

Drakesmith H, Chen N, Ledermann H, Scranton G, Townsend A, Xu XN, HIV-1 Nef down-regulates the haemochromatosis protein HFE, manipulating cellular iron homeostasis. Proc Natl Acad Sci USA 2005; 102: 11017-11022.

Nielsen P, Dejen O, Brummer J, Gabbe EE. Long-term survival in a patient with AIDS and hereditary haemochromatosis. Eur J Haematol 1999; 63: 202-204.

Brinkmann M, Teufel R, Laham N, Ehrlich R, Decker P, Lemmonier FA, Pascolo S. Expression of iron transport proteins divalent metal transporter-1, Ferroportin-1, HFE and transferrin receptor-1 in human monocyte-derived dendritic cells. Cell Biochem Funct 2007; 25: 287-296.

Kramer JL, Balthakis A, Alcantara OS, Boldt DH. Differentiation of functional dendritic cells and macrophages from human peripheral blood monocyte precursors is dependent on expression of p21 (WAFl/GIP1) and requires iron. Br J Haematol 2002; 117: 727-734.

De Sousa M. Lymphoid cell positioning: a new proposal for the mechanism of control of lymphoid cell migration. Symp Soc Exp Biol 1978; 32: 393-410.

De Sousa M. Lymphocyte Circulation. Experimental & Clinical Aspects. John Wiley & Sons, New York 1981: 201-216.

De Sousa M, Da Silva BM, Dörner M, Munn G, Nishiya K, Grady RW, Silverstone A. Iron and the lymphomyeloid system: rationale for considering iron as a target of “Innate Surveillance”. In: The Biochemistry and Physiology of Iron. Saltman P, Hegnauer J (Eds). Elservier North Holland, Inc. 1982: 687-697.

De Sousa M. T Lymphocytes and iron overload: novel correlations of possible significance to the biology of the immunological system. Mem Inst Oswaldo Cruz 1992; 87 Suppl 5: 23-29.

Walker EM, Walker SM. Effects of iron overload on the immune system. Ann Clin Lab Sci 2000; 30: 354-365.

Grady RW, Akbar AN, Giardina PJ, Hilgartner MW, de Sousa M. Disproportionate lymphoid cell subsets in thalassaemia major: the relative contributions of transfusion and splenectomy. Br J Haematol 1985; 59: 713-724.

Reimão R, Porto G, de Sousa M. Stability of CD4/CD8 ratios in man: new correlation between CD4/CD8 profiles and iron overload in idiopathic haemochromatosis patients. CR Acad Sci III 1991; 313: 481-487.

Porto G, Vicente C, Teixeira MA, Martins O, Cabeda JM, Lacerda R, Goncalves C, Fraga J, Macedo G, Silva BM, Alves H, Justicia B, de Sousa M. Relative impact of HLA phenotype and CD4/CD8 ratios on the clinical expression of hemochromatosis. Hepatology 1997; 25: 397-402.

Rodrigues P, Lopes C, Mascarenhas C, Arosio P, Porto G, De Sousa M. Comparative study between Hfe-/- and beta2m-/- mice: progression with age of iron status and liver pathology. Int J Patt Biol 2006; 8: 317-324.

Porto G, Reimão R, Gonçalves C, Vicente C, Justícia B, de Sousa M. Haemochromatosis as a window into the study of the immunological system: a novel correlation between CD8+ lymphocytes and iron overload. Eur J Haematol 1994; 52: 283-290.

