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Iron Metabolism in the Reticuloendothelial System

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ABSTRACT: Comprised mainly of monocytes and tissue macrophages, the reticuloendothelial system (RES) plays two major roles in iron metabolism: it recycles iron from senescent red blood cells and it serves as a large storage depot for excess iron. Although iron recycling by the RES represents the largest pathway of iron efflux in the body, the precise mechanisms involved have remained elusive. However, studies characterizing the function and regulation of Nramp1, DMT1, HFE, FPN1, CD163, and hepcidin are rapidly expanding our knowledge of the molecular aspects of RE iron handling. This review summarizes fundamental physiological and biochemical aspects of iron metabolism in the RES and focuses on how recent studies have advanced our understanding of these areas. Also discussed are novel insights into the molecular mechanisms contributing to the abnormal RE iron metabolism characteristic of hereditary hemochromatosis and the anemia of chronic disease.

KEY WORDS: CD163, DMT1, ferroportin1, hepcidin, HFE, Nramp1.

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I. INTRODUCTION

One of the most distinguishing features of iron metabolism is the degree to which body iron is conserved. Of the typical 3 to 4 g of iron contained in the normal adult human, only about 0.03% (or ~1 mg) is lost per day, mainly the result of obligatory losses of exfoliated mucosal cells, bile, and extravasated red cells. To replace these basal losses and remain in iron balance, the body must absorb a roughly equivalent amount of iron from the diet. This relatively small daily exchange of iron between body and environment contrasts sharply with the comparatively large exchange of this metal between internal organs. For example, each day the bone marrow utilizes approximately 24 mg of iron to produce over 200 billion new erythrocytes. To meet the demand for heme production necessary for erythropoiesis, iron must be recycled from senescent red cells; this process is carried out by macrophages of the reticuloendothelial system (RES). Despite this critical role of the RES in body iron conservation, iron recycling by the RE cell has remained one of the least well-understood areas of iron metabolism (Aisen, 1990). However, in the last decade five new genes involved in iron metabolism have been discovered: Nramp1 (Vidal *et*

al., 1993), HFE (Feder *et al*., 1996), DMT1 (Fleming *et al*., 1997; Gunshin *et al*., 1997), FPN1 (Abboud and Haile, 2000; Donovan *et al*., 2000; McKie *et al*., 2000), and CD163 (Kristiansen *et al*., 2001). These genes are abundantly (or exclusively) expressed in RE cells, and characterization of their functions is starting to reveal how the RES handles iron at the molecular level. Another noteworthy advance has been the identification of hepcidin, a serum peptide that appears to affect iron storage in the RES (Nicolas, 2001). A list of these new factors, along with other proteins known to participate in RE iron metabolism, is presented in Table 1. Here we review iron metabolism in the RES—from iron acquisition, intracellular iron processing, and iron release—highlighting how recent studies have contributed to our understanding of these highly dynamic processes.

II. THE RETICULOENDOTHELIAL SYSTEM (RES)

A. Definition and Functions

Also known as the "mononuclear phagocyte system" (Weinberg and Athens, 1993), the RES is composed of monocytes, macrophages, and their precursor cells. Monocytes arise from progenitor cells in the bone marrow and are released into the blood. After migration to different tissues, they differentiate into macrophages with characteristic morphologic and functional qualities. Studies using an antibody against the macrophage-specific antigen F4/80 show that mouse organs with the most macrophages are, in descending order, the liver, large intestine, small intestine, bone marrow, spleen, and kidney (Lee *et al*., 1985). Although RE cells residing in various tissues likely have different or highly specialized functions (*e.g*., immunoregulation, antimicrobial activity, antitumorical activity), one common task involves the clearance of particulate matter and damaged or effete cells. The removal of damaged or senescent erythrocytes, with the subsequent recycling of iron, directly links the RES and iron metabolism. This process is mainly carried out by RE cells of the spleen, liver, and bone marrow. The splenic red pulp appears to be one of the most active sites of red cell destruction. However, after splenectomy, red cell survival time does not increase (Athens, 1993), indicating that macrophages of the liver and bone marrow (or elsewhere) can rapidly compensate for this function of the spleen.

B. The Study of Iron Metabolism in the RES

A variety of systems are available for the study of iron metabolism in the RES, each with advantages and disadvantages. *In vivo* studies are clearly the most physiologic, but interpretation of the results can be complicated by the diffuse distribution and specialized functions of different RE cells. Pure primary cultures of liver macrophages (Kupffer cells) can be used, but their extraction from tissues is laborious and involves tissue disruption, producing cell populations with different degrees of activation and differentiation (Olynyk and Clarke, 1998). More readily available sources of macrophages include the lung and the peritoneum. Peripheral blood monocytes can also be relatively easily obtained and studied in culture, either before or after differentiation to macrophages. However, the extent to which monocytes or alveolar and peritoneal macrophages are involved in normal RE iron metabolism is

TABLE 1
Proteins Involved in RE Iron Metabolism*

Protein	Tissue and cellular expression	Subcellular localization	Function
CD163 (Hb scavenger receptor)	only in tissue Mϕ and monocytes	plasma membrane	scavenge Hp-Hb complexes from plasma
Ceruloplasmin (CP)	plasma	?	oxidize Fe^{2+} to Fe^{3+}, required for normal Fe release from RE cells
Divalent Metal Transporter1 (DMT1/NRAMP2/DCT1/ SLC11A2)	ubiquitous; highest in kidney	apical membrane of duodenum; endosomes and lysosomes	transport Fe into duodenal epithelia from gut lumen; transport Fe out of endosomes into cytosol
Ferritin	ubiquitous	cytosol	Fe storage
Ferroportin1 (FPN1/IREG1/MTP1/ SLC11A3)	liver, spleen, kidney, duodenum; highest in tissue Mϕ	basolateral membrane of duodenum; intracellular in RE cells?	transport Fe out of duodenal epithelia into portal blood; transport Fe out of RE cell?
Haptoglobin (Hp)	plasma	—	bind free Hb in plasma
Heme oxygenase (HMOX1)	liver, spleen, kidney	endoplasmic reticulum	catabolize heme to biliverdin, CO_2 and free Fe
Hepcidin (LEAP-1)	plasma	—	Fe regulatory hormone?
HFE	liver, duodenum	plasma membrane (extracellular)	associates with TfR; modulate Fe uptake?
Iron-regulatory proteins 1 and 2 (IRP1 and IRP2)	ubiquitous	cytosolic	regulate gene expression by binding to IREs of mRNA (*e.g.*, ferritin and TFR)
NRAMP1 (SLC11A1)	liver, spleen, lungs; Mϕ and monocytes	phagosomes	transport Fe into/out of phagolysosome
Transferrin (TF)	plasma	—	transport Fe in circulation
Transferrin receptor (TFR)	ubiquitous; highest in bone marrow	plasma membrane	bind TF and deliver TF-bound Fe to cells via endocytosis

*Abbreviations used: Hb, hemoglobin; Mϕ, macrophage.

unknown. In recent years, cell lines that display many of the key characteristics of bona fide macrophages are being used more frequently. It is worthwhile to note that the commonly used J774 and RAW264.7 macrophage cell lines do not express functional Nramp1 protein (Vidal *et al.*, 1996). Thus, iron metabolism studies using these cells must be interpreted carefully (see below). It is also difficult to compare results from different studies of RE iron metabolism because of the disparate forms of iron used (*e.g.*, erythrocytes, hemoglobin, heme, iron-transferrin, iron-transferrin-immune complex, iron dextran, ferric ammonium citrate, ferric nitrilotriacetic acid). Moreover, the metabolism of some of these iron compounds can differ depending on whether the iron is acquired via phagocytosis or endocytosis. Thus, gaining a comprehensive understanding of RES function has proven difficult.

