The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6


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Key Points

- Presence of the BMP type I receptor Alk3 is required for interleukin-6 to induce hepatic hepcidin gene expression.
- Alk3 contributes to the induction of hypoferremia by interleukin-6.

Increased IL-6 production induces, via STAT3 phosphorylation, hepatic transcription of the gene encoding the iron-regulatory hormone, hepcidin, leading to development of anemia of chronic disease (ACD). Inhibition of bone morphogenetic protein (BMP) signaling prevents the induction of hepcidin gene expression by IL-6 and ameliorates ACD. Using mice with hepatocyte-specific deficiency of Alk2 or Alk3, we sought to identify the BMP type I receptor that participates in IL-6–mediated induction of hepcidin gene expression. Mice were injected with adenovirus specifying IL-6 (Ad.IL-6) or control adenovirus. Seventy-two hours later, serum iron concentrations and hepatic levels of STAT3 phosphorylation and hepcidin messenger RNA were measured. Additional mice were injected with recombinant murine IL-6 (mIL-6) or vehicle, and hepatic hepcidin gene expression was measured 4 hours later. Deficiency of Alk2 or Alk3 did not alter the ability of Ad.IL-6 injection to induce hepatic STAT3 phosphorylation. Ad.IL-6 increased hepatic hepcidin messenger RNA levels and decreased serum iron concentrations in Alk2- but not Alk3-deficient mice. Similarly, administration of mIL-6 induced hepatic hepcidin gene expression in Alk2- but not Alk3-deficient mice. These results demonstrate that the ability of IL-6 to induce hepatic hepcidin gene expression and reduce serum iron concentrations is dependent on the BMP type I receptor Alk3. (Blood. 2014;123(14):2261-2268)

Introduction

Anemia of chronic disease (ACD), the second most prevalent form of anemia, is associated with chronic infections, autoimmune diseases, and neoplasias. ACD is characterized by increased levels of hepcidin, a hormone that induces degradation of the iron exporter ferroportin-1. Ferroportin-1 is responsible for intestinal absorption of dietary iron and for mobilization of iron from intracellular stores in enterocytes, macrophages, and hepatocytes. Increased hepcidin levels cause iron to remain inside cells, resulting in decreased serum iron concentrations and impairment of erythropoiesis.

Hepcidin production is transcriptionally regulated by inflammatory cytokines, including IL-6 and bone morphogenetic protein (BMP) signaling. Expression of the gene encoding hepcidin, Hmga-1, is also regulated by a variety of other stimuli, including iron, hypoxia, transforming growth factor-β, tumor necrosis factor-α, hemojuvelin (HJV, a BMP co-receptor), activin B, and the unfolded protein response. IL-6 induces hepcidin gene transcription, as well as the transcription of other target genes including the gene encoding heme oxygenase-1 (HO-1), Hmox1, via phosphorylation of the transcription factor STAT3. BMP signaling is activated by binding of BMP ligands to a BMP receptor complex, which consists of type II receptors (BMPR2, ActRIIa, and ActRIIb) and type I receptors (Alk1, Alk2, Alk3, and Alk6). Ligand binding induces the type II receptor to phosphorylate and activate the type I receptor. Activated type I receptors phosphorylate BMP-responsive SMAD proteins (SMADs 1, 5, and 8), which translocate together with SMAD4 into the nucleus, where they activate expression of multiple genes including those encoding hepcidin and Il-1.

Of the 4 BMP type I receptors, Alk1 is predominantly expressed in endothelial cells. Alk2 and Alk3 are highly expressed in hepatocytes, whereas Alk6 is expressed at much lower levels. We previously reported that hepatocyte-specific deletion of Alk2 or Alk3 in mice causes mild or severe iron overload phenotypes, respectively. In contrast, global deficiency of Alk6 does not alter serum iron concentrations or hepatic tissue iron levels in mice (P. J. Schmidt and M. D. Fleming, unpublished observations).

Inhibition of BMP signaling, using small molecule inhibitors of type I BMP receptor kinase activity (eg, LDN-193189) or recombinant proteins that scavenge BMP ligands (eg, noggin, Alk3-Fc, or HJV-Fc),...
reduces the ability of IL-6 to induce hepcidin gene expression and decrease serum iron concentrations, thereby, ameliorating ACD in animal models. In the current study, we sought to identify the BMP type I receptor(s) that contribute(s) to the induction of hepatic hepcidin gene expression by IL-6. We studied mice with hepatocyte-specific deficiency of Alk2 or Alk3 that were exposed to either sustained or transient increases in hepatic IL-6 levels induced by injection with an adenovirus specifying IL-6 (Ad.IL-6) or with recombinant murine IL-6 (mIL-6), respectively. We report that Alk3 is required for the IL-6-mediated induction of hepatic hepcidin gene expression and reduction in serum iron levels.

