The Nexus of Iron and Inflammation in Hepcidin Regulation: SMADs, STATs, and ECSIT


Abstract

Patients with alcoholic liver disease frequently exhibit iron overload in association with increased hepatic fibrosis. Even moderate alcohol consumption elevates body iron stores; however, the underlying molecular mechanisms are unknown. Hepcidin, a circulatory peptide synthesized in the liver, is a key mediator of iron metabolism. Ethanol metabolism significantly down-regulated both in vitro and in vivo hepcidin mRNA and protein expression. 4-Methylpyrazole, a specific inhibitor of the alcohol-metabolizing enzymes, abolished the effects of ethanol on hepcidin. However, ethanol did not alter the expression of transferrin receptor1 and ferritin or the activation of iron regulatory RNA-binding proteins, IRP1 and IRP2. Mice maintained on 10-20% ethanol for 7 days displayed down-regulation of liver hepcidin expression without changes in liver triglycerides or histology. This was accompanied by elevated duodenal divalent metal transporter1 and ferroportin protein expression. Injection of hepcidin peptide negated the effect of ethanol on duodenal iron transporters. Ethanol down-regulated hepcidin promoter activity and the DNA binding activity of CCAAT/enhancer-binding protein alpha (C/EBPα) but not beta. Interestingly, the antioxidants vitamin E and N-acetylcysteine abolished both the alcohol-mediated down-regulation of C/EBPα binding activity and hepcidin expression in the liver and the up-regulation of duodenal divalent metal transporter 1. Collectively, these findings indicate that alcohol metabolism-mediated oxidative stress regulates hepcidin transcription via C/EBPα, which in turn leads to increased duodenal iron transport.

The antimicrobial peptide hepcidin is produced primarily by hepatocytes and has key regulatory roles in iron homeostasis. The interaction of hepcidin with ferroportin, an iron export protein expressed on the surface of several cell types including enterocytes, macrophages, and hepatocytes, facilitates the internalization and degradation of ferroportin and may lead to decreased dietary iron absorption and to retention of iron in body stores.

Hepcidin expression can be up-regulated by high iron levels or during acute phase inflammatory responses. In mice, overexpression of hepcidin produces iron-deficiency anemia whereas hepcidin deficiency manifests as iron overload. Hereditary hemochromatosis is characterized by inappropriately low hepcidin expression in the face of increased body iron stores. Loss-of-function hepcidin gene mutations in humans result in severe hemochromatosis phenotypes. Similarly, mutations in genes encoding HFE, hemosjuelin (HJV), and transferrin receptor 2 (TFR2) may lead to hemochromatosis via effects on hepcidin expression.

Regulation of Hepcidin in Response to Inflammation. In both Hfe knockout mice and patients with HFE-related hemochromatosis, most data suggest that functional HFE is required to maintain basal hepatic hepcidin mRNA levels. In contrast, the weight of evidence indicates HFE is not needed for hepcidin up-regulation in response to inflammation. Lee and colleagues saw increased hepcidin expression in response to lipopolysaccharide (LPS)-induced inflammation in both Hfe knockout and wild-type mice. Interleukin-6 (IL-6) up-regulated hepcidin in hepatocytes cultured from either Hfe knockout or wild-type mice. Frazer and colleagues reported similar findings and proposed that hepatic hepcidin expression is stimulated by an HFE-dependent mechanism in response to iron but may be overcome in acute phase inflammatory responses, possibly through a pathway involving Toll-like receptor 4 (TLR-4) which is important in the innate immune response. Hepcidin is up-regulated in iron-deprived wild-type mice injected with LPS but not in Tlr-4−/−deficient mice, consistent with a TLR-4-dependent inflammatory pathway that overrides the iron-sensing pathway.

Consistent with potential roles in hepcidin regulation, TLR-4 is important in ferroportin down-regulation in...
response to LPS-induced inflammation or exposure to bacteria. In the liver, TLR-4 is involved in Kupffer cell activation and may be required for hepatic ischemia/reperfusion injury and hepatic injury following hemorrhagic shock, and may contribute to alcoholic liver disease.

Regulation of hepatocyte hepcidin expression through the iron-sensing pathway appears independent of either Kupffer or peripheral myeloid cells. However hepcidin regulation through the inflammatory pathway in response to bacterial pathogens could involve paracrine effects through Kupffer cells or might primarily act through TLR-4 expressed on peripheral myeloid cells (macrophages, neutrophils).

Signaling through TLR-4 activates nuclear factor κB (NF-κB), leading to IL-6 production. This may represent one mechanism whereby activation of Kupffer or other myeloid cells and signaling through TLR-4 could trigger endocrine responses, altering hepatic hepcidin expression in liver injury and disease via TLR-4. Hepcidin is down-regulated in alcoholic liver injury, suggesting it may also be regulated through TLR-2 or TLR-3, which down-regulates signaling through TLR-4 to reduce hepatic injury in response to LPS. Although most hepcidin studies used LPS, which is a model for Gram-negative bacterial infection, other stimuli trigger different Toll pathways. But if TLR can regulate hepcidin expression, how does this interact with the iron sensing pathway?

Regulation of Hepcidin in Response to Iron. Basal hepcidin gene expression is lower in HFE-related HH or Hfe knockout mice, although it may be increased above basal levels in response to altered iron status. The iron-sensitive mechanism for up-regulating hepcidin expression in response to iron overload appears to involve TFR2 and HJV. Body iron status may be sensed through dipherric transferrin, which has been proposed to regulate the interactions of HFE with either TFR1 or TFR2 and subsequent downstream signaling to hepcidin.