III. IRON ACQUISITION BY THE RES

A. Erythrophagocytosis

Macrophages of the RES acquire most of their iron by phagocytosing senescent red blood cells. With each red cell ingested, the macrophage accrues approximately one billion iron atoms. It has been estimated that fixed macrophages of rat liver, spleen, and bone marrow phagocytose an average of one red cell per macrophage per day (Kondo *et al*., 1988). Interestingly, the cellular and molecular mechanisms of the seemingly simple clearance of effete erythrocytes from the circulation remains the subject of a great deal of controversy (reviewed by Bratosin *et al*., 1998). After erythrophagocytosis, hydrolytic enzymes present in the phagolysosome degrade the red blood cell. Proteolytic digestion of hemoglobin liberates heme, which is assumed to cross the phagolysosomal membrane either by diffusion or by a specific transporter in order to reach heme oxygenase (HMOX). Three isoforms of HMOX have been described in mammals: an inducible HMOX1; a constitutively active but uninducible HMOX2; and HMOX3, a form nearly devoid of catalytic capability (Elbirt and Bonkovsky, 1999). HMOX2 appears predominant in all organs measured, except for the rat spleen, which normally expresses five times more HMOX1 than HMOX2 (Braggins *et al*., 1986). The strong splenic HMOX1 expression likely reflects the high concentration of erythrophagocytosing RE cells in this organ. Although HMOX1 appears to be largely responsible for heme catabolism in RE cells, studies of mice lacking HMOX1 reveal the existence of other significant, but less-efficient pathways of heme degradation (Poss and Tonegawa, 1997).

HMOX proteins are localized to the endoplasmic reticulum (ER), where they catabolize heme to produce biliverdin, carbon monoxide, and Fe^{2+} (Maines, 1997). The iron thus liberated is then either released from the macrophage or stored (see below). Baranano *et al*. (2000) propose that the iron freed from heme transiently becomes part of the cytoplasmic labile iron pool before being transported to the lumenal side of the ER by a novel ATPase. This iron-inducible ATP-dependent transporter localizes with HMOX1 to microsomal membranes and is greatly enriched in the spleen (Baranano *et al*., 2000), but rigorous identification of a gene product is still needed. An alternative site of heme catabolism is suggested by recent analyses of J774 macrophages in the process of erythrophagocytosis. Using electron microscopy and two-dimensional gel electrophoresis, Gagnon *et al*. (2002) provide compelling evidence that part of the phagosomal membrane is derived from ER. This observation raises the intriguing possibility that ER-associated HMOX proteins may catalyze heme degradation and iron liberation within the phagolysosome. If so, a nonheme iron transporter would be required to translocate iron into the cytosol. Future studies need to explore whether HMOX proteins are recruited to the phagolysosomal membrane after erythrophagocytosis to determine the exact site of heme catabolism.

B. Receptor-Mediated Uptake of Hemoglobin

From kinetic studies of hemoglobin turnover in humans, it has been calculated that 10 to 20% of normal erythrocyte destruction occurs intravascularly, resulting in the release of hemoglobin (Garby and Noyes, 1959a). Under normal circumstances, all of

this hemoglobin is rapidly bound by haptoglobin, which is then cleared from the circulation by parenchymal cells of the liver (Deiss, 1999). However, recent studies have identified a hemoglobin scavenger receptor, CD163, expressed exclusively on monocytes and macrophages (Kristiansen *et al*., 2001). Found in the highest concentrations in the spleen and the liver, CD163 scavenges hemoglobin by mediating endocytosis and subsequent degradation of the hemoglobin-haptoglobin complex (Kristiansen *et al*., 2001). Thus, uptake of hemoglobin-haptoglobin via CD163 may represent a significant pathway of normal iron acquisition by the RES. Under conditions associated with increased intravascular hemolysis (*e.g*., hemolytic anemia, thalassemia, and certain bacterial infections), the hemoglobin-binding capacity of haptoglobin can be exceeded such that free hemoglobin appears in the plasma. Some of the circulating free hemoglobin degrades and releases heme, which then becomes bound to the plasma glycoprotein hemopexin. Specific hemopexin receptors on hepatocytes clear the heme-hemopexin complex from the circulation (Alam and Smith, 1989). The detection of hemopexin receptors on human monocytic cell lines (Alam and Smith, 1989; Taketani *et al*., 1990) also suggests that the RES is able to acquire heme from this pathway, but the amount taken up is probably not significant under normal circumstances.

C. Receptor-Mediated Uptake of Transferrin

Iron is delivered to most tissues via endocytosis of the plasma iron-binding protein transferrin bound to its cell surface receptor. The transferrin receptor is a dimer of 90-kDa subunits that associates with a regulatory molecule called HFE (Parkkila *et al*., 1997; Feder *et al*., 1998). Isolated human monocytes express transferrin receptors (Bjorn-Rasmussen *et al*., 1985) and are able to take up iron from transferrin (Sizemore and Bassett, 1984). When cultured monocytes differentiate into macrophages, the expression of transferrin receptor increases greatly (Andreesen *et al*., 1984). Transferrin-binding activity has also been demonstrated in various macrophages from mice (Hamilton *et al*., 1984), rats (Nishisato and Aisen, 1982; Kumazawa *et al*., 1986), and humans (Andreesen *et al*., 1984; Testa *et al*., 1987; Testa *et al*., 1989; Montosi *et al*., 2000). Although macrophages in culture can acquire iron from transferrin, the extent to which this occurs *in vivo* remains unknown. Human studies have failed to find evidence of significant iron uptake by RE cells after injection of radiolabeled transferrin-bound iron (Finch *et al*., 1970).

IV. INTRACELLULAR IRON METABOLISM IN THE RES

A. Iron Homeostasis and IRE-IRP

Cellular iron homeostasis is regulated posttranscriptionally by two cytoplasmic iron regulatory proteins, IRP-1 and IRP-2. IRPs control cellular iron uptake and storage by binding to iron-responsive elements (IRE) present in mRNAs of factors involved in iron metabolism, and in particular, transcripts for the transferrin receptor and ferritin. When cytoplasmic iron concentrations are low, IRPs bind to IRE and coordinately increase the stability of transferrin receptor mRNA and decrease the translation of ferritin. Conversely, when iron is plentiful IRPs do not bind to IRE, and transferrin receptor mRNA is degraded while iron storage in ferritin predominates. The many factors that

influence IRP function have been reviewed elsewhere in detail (Eisenstein, 2000). In human peripheral blood monocytes, IRE-IRP binding activities increase in response to iron depletion and decrease with iron loading (Cairo *et al*., 1997). Similar regulation of IRE-IRP binding activities has been demonstrated in THP-1 cells (Weiss *et al*., 1996), mouse peritoneal macrophages (Kuriyama-Matsumura *et al*., 1998), J774 cells (Recalcati *et al*., 1998; Pinero *et al*., 2001), and RAW264.7 cells (Kim and Ponka, 1999; Kim and Ponka, 2000; Wardrop and Richardson, 2000). Accordingly, increased transferrin receptor mRNA levels are associated with increased IRE-IRP binding (Kim and Ponka, 1999; Kim and Ponka, 2000; Wardrop and Richardson, 2000). Thus, it appears that the IRE-IRP regulatory system functions in RE cells as it does in other cell types.