Methods

Cells and media

Human hepatoma cells (HepG2, American Type Culture Collection, Manassas, VA) were cultured in Eagle’s Minimal Essential Medium supplemented with 10% fetal bovine serum, penicillin, streptomycin, and L-glutamine. Cells were transferred to 6-well dishes and starved with Eagle’s Minimal Essential Medium containing no fetal bovine serum for at least 6 hours before exposure to human IL-6.

Animals

All mouse experiments were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital, where the experiments were performed. Mice with floxed Alk2 allele (Alk2<sup>fl/fl</sup>) on a mixed C57BL/6; SV129 background or mice with floxed Alk3 allele (Alk3<sup>fl/fl</sup>) on a C57BL/6 background<sup>23,26</sup> were bred with B6.Cg-Tg(Alb-Cre)<sup>21Mge1I</sup> mice (Jackson Labs). Heterozygous animals were intercrossed to obtain animals homozygous for the Alk<sup>2</sup> or Alk<sup>3</sup> alleles with or without the transgene specifying Cre recombinase under the control of the hepatocyte-specific albumin gene promoter (Alb-Cre), as described previously.<sup>21</sup> Mice with hepatocyte-specific Alk2 deficiency (Alk2<sup>fl/fl</sup>, Alb-Cre) were compared with Alk2<sup>fl/fl</sup> mice that did not carry the Alb-Cre transgene. Similarly, mice with hepatocyte-specific Alk3 deficiency (Alk3<sup>fl/fl</sup>, Alb-Cre) were compared with Alk3<sup>fl/fl</sup> mice that did not carry the Alb-Cre transgene. In accordance with the recently published ARRIVE guidelines (http://www.ncrfs.org/ARRIVE), mice were randomized according to their genotypes to either treatment or control group. The investigators, who measured iron concentrations in serum and tissues and who measured messenger RNAs (mRNAs) and protein levels, were blinded as to the experimental groups to which the mice were assigned. The experimental procedure is explained for each method in detail below (e.g., adenovirus or recombinant protein injection). Male mice at the age of 8 to 12 weeks were studied, as indicated for each experiment. The average weight of these mice was 20 g. Mice were fed a standard, iron-replete diet (Prolab SP75 Isopro 3000, 380 ppm iron) and not more than 4 to 5 siblings were housed in each cage.

Adenoviruses

Recombinant adenovirus specifying GFP was purchased from Vector Biolabs (Ad.GFP, ready-to-use human adenovirus type 5 [dE1/E3], catalog No. 1060, gene name: eGFP, promoter: cytomegalovirus, titer 1 × 10<sup>10</sup> PFU/mL). Recombinant Ad.IL-6 was purchased from Vector Biolabs (Ad.IL-6, ready-to-use human adenovirus type 5 [dE1/E3], catalog No. 1380, gene name: IL6, promoter: cytomegalovirus, titer 1 × 10<sup>10</sup> PFU/mL). Ad.GFP or Ad.IL-6 were propagated in 293 cells and harvested from the cells just before spontaneous lysis. The virus was purified from cell extracts with an Adenovirus Standard Purification Virakit (Virapur). The viral titer was estimated from the optical density at 260 nm.

Mice without and with hepatocyte-specific Alk2 or Alk3 deficiency were injected with 1 × 10<sup>11</sup> virus particles (in 100 µL of saline) of either Ad.GFP or Ad.IL-6 via a tail vein. Blood and tissue samples were collected 72 hours after injection.

Injections of mice with recombinant mIL-6

Mice received a single intraperitoneal injection of recombinant mouse IL-6 (#406-ML-025, 100 ng/g; R&D Systems) or vehicle (0.1% bovine serum antigen [BSA] in phosphate-buffered saline [PBS]). Wild-type mice were anesthetized at 2 and 4 hours after injection, and blood was collected. Mice were euthanized and tissue samples were harvested. Four hours after injection with mIL-6 or vehicle, blood and tissues were harvested from mice with and without hepatocyte-specific Alk2 or Alk3 deficiency.

