

## Is HFE Involved in Increased Hepcidin Expression and Hypoferremia in Inflammation and Anemia of Chronic Disease?

Roy CN, Custodio AO, de Graaf J, Schneider S, Akpan I, Montross LK, Sanchez M, Gaudino A, Hentze MW, Andrews NC, Muckenthaler MU. An Hfe-dependent pathway mediates hyposideremia in response to lipopolysaccharide-induced inflammation in mice. *Nature Genetics* 2004;36:481-5. <http://www.nature.com/ng/index.html>. (Reprinted by permission of Nature Publishing Group.)

### Abstract

**Inflammation influences iron balance in the whole organism. A common clinical manifestation of these changes is anemia of chronic disease (ACD; also called anemia of inflammation). Inflammation reduces duodenal iron absorption and increases macrophage iron retention, resulting in low serum iron concentrations (hyposideremia). Despite the protection hyposideremia provides against proliferating microorganisms, this "iron withholding" reduces the iron available to maturing red blood cells and eventually contributes to the development of anemia. Hepcidin antimicrobial peptide (Hamp) is a hepatic defensin-like peptide hormone that inhibits duodenal iron absorption and macrophage iron release. Hamp is part of the type II acute phase response and is thought to have a crucial regulatory role in sequestering iron in the context of ACD. Mice with deficiencies in the hemochromatosis gene product, Hfe, mounted a general inflammatory response after injection of lipopolysaccharide but lacked appropriate Hamp expression and did not develop hyposideremia. These data suggest a previously unidentified role for Hfe in innate immunity and ACD.**

### Comments

Hepcidin (also called Hamp) is an antimicrobial peptide hormone synthesized by the liver,<sup>1</sup> that has been likened to the defensins, small neutrophil-derived peptides with roles in innate and adaptive immunity. There is evidence from both human and mouse models that hepcidin downregulates gastrointestinal iron absorption and serum iron by inhibiting both intestinal iron absorption and iron release from reticuloendothelial macrophage storage sites.<sup>2</sup> This inhibition can lead to abnormally low serum iron concentrations (hypoferremia or hyposideremia).

Low serum iron in the face of high body iron stores is a characteristic diagnostic feature of anemias of chronic disease (ACD),<sup>3,4</sup> prompting suggestions that hepcidin is important in the pathogenesis of ACD.<sup>5,6</sup> These anemias,

also called anemias of inflammation, comprise a spectrum of anemias associated with chronic diseases such as malignancy, arthritis, infections and other inflammatory disorders.<sup>3,4,7</sup>

The evidence that hepcidin modulates iron status in both iron deficiency and iron overload has been recently reviewed.<sup>8,9</sup> Briefly, the hepatic expression of mouse hepcidin messenger RNA (mRNA) responds to iron status, being increased during iron overload and decreased during iron deficiency.<sup>10,11</sup> Overexpression of hepcidin in mice leads to severe iron deficiency anemia<sup>12</sup> and inhibits liver iron accumulation in the *Hfe*-knockout mouse model of hemochromatosis.<sup>13</sup> On the other hand, upstream stimulatory factor 2 (USF2) knockout mice, in which endogenous hepcidin expression is disrupted, have severe tissue iron overload which resembles that in *Hfe*-knockout mice.<sup>2</sup> In both these mouse models, this overload is thought to be due to increased intestinal absorption of iron in conjunction with impaired retention in reticuloendothelial macrophages. In humans, mutations in the hepcidin gene that disrupt the function of hepcidin can result in juvenile hemochromatosis.<sup>14</sup> Conversely, excessive expression of hepcidin from large hepatic adenomas is associated with severe iron refractory anemia.<sup>5</sup> These studies along with others support the contention that hepcidin downregulates serum iron both by reducing uptake of dietary iron and by inhibiting release from body iron stores.

One protein involved in regulating hepcidin expression is HFE. Bridle et al.<sup>15</sup> observed decreased hepatic hepcidin mRNA levels in both *Hfe*-knockout mice and in untreated patients with HFE-related hemochromatosis.<sup>15</sup> Similar results have been reported by various other studies.<sup>16-19</sup> Notably microarray analyses of hepatic gene expression also suggest hepcidin mRNA expression is specifically decreased in mouse models of HFE-related hemochromatosis whereas, in contrast, hepcidin expression is appropriately increased in mice with secondary iron overload from iron-dextran injection.<sup>7,18</sup>

These findings raised the possibility that HFE might be required for upregulation of hepcidin and consequent hypoferremia in response to inflammation. Roy et al.<sup>7</sup> investigated this question by examining expression of hepcidin, interleukin 6 (IL-6) and tumor necrosis factor (TNF) in wild-type and *Hfe*-knockout mice 1.5 hours after lipopolysaccharide (LPS) treatment. At this time point, IL-6

and TNF expression were elevated in both wild-type and *Hfe*-knockout mice. However, despite the normal cytokine response in *Hfe*-knockout mice in response to LPS-induced inflammation, increases in hepatic hepcidin mRNA levels and hypoferrremia were not observed in this study.<sup>7</sup> These observations lead to the conclusion that the lack of hepcidin up-regulation in *Hfe*-knockout mice was “not due to an insufficient acute phase response” and HFE must “act downstream, or independently of, IL-6 in the inflammatory cascade” that upregulates hepcidin expression.<sup>7</sup> Roy and colleagues therefore suggested that preventing hypoferrremia through “inhibition of HFE function may be a new therapeutic approach to treating ACD”.<sup>7</sup>

In contrast, two subsequent studies<sup>19,20</sup> using comparable mouse models have reported that hepcidin upregulation during inflammation does not require *Hfe*. Frazer et al.<sup>19</sup> found that 16 hours after inducing an acute phase inflammatory response by injecting Freund’s Complete Adjuvant, hepatic hepcidin mRNA expression was significantly increased in *Hfe*-knockout mice and to an extent similar to increases seen in wild-type mice. Hypoferrremia (indicated by decreased transferrin saturation) occurred in both types of mice. Similarly Lee et al.<sup>20</sup> demonstrated that LPS treatment induced hepcidin expression in both *Hfe*-knockout and wild-type mice and that IL-6 caused equivalent upregulation of hepcidin mRNA levels in isolated hepatocytes from both types of mice.

The results of these two studies<sup>19,20</sup> argue that HFE is not necessary for either hepcidin induction or hypoferrremia in response to inflammation. Frazer et al.<sup>19</sup> propose that hepatic hepcidin expression is usually regulated by an HFE-dependent mechanism that can be stimulated directly or indirectly in response to iron in the intact animal. However, during acute phase inflammatory response, this mechanism can be overridden, allowing hepcidin to be upregulated independent of HFE, possibly by a pathway involving Toll-like receptor 4.<sup>19</sup>

The reasons for the disparities between Roy et al.<sup>7</sup> and the other two studies<sup>19,20</sup> are not clear. One possibility is that there may be short-term actions of the HFE-dependent pathway on inflammation induced expression of hepcidin that were detectable at the time used by Roy et al.<sup>7</sup> (1.5 hours after LPS injection; cytokine and hepcidin responses returned to baseline by 24 hours) that might have been missed by the other studies (6 hours<sup>20</sup> or 16 hours<sup>19</sup> after inflammatory stimuli). However, while short-term actions may be important in other contexts, long-term actions are more relevant to ACD, which is typically associated with chronic disease and inflammation.

It remains unknown how modulating the levels of HFE or hepcidin will affect inflammatory responses and iron status over longer periods when superimposed on a coexisting, chronic, inflammatory disease or infection. It will be important to examine these questions in models more closely representing human anemias of infection and chronic disease.

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