B. Cellular Iron Transport: Roles of Nramp1 and Nramp2 (DMT1)

Two proteins of the NRAMP (natural resistance associated macrophage protein) family have been identified: Nramp1 (Vidal *et al*., 1993) and Nramp2 (Gruenheid *et al*., 1995). Nramp1 is a highly hydrophobic 56-kDa protein with 12 predicted transmembrane regions that is expressed exclusively in monocytes and macrophages. The protein sequence of Nramp1 shares 64% amino acid sequence identity with Nramp2, which is ubiquitously expressed. The association between Nramp2 and iron transport was established by Fleming *et al*. (1997) and Gunshin *et al*. (1997). Also known as DCT1 (divalent cation transporter1), Nramp2 is now more commonly referred to as DMT1 (divalent metal transporter1).

Nramp1 localizes to lysosomes and late endosomes and is rapidly recruited to membranes of maturing phagosomes (Gruenheid *et al*., 1997; Searle *et al*., 1998; Govoni *et al*., 1999). As its name implies, Nramp1 is involved in determining the ability of inbred mouse strains to resist infection with certain intracellular pathogens. Susceptibility is associated with a single G169D substitution in the protein (Vidal *et al*., 1993): mice expressing the wild-type Nramp1^{G169} allele are resistant, whereas those expressing the Nramp1^{D169} allele are susceptible. The Nramp1^{D169} allele encodes a nonfunctional protein that is rapidly degraded in macrophages (Vidal *et al*., 1995). A role for Nramp1 in intracellular iron transport was established in studies using the Nramp1-deficient RAW264.7 macrophage cell line. The transport of iron into phagosomes containing latex beads (Kuhn *et al*., 1999) or mycobacteria (Zwilling *et al*., 1999; Kuhn *et al*., 2001) was shown to be higher in RAW264.7 cells transfected with Nramp1^{G169} than in cells expressing Nramp1^{D169}. These observations are consistent with the hypothesis that Nramp1 functions to transport iron into the bacterium-containing phagosome and thereby limit mycobacterial growth by catalyzing the formation of reactive oxygen species. In contrast, data from other studies in intact cells have been interpreted to suggest that Nramp1 transports metals out of the phagosome (Atkinson and Barton, 1999; Barton *et al*., 1999; Jabado *et al*., 2000), a function that may restrict the growth of phagocytosed pathogens by decreasing the availability of iron as an essential nutrient. These two seemingly contradictory functions of Nramp1 may be reconciled by a recent study characterizing Nramp1 transport activity. When expressed in *Xenopus* oocytes, Nramp1 is capable of transporting iron bidirectionally, depending on pH (Goswami *et al*., 2001). Clearly, more work is needed to define Nramp1 function in intracellular iron transport. For a more detailed review of Nramp1

and macrophage iron metabolism, the reader is referred to Wyllie *et al.* (2002).

The recruitment of Nramp1 to the phagolysosome has fostered speculation that Nramp1 may function to transport erythrocyte-derived iron into the cytosol (Fleming *et al.*, 1998; Atkinson and Barton, 1999). This idea, however, presupposes that iron is released from heme inside the phagolysosome, and this seems unlikely if HMOX1 functions in the ER (Tenhunen *et al.*, 1968). Moreover, if Nramp1 were the sole mediator of erythrocyte iron transport out of the phagolysosome, then inbred mouse strains homozygous for the mutant Nramp1^{D169} allele (*e.g.*, BALB/c and C57BL/6) would be expected to have iron-deficiency anemia due to inefficient iron recycling. The demonstration that these mice have normal hematological profiles (Leboeuf *et al.*, 1995) suggests that lack of Nramp1 does not disrupt this process.

The effect of iron status on Nramp1 mRNA and protein levels has been investigated recently using *in vitro* and *in vivo* systems. Nramp1 mRNA levels increased in bone marrow-derived cells exposed to hemin (Biggs *et al.*, 2001) or ferric ammonium sulfate (Baker *et al.*, 2000), whereas no change was observed in RAW267.4 or J774 cells treated with ferric ammonium citrate or the iron chelator desferrioxamine (Wardrop and Richardson, 2000). At the protein level, Nramp1 increased in bone marrow-derived macrophages in response to ferric ammonium sulfate (Baker *et al.*, 2000) and after treating splenic cells with red blood cells (Biggs *et al.*, 2001). Using immunohistochemistry, Biggs *et al.* (2001) did not detect changes in Nramp1 protein levels in splenic macrophages in mice given an intraperitoneal injection of iron dextran. Discordant findings between these studies may reflect inherent variability among different types of macrophages or the use of different chemical forms of iron.

Although discovered after Nramp1, DMT1 has been more fully characterized in terms of its biochemical function (Gunshin *et al.*, 1997). Studies in HEp-2, HeLa, and COS-7 cells reveal that DMT1 localizes to recycling endosomes where it transports iron from transferrin into the cytosol (Fleming *et al.*, 1998; Tabuchi *et al.*, 2000). Because this transporter co-localizes with transferrin in RAW 264.7 macrophages (Gruenheid *et al.*, 1999), it is likely that DMT1 performs a similar function in RE cells. The observation that DMT1 also becomes associated with the phagolysosome in J774 macrophages (Gruenheid *et al.*, 1999) suggests that it may transport erythrocyte-derived iron into the cytosol. As with Nramp1, this model would require iron liberation from heme inside the phagolysosome.

Of the two splice variants of DMT1 that have been identified, one has an atypical IRE in its 3'UTR, suggesting that it may be regulated like transferrin receptor—that is, DMT1 mRNA would be stabilized under low iron conditions and degraded under iron loading. Studies of intestinal and liver cell lines support this idea (Gunshin *et al.*, 2001). However, studies of macrophage cell lines (RAW264.7 and J774) indicate that DMT1 transcript levels do not change in parallel with changes in transferrin receptor mRNA (Wardrop and Richardson, 2000). The lack of iron responsiveness of macrophage DMT1 mRNA levels is consistent with results from studies of cultured fibroblasts and erythroleukemic cells (Wardrop and Richardson, 1999). To better characterize DMT1's function in RE iron metabolism, future studies need to determine the effect of iron status on DMT1 protein levels.

C. Iron Storage

The main sites of body iron stores are the hepatic parenchyma and the RES, par-

ticularly the RE cells of the bone marrow, spleen, and liver. The liver and the total bone marrow each contain approximately 100 to 300 mg of storage iron in healthy Western individuals (Gale *et al*., 1963; Bothwell *et al*., 1979). The concentrations of iron in liver and bone marrow have been shown to correlate well over a wide range (up to 9000 μg/g tissue) (Gale *et al*., 1963).