Cardoso EM, Porto G, de Sousa M. H. pylori infection and growth by Mycobacterium tuberculosis within human macrophages. J Leukoc Biol 2007; 81: 195-204.
C282Y homozygosity: relationship to severity of iron overload and HLA-A and -B alleles and haplotypes. BMC Blood Disord 2005; 5: 5
79 Fabio G, Zaranontello M, Mocellin C, Bonara P, Corengia C, Fargion S, Fiorelli G. Peripheral lymphocytes and intracellular cytokines in C282Y homozygous hemochromatosis patients. J Hepatol 2002; 37: 753-761
80 Cardoso C, Porto G, Lacerda R, Resende D, Rodrigues P, Bravo F, Oliveira JC, Justiça B, de Sousa M. T-cell receptor repertoire in hereditary hemochromatosis: a study of 32 hemochromatosis patients and 274 healthy subjects. Hum Immunol 2001, 62: 488-499
81 Arosa FA, da Silva AJ, Godinho IM, ter Steege JC, Porto G, Rudd CE, de Sousa M. Decreased CD8-p56lck activity in peripheral blood T-lymphocytes from patients with hereditary haemochromatosis. Scand J Immunol 1994; 39: 426-432
82 Cruz E, Melo G, Lacerda R, Almeida S, Porto G. The CD8+ T-lymphocyte profile as a modifier of iron overload in HFE hemochromatosis: an update of clinical and immunological data from 70 C282Y homozygous subjects. Blood Cells Mol Dis 2006; 37: 33-39
83 Cruz E, Vieira J, Goncalves R, Alves H, Almeida S, Rodrigues P, Lacerda R, Porto G. Involvement of the major histocompatibility complex region in the genetic regulation of circulating CD8 T-cell numbers in humans. Tissue Antigens 2004; 64: 25-34
84 Cruz E, Vieira J, Almeida S, Lacerda R, Gartner A, Cardoso CS, Alves H, Porto G. A study of 82 extended HLA haplotypes in HFE-C282Y homozygous hemochromatosis subjects: relationship to the genetic control of CD8+ T-lymphocyte numbers and severity of iron overload. BMC Med Genet 2006; 7: 16
85 Rothenberg BE, Voland JR. beta2 knockout mice develop parenchymal iron overload: A putative role for class I genes of the major histocompatibility complex in iron metabolism. Proc Natl Acad Sci USA 1996; 93: 1529-1534
86 Santos M, Schilham MW, Rademakers LH, Marx JJ, de Sousa M, Clevers H. Defective iron homeostasis in beta 2-microglobulin knockout mice recapitulates hereditary hemochromatosis in man. J Exp Med 1996; 184: 1975-1985
87 Koller BH, Marrack P, Kappler JW, Smithies O. Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. Science 1990; 248: 1227-1230
88 Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. Beta 2-microglobulin deficient mice lack CD4+8+ cytolytic T cells. Nature 1990; 344: 742-746
89 Santos M, Clevers H, de Sousa M, Marx JJ. Adaptive response of iron absorption to anemia, increased erythropoiesis, iron deficiency, and iron loading in beta2-microglobulin knockout mice. Blood 1998; 91: 3059-3065
80 Muckenthaler MU, Rodrigues P, Macedo MG, Minana B, Brennan K, Cardoso EM, Hentze MW, de Sousa M. Molecular analysis of iron overload in beta2-microglobulin-deficient mice. Blood Cells Mol Dis 2004; 33: 125-131
91 Levy JE, Montross LK, Andrews NC. Genes that modify the hemochromatosis phenotype in mice. J Clin Invest 2000; 105: 1209-1216
92 Miranda CJ, Makui H, Andrews NC, Santos MM. Contributions of beta2-microglobulin-dependent molecules and lymphocytes to iron regulation: insights from HfeRag1(-/-) and beta2mRag1(-/-) double knock-out mice. Blood 2004; 103: 2847-2849
93 Ten Elshof AE, Brittenham GM, Chorney KA, Page MJ, Gerhard G, Cable EE, Chorney MJ. Gamma delta intraepithelial lymphocytes drive tumor necrosis factor-alpha responsiveness to intestinal iron challenge: relevance to hemochromatosis. Immunol Rev 1999; 167: 223-232
94 Perarau B, Saron RF, Reina San Martin B, Bervas N, Ong H, Soloski MJ, Smith AG, Ure JM, Gairin JE, Lemonnier FA. Single H2Kb, H2Db and double H2KbDb knockout mice: peripheral CD8+ T cell repertoire and anti-lymphocytic choriomeningitis virus cytolytic responses. Eur J Immunol 1999; 29: 1243-1252
95 de Sousa M, Porto G. The immunological system in hemochromatosis. J Hepatol 1998; 28 Suppl 1: 1-7
96 De Sousa M. Iron and the Lymphomyeloid System: A Growing Knowledge. In: De Sousa M and Brock J eds. Iron in Immunity, Cancer and Inflammation. John Wiley & Sons Ltd. 1989: 3-16
97 Ahmad KA, Ahmann JR, Migas MC, Waheed A, Britton RS, Bacon BR, Sly WS, Fleming RE. Decreased liver hepcidin expression in the Hfe knockout mouse. Blood Cells Mol Dis 2002; 29: 361-366
98 Wallace DF, Summerville L, Lusby PE, Subramaniam VN. Prohepcidin localises to the Golgi compartment and secretory pathway in hepatocytes. J Hepatol 2005; 43: 720-728

S-Editor Liu Y  L-Editor Alpini GD  E-Editor Lu W