New insights into the iron-sensing pathway have emerged from studies by Wang and colleagues in mice with liver-specific disruption of SMAD4, an important mediator of the transforming growth factor β (TGF-β) super-family signaling pathway. These mice have markedly decreased hepcidin levels and accumulate iron in liver and other organs and exhibit increased expression of other iron-related genes including ferroportin, Dcytb and DMT1. Hepatocyte up-regulation of hepcidin in response to both iron and the pro-inflammatory cytokine IL-6 required SMAD4, suggesting this molecule may be a nexus between the iron-responsive and the inflammatory regulatory pathways. Wang and colleagues found hepcidin was up-regulated by TGF-β and another member of the TGF-β super-family, bone morphogenetic protein (BMP), which both signal through SMAD-dependent pathways. In each case, up-regulation was abrogated in SMAD4-deficient hepatocytes. Another group failed to find a TGF-β effect but confirmed that BMP can up-regulate hepatic hepcidin expression through a pathway likely to involve SMADs. This was enhanced by HJV, proposed to be a BMP co-receptor. HJV belongs to the repulsive guidance molecule (RGM) family, a subfamily of the TGF-β superfamily, and 2 other RGMs are BMP co-receptors. A third study showed that regulation of hepcidin by BMP2, BMP4, and BMP9 is independent of HFE, TFR2, and IL-6.

These observations suggest an integrated mechanism for iron-responsive hepcidin regulation. The involvement of BMPs raises intriguing possibilities for mechanisms underlying arthritis and osteopenia in iron overload. Yet much remains to be resolved. The in vivo data on SMAD phosphorylation, which occurs at substantial levels even in Hjv−/− mice, and the data on hepcidin expression in hepatocytes from Hjv−/− mice suggest HJV is responsible only for a fraction of the BMP effect on hepatic hepcidin expression. It is also not yet clear how the proposed pathway relates other putative mechanisms for hepcidin regulation through HJV and TFR2. Neogenin, a high-affinity RGM receptor, may also be a hepatocellular HJV receptor, and it is possible that different receptor systems may dominate in different in vivo scenarios.

Coordination of Hepcidin Regulation. Although the role of HJV needs clarification, we suggest the 2 sides of the story involving TLR-4 and BMP may be linked through the protein ECSIT (evolutionarily conserved signaling intermediate in Toll pathways), proposed to mediate cross-talk between the BMP/SMAD and Toll pathways and apparently essential for SMAD4 transcriptional effects. Another nexus between the iron and inflammatory pathways may involve members of the signal transducer and activator of transcription (STAT) family. These are reported to be necessary and sufficient for IL-6 effects on hepcidin and probably also contribute to hepcidin responses to interferon-beta and other cytokines such as leukemia inhibitory factor (LIF) that act primarily through JAK-STAT mechanisms. There are various STAT/SMAD interactions. The transcriptional co-activator CBP/p300 forms a physical bridge between STAT3 and SMAD1, interacting with STAT3 in a cytokine stimulation–dependent manner and with SMAD1 in a cytokine stimulation–independent manner and can modulate STAT/SMAD interactions. A protein inhibitor of activated STAT (PIAS) represses BMP signaling
through SMAD interactions, probably by effects on CBP/p300.28

The proposed pathways can be broadly summarized as follows. Iron-related stimuli such as HJV may act through BMP receptors and possibly also TGF-β receptors, both of which signal through complexes of SMADs that, once activated, translocate to the nucleus to alter gene expression. Inflammatory stimuli (e.g., LPS) signal through Toll-like receptors (TLR), activating kinase pathways that include cytokine-related signaling factors (e.g., interleukin-1 receptor associated kinase IRAK and tumor necrosis factor receptor associated factors TRAFs). These pathways activate NF-κB and c-Jun N-terminal kinase (JNK), which also translocate to the nucleus. ECSIT is proposed to interact with TRAF6 and mitogen-activated protein kinase/extracellular signal regulated kinase kinase 1 (MEKK1) in the cytoplasm and an alternative splice form, ECSIT2, can localize to the nucleus where it binds SMADs.19 If ECSIT itself binds cytoplasmic SMADs, this raises the possibility of bidirectional cross-talk between the 2 receptor systems. In addition, SMADs may also cross-talk with JAK-STAT pathways downstream of cytokine receptors, possibly by CBP/300 family members physically linking SMADs and STATs, which might be blocked by PIAS family members. Ultimately, these pathways will cause net changes in expression of cytokines, and probably also other proteins, that in turn regulate hepcidin gene expression. It is presently unclear as to whether these systems are in operation in humans. Kemna and colleagues have now injected LPS into 10 human volunteers and found increases in IL-6 and urinary hepcidin within 6 hours with rapid decreases in serum iron levels,29 consistent with LPS actions in mice. However, other studies in humans suggest that inflammation does not invariably countermand the effects of iron status. Beutler and colleagues postulated that C282Y homozygotes with higher levels of inflammation in general, and IL-6 in particular, may have greater hepcidin up-regulation, restoring hepcidin to more normal levels and reducing iron stores in these individuals, contributing to the variable penetrance of the C282Y mutation. But instead they found there was no significant relationship between iron stores (determined by quantitative phlebotomy) and IL-6 or plasma C-reactive protein, a marker of inflammation, in 19 male and 13 female HFE C282Y homozygotes.30 Another study found liver hepcidin mRNA levels correlate with iron but not inflammation in hepatitis C patients.31

In summary, inflammation may not inevitably override iron signaling in humans. This may partly reflect the stimuli used because human responses to LPS (modeling inflammation due to bacterial infection) appear similar to those in mice while responses to inflammation in hepatitis or hemochromatosis differed from LPS responses in humans or mice. The story clearly has more installments to come.

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