Iron in the RES most likely accumulates secondary to the catabolism of red cell heme. RE iron acquired via erythrophagocytosis that is not utilized or released is first destined for storage in ferritin, a cytosolic protein comprised of 24 subunits of two types, H and L. In RE cells, ferritin is comprised mainly of the L-subunit (Invernizzi *et al*., 1990), the form most associated with iron storage (Levi *et al*., 1994). Cell culture studies using monocytes and macrophages document the formation of ferritin protein within hours after red cell ingestion (Custer *et al*., 1982; Bornman *et al*., 1999). Three hours after erythrophagocytosis, both H- and L-subunits of ferritin are upregulated in equal amounts (Bornman *et al*., 1999), whereas after 18 , the L-form predominates (Raha-Chowdhury *et al*., 1993). Although ferritin synthesis after red cell ingestion can be regulated via IRP-IRE interactions effected by changes in iron levels, some evidence indicates that reactive oxygen species formed during phagocytosis may also play a role (Bornman *et al*., 1999), perhaps through upregulation of ferritin transcription (Tsuji *et al*., 2000). Recent serial analyses of gene expression in human monocyte-derived macrophages highlight the importance of ferritin in the RE cell (Hashimoto *et al*., 1999). Out of 35,000 genes identified by this method, ferritin L- and H-chains were the first and third most abundant mRNA species, representing nearly 5% of all transcripts. Understandably, targeted deletion of the murine H-ferritin gene in $Fth^{-/-}$ mice leads to early embryonic death (Ferreira *et al*., 2000), but it is of interest that heterozygous $Fth^{+/-}$ mice, which have markedly increased ratios of L-to-H subunits, show no abnormalities in iron metabolism, including no changes in splenic iron stores (Ferreira *et al*., 2001).

The storage of iron from the uptake of hemoglobin appears to be influenced by genetic polymorphisms in haptoglobin. Of the three haptoglobin polymorphisms in humans (Langlois and Delanghe, 1996), the multimeric Hp2-2 phenotype has the highest functional affinity for the hemoglobin scavenger receptor, CD163 (Kristiansen *et al*., 2001). In a study of 717 healthy Caucasian subjects, males with the Hp2-2 phenotype had significantly increased serum iron levels and twofold higher monocyte L-ferritin concentrations than other Hp phenotypes (Langlois *et al*., 2000). These associations, along with observations from early studies of hemoglobin iron metabolism (Garby and Noyes, 1959b), have led to the hypothesis that hemoglobin iron acquired via CD163 on RE cells is shunted into slowly exchanging storage compartments normally bypassed by iron recycling pathways (Delanghe and Langlois, 2002). More work will be needed to better define the quantitative contribution of hemoglobin to iron stores within the RES.

As the amount of iron in the cell increases, a larger percentage deposits in hemosiderin, an insoluble, aggregated form of partially digested ferritin. Diversion of excess iron into hemosiderin permits storage of more iron per unit volume in the cell, and, in fact, the highest concentrations of hemosiderin in the body are found in the RES (Bothwell *et al*., 1979).

V. IRON RELEASE BY THE RES

A. Iron Release and Plasma Iron

Normal adult human plasma contains about 3 to 4 mg of iron, essentially all bound

to transferrin. About 80% of the circulating iron is en route between the RES and the bone marrow. Small amounts of plasma iron are contributed by hepatic iron stores and by the absorption of dietary iron from the duodenum, but most circulating iron is contributed by the RES through the release of iron from catabolized senescent red cells (Figure 1). Cyclic fluctuations in RE iron release appear to cause the pronounced circadian variation in plasma iron concentrations (Fillet *et al*., 1974). Neither the mechanism nor the significance of this diurnal variation in iron output from the RES is known.

B. Kinetics and Chemical Forms of Released Iron

In vivo ferrokinetic studies have characterized RE iron release using trace amounts of ^{59}Fe heat-damaged red blood cells (^{59}FeHDRBCs). After injection into the circulation, ^{59}FeHDRBCs are rapidly scavenged and processed by the RES. Studies in dogs (Fillet *et al*., 1974) and humans (Fillet *et al*., 1989) show that iron given in this manner is released in two distinct phases: an early phase, in which two-thirds of the iron freed from hemoglobin is returned to the plasma within the first few hours, and a late phase, in which the remainder is released from RE stores over days and weeks. A similar biphasic pattern of iron release after erythrophagocytosis has been observed in isolated human monocytes (Moura *et al*., 1998b) and macrophages (Custer *et al*., 1982), cultured rat peritoneal macrophages (Saito *et al*., 1986), and Kupffer cells (Kondo *et al*., 1988). The efficient release of erythrocyte-derived iron appears to require heme catabolism by HMOX1, as mice lacking this enzyme develop iron-deficiency anemia (Poss and Tonegawa, 1997).

Most of the iron released into the plasma is bound by transferrin. Studies of cultured macrophages confirm that iron is released as a low-molecular-weight species that readily binds to plasma transferrin (Haurani and O'Brien, 1972; Kondo *et al*., 1988; Rama *et al*., 1988; Moura *et al*., 1998b). A number of studies also indicate that RE cells release significant amounts of erythrophagocytosed iron in the form of hemoglobin (Custer *et al*., 1982; Saito *et al*., 1986; Kondo *et al*., 1988; Costa *et al*., 1998; Moura *et al*., 1998b), heme (Kleber *et al*., 1981; Costa *et al*., 1998), or ferritin (Kleber *et al*., 1981; Custer *et al*., 1982; Kondo *et al*., 1988; Rama *et al*., 1988; Moura *et al*., 1998b). It has been speculated that hemoglobin release results from macrophage cell death after the ingestion of too many erythrocytes (Kondo *et al*., 1988), whereas others argue that hemoglobin release represents a normal physiological process (Custer *et al*., 1982; Moura *et al*., 1998b). Interestingly, Moura *et al*. (1998b) note that most early release consists of hemoglobin, whereas ferritin and low-molecular-weight iron are the main forms released subsequently.

C. Effect of Transferrin and Ceruloplasmin

Because most of the iron recycled by the RES after erythrophagocytosis binds to circulating transferrin, studies have addressed whether the iron-binding capacity of transferrin affects iron mobilization. Increasing plasma iron-binding capacity by injecting apotransferrin into rats does not affect the release of radioiron after infusion of ^{59}FeHDRBCs (Lipschitz *et al*., 1971). Similarly, apotransferrin had no effect on iron release after erythrophagocytosis by isolated rat peritoneal macrophages (Saito *et al*., 1986). In other studies, however, the presence of apotransferrin slightly increased

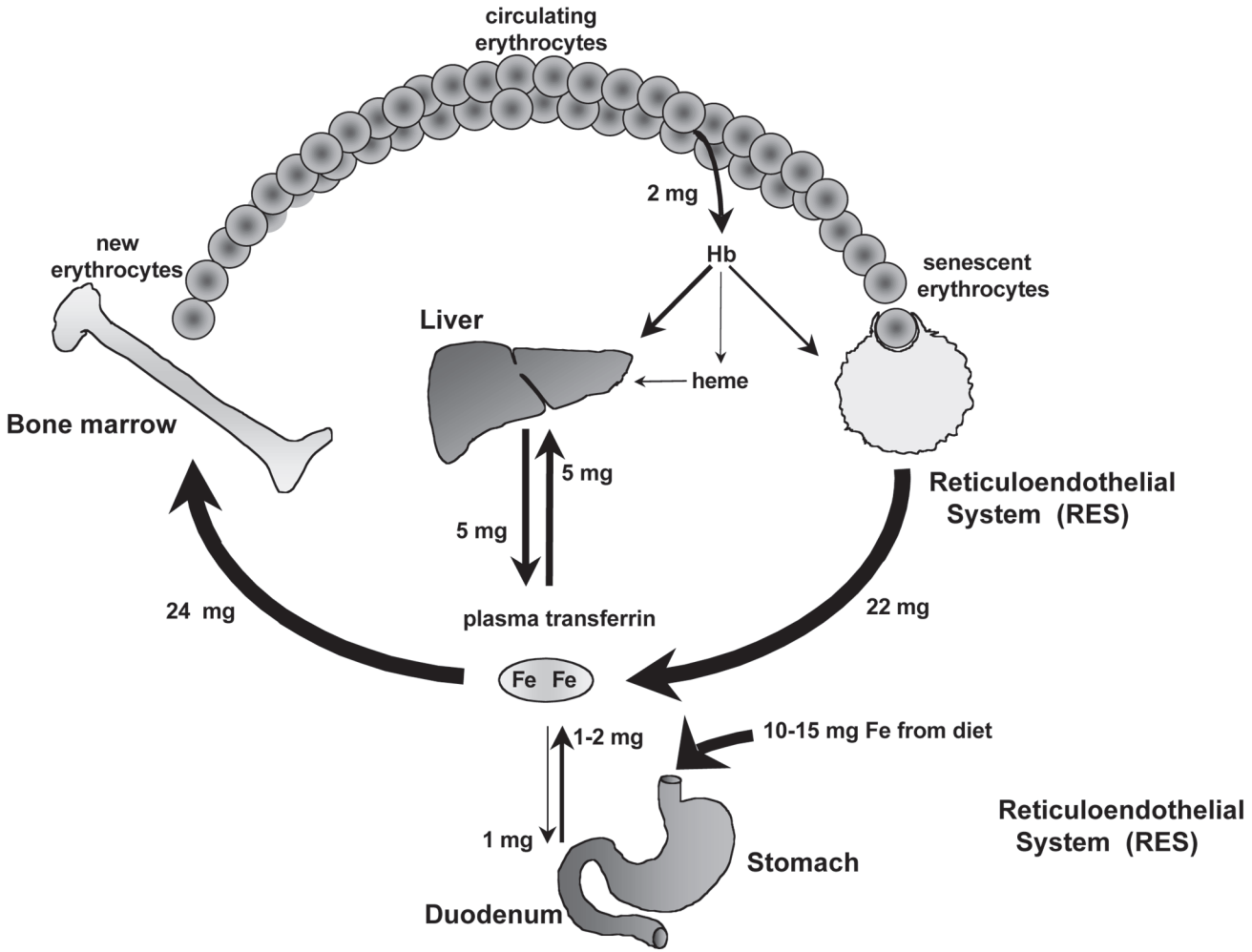

FIGURE 1. Recycling of erythrocyte iron by the RES in relation to other pathways of internal iron exchange. Phagocytosis of senescent erythrocytes is performed primarily by RE macrophages located in the spleen, liver, and bone marrow. Numbers indicate the approximate daily flow of iron through each pathway (Bothwell *et al.*, 1974).

iron efflux from rat Kupffer cells (Kondo *et al*., 1988) or dramatically enhanced iron release from rat bone marrow macrophages (Rama *et al*., 1988). Decreasing plasma iron-binding capacity by intravenous iron infusion before administering ^{59}FeHDRBCs has been shown to reduce RE iron release in most (Lipschitz *et al*., 1971; Bergamaschi *et al*., 1986; Siegenberg *et al*., 1990) but not all studies (Fillet *et al*., 1974). A slight suppression in iron release from isolated rat bone marrow macrophages incubated with saturated transferrin has also been reported (Rama *et al*., 1988). Thus, although these studies do not allow definitive conclusions to be drawn regarding the effect of iron-binding capacity of transferrin on iron release, it should be noted that neither the protein nor its iron-binding capacity appears to be essential for RE iron release. Cultured macrophages can release iron in the absence of apotransferrin in the culture media (Saito *et al*., 1986; Kondo *et al*., 1988; Moura *et al*., 1998b), and patients with hemochromatosis or bone marrow aplasia can release iron despite transferrin saturation (Fillet *et al*., 1989). Moreover, the conspicuous lack of iron accumulation in the spleen of hypotransferrinemic mice, which can survive at least 9 months without exogenous transferrin injections (Trenor *et al*., 2000), also suggests that iron is recycled through this organ in the virtual absence of transferrin.

Normal iron release does seem to require ceruloplasmin, a multicopper ferroxidase. Early studies showed that copper-deficient pigs developed iron-deficiency anemia despite having normal or elevated iron stores (Lee *et al*., 1968). The iron deficiency appeared to result from inefficient release of iron from the RES because serum iron concentrations did not increase significantly after intravenous administration of damaged erythrocytes. The subsequent observation that the defective iron mobilization in copper deficiency could be promptly corrected by the intravenous administration of ceruloplasmin (Ragan *et al*., 1969) indicated that this protein plays a role. Ceruloplasmin appears to mobilize iron from storage sites by catalyzing the oxidation of ferrous iron to the ferric form, which can be incorporated into apotransferrin (Osaki *et al*., 1971). More direct evidence for ceruloplasmin's role in RE iron release is provided by studies of aceruloplaminemic ($Cp^{-/-}$) mice (Harris *et al*., 1999), which have normal copper metabolism (Meyer *et al*., 2001). As in copper-deficient animals, serum iron concentrations of $Cp^{-/-}$ mice do not change significantly after the administration of damaged red cells, but do increase after the administration of ceruloplasmin and not apoceruloplasmin (Harris *et al*., 1999). The observation that Kupffer cells of $Cp^{-/-}$ mice display markedly increased iron levels (Harris *et al*., 1999) is also consistent with a role for ceruloplasmin in RE iron release. Curiously, $Cp^{-/-}$ mice do not develop iron-deficiency anemia, indicating that other sources of ferroxidase activity capable of mobilizing iron from storage sites exist in this animal model. Patients with aceruloplasminemia consistently present with mild-to-moderate iron-deficiency anemia (Miyajima *et al*., 1987; Yoshida *et al*., 1995), sometimes associated with iron loading in Kupffer cells (Logan *et al*., 1994; Bosio *et al*., 2002; Hellman *et al*., 2002). Future work is needed to determine whether ceruloplasmin promotes RE iron release extracellularly or intracellularly.

Recent gene mapping studies have identified a ceruloplasmin homologue, hephaestin, that is expressed predominantly in the small intestine (Vulpe, 1999). Mutations in hephaestin result in impaired iron export from the duodenum into the portal circulation, producing the phenotype of the sex-linked anemia (*sla*) mouse. Although hephaestin mRNA has been detected in the

spleen (Frazer *et al*., 2001), its potential involvement in iron export from the RES has not been studied. The observation that the anemia of the *sla* mouse is rapidly corrected by a single intraperitoneal injection of iron dextran (Bannerman and Cooper, 1966), which is taken up and recycled by the RES, suggests that hephaestin is not necessary for RE iron release.

D. Potential Roles for Ferroportin1 and Nramp1

Ferroportin1 (FPN1) is a 62-kDa iron-export protein with 9 or 10 predicted transmembrane regions (Donovan *et al*., 2000). The protein is also known as iron-regulated transporter 1, IREG1 (McKie *et al*., 2000) and metal transporter protein 1, MTP1 (Abboud and Haile, 2000). FPN1 mRNA contains an IRE sequence in the 5'UTR, suggesting that iron regulates its expression in a manner similar to ferritin. Northern blot analyses of the tissue distribution of human and mouse FPN1 mRNA reveals most abundant expression in liver, spleen, kidney, placenta, and duodenum (Abboud and Haile, 2000; Donovan *et al*., 2000; McKie *et al*., 2000). Immunostaining indicates particularly strong FPN1 expression in hepatic Kupffer cells and splenic macrophages (Abboud and Haile, 2000; Donovan *et al*., 2000). Recent double immunofluorescence staining using antibodies to FPN1 and F4/80, a macrophage-specific cell surface antigen, has confirmed the localization of FPN1 to RE cells in liver, spleen, and bone marrow (Yang *et al*., 2002).

Several lines of evidence indicate that FPN1 functions as an iron exporter in various cell types. First, in duodenal mucosal cells and syncytiotrophoblasts of the placenta, FPN1 localizes to the site of iron export, the basolateral membrane. Second, FPN1 mutations in zebrafish are associated with severe iron-deficiency anemia, which can be partially rescued by exogenous expression of wild-type FPN1 (Donovan *et al*., 2000). Third, iron-loaded *Xenopus* oocytes injected with FPN1 cRNA display increased iron release (Donovan *et al*., 2000; McKie *et al*., 2000), and HEK293T cells transfected with FPN1 cDNA have decreased levels of cytosolic iron (Abboud and Haile, 2000). Incidentally, it should be noted that increased iron efflux after FPN1 expression in *Xenopus* required the presence of either ceruloplasmin (McKie *et al*., 2000) or high concentrations of transferrin (Donovan *et al*., 2000) in the culture media.

The expression profile of FPN1 in macrophages of the liver, spleen, and bone marrow implicates the protein in iron recycling by the RES. Consistent with this possibility is the observation that loading the RES with iron dextran enhances mouse Kupffer cell FPN1 expression (Abboud and Haile, 2000). In this case, the increased FPN1 expression may serve to export the acquired iron. However, it remains to be determined how FPN1 expression changes in response to erythrophagocytosis. Causal relationships between RE iron release and FPN1 expression also need to be demonstrated. Nonetheless, recent clinical reports continue to strengthen the link between FPN1 and RE iron metabolism. Patients with FPN1 mutations exhibit an autosomal dominant form of hemochromatosis (Montosi *et al*., 2001; Njajou *et al*., 2001; Devalia *et al*., 2002; Roetto *et al*., 2002) in which hepatic iron loads primarily in Kupffer cells (Devalia *et al*., 2002; Pietrangelo, 2002; Roetto *et al*., 2002). One caveat is that an iron export protein would be expected to reside exclusively at the plasma membrane, whereas the available immunofluorescence data in Kupffer cells and RAW267.4 cells indicate an intracellular distribution of FPN1. Therefore, it is possible that FPN1 mediates intra-

cellular transit of iron released by heme catabolism after erythrophagocytosis.

As discussed above, Nramp1 has also been implicated in iron release. This possible function has been studied in RAW264.7 macrophage lines stably transfected with either wild-type Nramp1^{G169} or nonfunctional mutant Nramp1^{D169}. No differences in iron release were found between the two cell lines after loading with iron using ^{55}Fe-citrate (Kuhn *et al*., 1999), ^{59}Fe-transferrin (Mulero *et al*., 2002a), or ^{59}Fe-transferrin-anti-transferrin immune complex (Biggs *et al*., 2001; Mulero *et al*., 2002a), which is phagocytosed by the macrophage. However, after loading with ^{59}Fe-transferrin-anti-transferrin immune complex and treatment with interferon-γ and lipopolysaccharide, macrophages expressing functional Nramp1 released significantly more iron than those expressing nonfunctional Nramp1 (Biggs *et al*., 2001; Mulero *et al*., 2002a). Interestingly, the release of iron from the phagocytosed immune complex is reduced if the activity of inducible nitric oxide synthase (iNOS) is inhibited (Biggs *et al*., 2001; Mulero *et al*., 2002a; Mulero *et al*., 2002b). Bone marrow macrophages from mice lacking iNOS also have reduced iron release (Mulero *et al*., 2002b), further indicating that NO influences iron efflux. Although these studies suggest a role for Nramp1 in iron release, the localization of this protein to the phagolysosome makes it an unlikely candidate for performing the ultimate step in iron export from the RE cell. Moreover, it remains to be determined if the RE cell handles the transferrin-anti-transferrin immune complex iron in the same fashion as erythrophagocytosed iron, which must first be liberated by ER-bound HMOX. As these studies indicate, the precise roles of FPN1 and Nramp1 in iron release from the RES remain to be better defined.

E. Regulation of Iron Release

Marrow iron requirements appear to be an important factor in the physiological regulation of iron release from the RES. When body (marrow) requirements increase, as in iron deficiency or venesection, iron release increases (Noyes *et al*., 1960; Beamish *et al*., 1971; Lipschitz *et al*., 1971). Within hours after being given ^{59}FeHDRBCs, iron-deficient individuals released 100% of the iron, whereas normal subjects had a mean release of 64% (Fillet *et al*., 1989). Conversely, decreased marrow requirements resulting from either hypertransfusion (Finch *et al*., 1982) or bone marrow aplasia (Fillet *et al*., 1989) are associated with decreased iron release. Interestingly, patients with aplasia and suppressed erythropoiesis still release 22% of iron in the early phase. As noted by Fillet *et al*. (1989), this release may represent the limit of the RES to retain iron from recycled erythrocytes. How a distant stimulus from the bone marrow regulates RE iron release is not understood. Recently, Pietrangelo (2002) has proposed that the extent of transferrin saturation relays information about the iron status of the bone marrow to the RES. Another model with a signaling role of transferrin saturation, in combination with levels of soluble transferrin receptor in plasma, has been suggested by Townsend and Drakesmith (2002).

VI. PERTURBATIONS OF RE IRON METABOLISM

A. Hereditary Hemochromatosis (HH)

In normal individuals, any dietary iron absorbed in excess deposits in roughly equal

amounts in parenchymal cells of the liver and RE cells. In contrast, the abnormally elevated iron absorption of HH patients leads to preferential iron accumulation in the parenchymal cells of the liver; it is only late in the disease that iron starts to accrue in the Kupffer cells of the liver and RE cells of the bone marrow (Bothwell *et al*., 1965; Valberg *et al*., 1975; Brink *et al*., 1976). This unique pattern of iron deposition in HH suggests that there is a defect in the RE cell's ability to accumulate iron. Indeed, because intestinal iron absorption is inversely related to RE iron stores (Rosenmund *et al*., 1980), abnormal iron handling by RE cells might be responsible for both excess deposition in parenchymal cells and the lack of feedback regulation of duodenal iron uptake (Valberg, 1978). Such a defect could result from an altered ability of the RE cell to acquire, store, or release iron. Decreased erythrophagocytosis, which has been observed in cultured monocyte-derived macrophages from HH patients (Moura *et al*., 1998b; Moura *et al*., 1998a), may indicate a defect in iron acquisition from senescent erythrocytes. No abnormalities in iron acquisition from transferrin have been identified in studies of HH monocytes (Jacobs and Summers, 1981; Sizemore and Bassett, 1984).

Given that one of the normal functions of the RES is to store iron, it has been speculated that the low RE iron levels in HH result from defective synthesis of the iron storage protein ferritin. Studies of HH monocytes incubated with transferrin-bound iron, however, have failed to detect any abnormalities in their ability to synthesize ferritin or to incorporate iron into the storage protein (Jacobs and Summers, 1981; Bassett *et al*., 1982). Recently, Cairo *et al*. (1997) have studied the activity of IRP, the intracellular regulator of ferritin synthesis, in monocytes from HH patients. Unexpectedly, they found that HH monocyte IRP activity was 50% higher than normal. The increased activity does not appear to be due to an inherent defect in IRP control, because changes in cellular iron status modulated IRP activities similarly in HH monocytes as in controls. As noted by Cairo *et al*. (1997), the increased IRP activity likely reflects a reduction in the labile iron pool, which could be due to either decreased iron uptake or increased release. The increase in IRP activity would be expected to decrease ferritin mRNA translation and thus may contribute to the inability of the RE cell to store iron in ferritin.

Fillet *et al*. (1989) used ^{59}FeHDRBCs to study *in vivo* iron release from the RES of HH patients. In these patients, the early iron-release phase was similar to that of healthy individuals, but did not negatively correlate with iron stores as it did in normal subjects. This result suggests that the RES in HH is unable to efficiently downregulate iron release in the face of high iron stores. Abnormally elevated iron release from the RES thus may contribute to the high serum iron levels characteristic of HH. Using isolated monocytes from HH patients, Moura *et al*. (1998b) investigated iron efflux after erythrophagocytosis. Similar to the *in vivo* studies of Fillet *et al*. (1989), iron release was identical in control and HH monocytes; however, HH monocytes released twice as much iron in a low-molecular-weight form as did control cells. Moura *et al*. (1998b) speculate that the released low-molecular-weight iron, which readily binds to transferrin, may contribute to the high plasma transferrin saturation and nontransferrin bound iron observed in HH patients. Significantly increased ferritin release by HH monocytes has also been reported (Flanagan *et al*., 1989).

The recent discovery of the genetic basis of HH is providing insight into the abnormal RE iron metabolism in the disease. The majority of HH cases are caused by a mutation of amino acid 282 (C282Y) in the

HFE gene (Feder *et al*., 1996). HFE encodes a protein similar in structure to MHC class I molecules in that it associates with β2-microglobulin at the cell surface. The C282Y mutant protein demonstrates diminished binding with β2-microglobulin and decreased cell-surface expression (Waheed *et al*., 1997). Functional HFE protein appears to be required for normal iron deposition in the RES, as mice without HFE (Zhou *et al*., 1998; Levy *et al*., 1999) or with C282Y HFE (Levy *et al*., 1999) do not accumulate appreciable amounts of iron in Kupffer cells and in the spleen, despite hepatic iron overload. HFE protein is abundantly expressed in monocytes (Parkkila *et al*., 1997), tissue macrophages (Parkkila *et al*., 1997), and Kupffer cells (Bastin *et al*., 1998; Griffiths *et al*., 2000). In monocytes from HH patients, the C282Y protein is detectable by immunohistochemistry, but at reduced levels (Parkkila *et al*., 2000). Although the exact function of HFE remains unknown (Philpott, 2002), its association with the transferrin receptor (Parkkila *et al*., 2000) implicates its involvement in the metabolism of transferrin-bound iron. Support for this role is provided by a study showing that monocyte-derived macrophages from HH patients accumulate less iron from transferrin than macrophages from normal individuals (Montosi *et al*., 2000). The additional demonstration that the HH macrophages accumulated 50% more transferrin-iron after transfection with wild-type HFE directly implicates a role for HFE in RE iron accumulation. These findings suggest that, in these cells, HFE either enhances the uptake of iron or decreases its release. Townsend and Drakesmith (2002) have proposed a model in which HFE not associated with the transferrin receptor inhibits RE iron release by inhibiting FPN1.

B. Anemia of Chronic Disease (ACD)

Patients with infection, inflammation, or other chronic diseases often develop a mild-to-moderate anemia after several months. This type of anemia is most commonly known as ACD; other designations include "anemia of chronic disorders" (Lee, 1993), "anemia of inflammation (Schilling, 1991)", "primary defective iron-reutilization syndrome (Besa *et al*., 2000)", and "hypoferremic anemia with reticuloendothelial siderosis" (Cartwright and Lee, 1971). The low serum iron concentrations and anemia of ACD appear to result primarily from the decreased flow of iron from cells to plasma. Although diminished iron flux occurs in enterocytes (Cortell *et al*., 1967) and hepatocytes (Hershko *et al*., 1972), the decreased iron flow from RE cells is most important quantitatively. Impaired RE iron release from ^{59}FeHDRBCs has been observed in rat models of acute infection (Kampschmidt *et al*., 1964) and inflammation (Konijn and Hershko, 1977). Similarly, ferrokinetic studies using ^{59}FeHDRBCs in patients with inflammation demonstrate that the early iron release phase is decreased about 20% (Fillet *et al*., 1989). This modest decrease in RE iron release may account for the mild and nonprogressive nature of the anemia in ACD. The inhibition of iron release *in vitro* has also been observed in inflammatory mouse peritoneal macrophages (Esparza and Brock, 1981) and J774 macrophages treated with lipopolysaccharide (Mulero and Brock, 1999).

The molecular mechanisms responsible for the decreased iron release from the RES remain unidentified. Early studies suggested that ferritin levels, which increase markedly in inflammatory and malignant conditions, impair release by diverting iron into storage (Konijn and Hershko, 1977). However,

studies in mouse peritoneal macrophages found that the reduced iron release after injection of an inflammatory agent was associated with decreased ferritin synthesis (Alvarez-Hernandez *et al*., 1986). The diversion of iron into more inert storage forms such as hemosiderin, which did increase after inflammation, was thus proposed as a mechanism for impaired release (Alvarez-Hernandez *et al*., 1986). Various cytokines, especially tumor necrosis factor alpha and interleukin-1β, have also been implicated in the impairment of RE iron release, but results from different groups are inconsistent (Kondo *et al*., 1988; Alvarez-Hernandez *et al*., 1989; Uchida *et al*., 1991; Mabika and Laburn, 1999). Recent studies suggest that downregulation of FPN1 plays a role. Using a model of acute inflammation in mice, Yang *et al*. (2002) found that treatment with lipopolysaccharide resulted in a downregulation of FPN1 expression in RE cells of the spleen, liver, and bone marrow. Time course experiments revealed that the LPS-induced hypoferremia preceded the downregulation of splenic FPN1 protein levels, indicating that the initial hypoferremia results from mechanisms other than FPN1 in the spleen. Yang *et al*. (2002) speculate that downregulation of splenic FPN1 may serve to maintain the hypoferremia rather than induce it.

C. Possible Role of Hepcidin

It is interesting to note that the perturbations in RE iron metabolism in HH and ACD are, for the most part, exactly opposite. Recent studies have led to the proposal that this reciprocal regulation may be mediated a novel plasma peptide called hepcidin (Fleming and Sly, 2001). Also known as LEAP-1 (liver-expressed antimicrobial peptide) (Krause *et al*., 2000), hepcidin is synthesized by the liver in the form of an 84 amino acid propeptide and is detected in the plasma as a peptide of 25 amino acids (Krause *et al*., 2000). A link between hepcidin and iron metabolism was first made by Pigeon *et al*. (2001), who demonstrated that hepatic hepcidin mRNA levels increased with various forms of iron loading and decreased with iron deprivation. Subsequently, Nicolas *et al*. (2001) observed that mice lacking hepcidin develop severe tissue iron overload. Based on these two studies, Nicolas *et al*. (2001) proposed that hepcidin may serve as an iron-status signaling molecule between tissues involved in iron mobilization. According to this model, an iron-loaded liver would secrete increased amounts of hepcidin into the plasma, which in turn would signal the intestine to downregulate iron absorption and the RES to downregulate iron release. The demonstration that lipopolysaccharide, a classic inducer of the inflammatory response, also enhanced hepatic hepcidin mRNA expression raised the possibility that the diminished iron absorption and impaired RE iron release of ACD are mediated through changes in plasma hepcidin levels. This connection has been strengthened by recent studies showing increased hepatic hepcidin mRNA levels in animal models of infection (Shike *et al*., 2002) and in anemic patients with hepatic adenomas (Weinstein *et al*., 2002). On the other hand, in the absence of hepcidin, intestinal iron absorption and RE iron release would be expected to continue unabated as liver iron accumulates. Over time, this would recapitulate the cardinal features of HH: abnormally increased iron absorption, elevated plasma iron levels, and increased iron deposition in the hepatic parenchymal cells, but not in the RES. Indeed, all of these features are displayed by mice lacking hepcidin (Nicolas *et al*., 2001; Nicolas *et al*., 2002). Although these studies are consistent with the hypothesis that plasma hepcidin can mediate the perturbations of

iron metabolism characteristic of both HH and ACD, future studies will need to determine plasma hepcidin levels in these disease states affecting the RES.

VI. UNANSWERED QUESTIONS

Figure 2 summarizes our understanding of the major pathways of iron handling by the RE cell. As indicated by the figure and throughout this review, several key questions remain:

- *Where is iron liberated from heme?* If liberated at the endoplasmic reticulum, do intracellular heme transporters exist? If freed within the phagolysosome, what transporter is responsible for the efflux of iron into the cytosol (Nramp1, DMT1, FPN1, or some other factor)?
- *How is iron released?* Does FPN1 export iron from the RE cell as it appears to do for other cell types? Does FPN1 act at the plasma membrane or does it function within the cell? Does the release of hemoglobin, heme, and ferritin represent a normal physiologic process? If so, how significant is their release in quantitative terms?
- *How is iron release coordinated with body iron status?* Do the plasma proteins transferrin or hepcidin serve as signaling molecules between the bone marrow and the RE cell? If hepcidin plays such a role, what changes does it elicit in the RE cell? Does it interact with or regulate FPN1, HFE, and/or transferrin receptor?
- *What molecular mechanisms mediate the perturbations in RE iron metabolism that characterize HH and ACD?* What iron release pathways are upregulated in HH and downregulated in ACD? Is hepcidin a marker or a mediator of these changes?

While the recent discoveries of Nramp1, DMT1, HFE, FPN1, CD163, and hepcidin have significantly advanced our knowledge of iron metabolism in the RES, it is clear that these outstanding questions (and others) need to be addressed. Given the rapid advances in characterizing the proteins responsible for iron transport, the molecular pathways mediating the movement of iron into and out of the RES should soon be revealed.

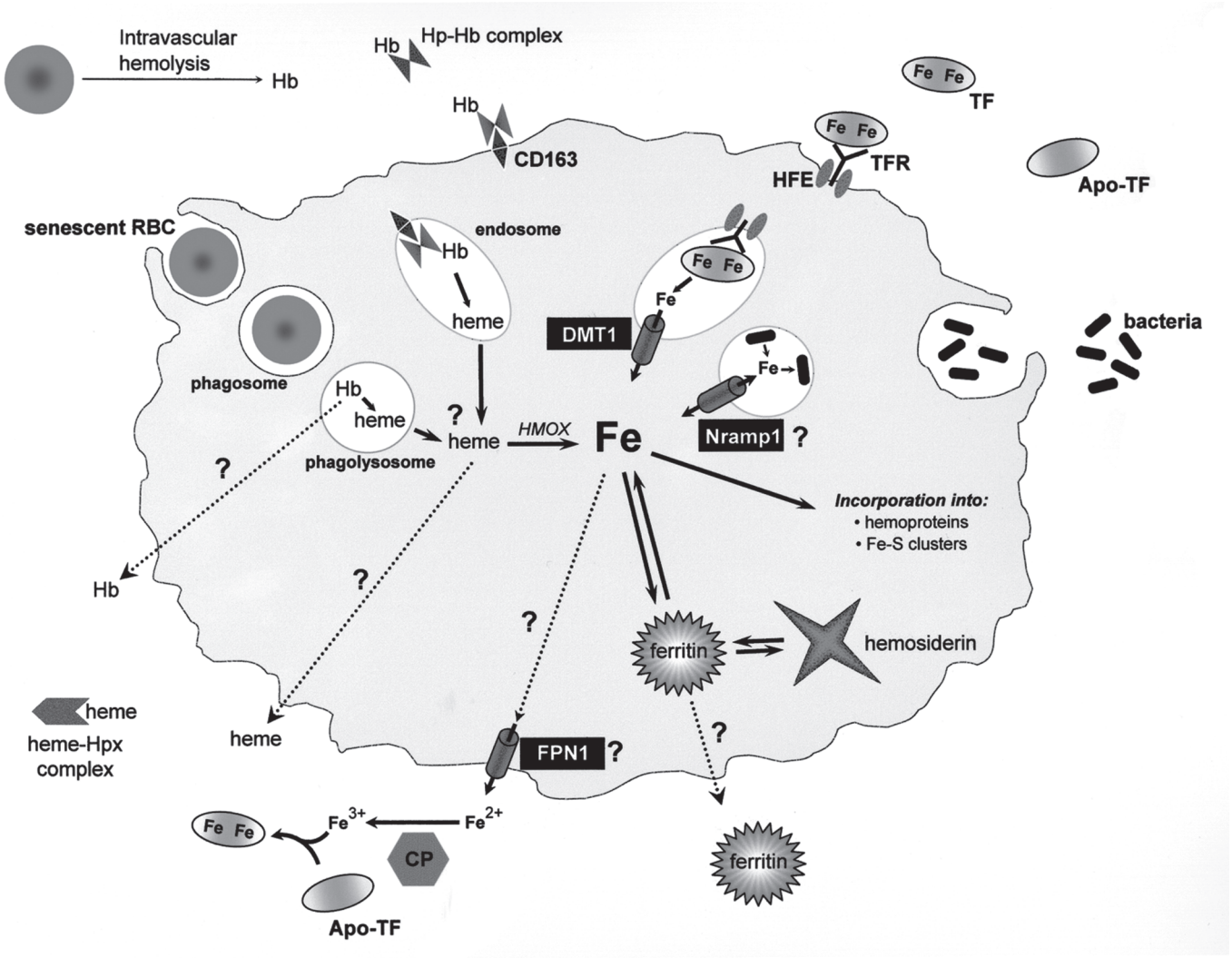

FIGURE 2. Iron handling by the RE cell. Question marks indicate that either the pathway or the transport mechanism has not been elucidated. Arrows with dotted lines indicate forms of iron released by the cell. **CD163**, hemoglobin scavenger receptor; **CP**, ceruloplasmin; **DMT1**, divalent metal transporter 1; **FPN1**, ferroportin; **Hb**, hemoglobin; **Hp**, haptoglobin; **HMOX**, heme oxygenase; **Nramp1**, natural resistance-associated macrophage protein 1; **TF**, transferrin; **TFR**, transferrin receptor.

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