Hematologic and iron parameters

Blood for measurement of serum iron parameters was obtained by retroorbital puncture, as described previously.<sup>18</sup> Serum iron concentrations and transferrin saturations were determined using the Iron/UBC Kit (IRON-SL, #157-30; Thermo Fisher Scientific and SEKISUI Diagnostics) following the manufacturers’ protocols. After mice were euthanized liver and spleen tissues were harvested, and non–heme tissue iron levels were determined, as previously described.<sup>18</sup>

Hepatic mRNA levels

Total RNA was extracted from liver tissues using Trizol (Invitrogen), as previously described.<sup>21</sup> Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed on a Mastercycler Realplex2 (Eppendorf) using KAPA SYBR FAST (Kapa Biosystems) for conventional Sybr primers or KAPA PROBE FAST (Kapa Biosystems) for hydrolysis probes (TaqMan Gene Expression Assays, Applied Biosystems, Life Technologies). The relative Ct method was used to normalize the levels of target transcripts to 18S rRNA levels. Primer pairs used for quantitative RT-PCR are listed in supplemental Table 1 (available on the Blood Web site) and are described in our previous publication.<sup>21</sup>

Measurement of phosphorylated STAT3 and BMP-responsive SMAD levels

HepG2 cells and liver tissues were harvested in radio-immunoprecipitation assay (RIPA) buffer supplemented with protease and phosphatase inhibitors (Sigma-Aldrich). Extracted proteins were separated by electrophoresis using 4% to 12% bis-Tris gels (NuPAGE, Invitrogen) and transferred to polyvinylidene fluoride membranes (Immobilon-FL; Millipore). Membranes were incubated with antibodies directed against total STAT3 or the phosphorylated form (P-STAT3, Cat. No. 9145L; Cell Signaling Technology), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cat. No. 5174s; Cell Signaling Technology). After washing, membranes were incubated with horseradish peroxidase–linked anti-rabbit IgG or anti-mouse IgG (Cell Signaling Technology). Membranes were incubated with ECL-Plus (GE Healthcare), and chemiluminescence was detected with a Versadoc 4000MP imager (Bio-Rad).

Statistical methods

All values were expressed as mean ± standard deviation. Data were analyzed using the Student t test and one-way analysis of variance (ANOVA) Kruskal-Wallis test with Dunn’s correction for multiple comparisons, when applicable. Statistical significance was considered for P values <.05.

Results

The effect of hepatocyte-selective deletion of BMP type I receptors on IL-6–mediated changes in iron homeostasis

We sought to determine which of the 2 BMP type I receptors involved in hepatic iron signaling, Alk2 or Alk3, contributes to the
ability of IL-6 to induce hepcidin gene expression and reduce serum iron concentrations. Hepatocyte-specific deletion of a BMP type I receptor was performed by generating mice homozygous for Alk2 or Alk3 alleles flanked by loxP sequences that also carry a transgene in which the albumin promoter directs expression of Cre recombinase. To achieve sustained increases in hepatic IL-6 expression, mice were injected with a recombinant adenovirus (type 5) specifying IL-6 (Ad.IL-6). In pilot studies of wild-type mice injected with Ad.IL-6, serum iron concentrations and transferrin saturations were higher in Ad.GFP-injected mice than in Ad.GFP-injected Alk2fl/fl mice (Figure 1A-B). These results were consistent with our previous observations that serum iron concentrations are elevated in untreated Alk2fl/fl, Alb-Cre mice.

To determine the impact of hepatocyte-specific Alk3 deficiency on the ability of IL-6 to modulate serum iron concentrations, Alk3fl/fl and Alk3+/fl mice were injected with either Ad.IL-6 or Ad.GFP. In Ad.GFP-injected Alk3fl/fl, Alb-Cre mice, serum iron levels and transferrin saturations were higher than in Ad.GFP-injected Alk3fl/fl mice (Figure 1C-D). These results were consistent with our previous observations that serum iron concentrations are elevated in untreated Alk2fl/fl, Alb-Cre mice. Exposure to IL-6 for 72 hours decreased serum iron levels and transferrin saturations in both Alk2fl/fl and Alk3fl/fl, Alb-Cre mice (Figure 1A-B). These results suggest that Alk2 is not required for IL-6 to reduce iron levels and transferrin saturations. However, it is of note that the percent reduction in serum iron levels caused by Ad.IL-6 was greater in Alk2fl/fl mice than in Alk2fl/fl, Alb-Cre mice.
Impact of BMP type I receptor deletion on IL-6-mediated induction of hepcidin gene expression

To test whether IL-6 requires Alk2 or Alk3 to increase hepcidin gene expression, we measured hepatic hepcidin mRNA levels in Alk2\(^{fl/fl}\) and Alk3\(^{fl/fl}\); Alb-Cre mice, as well as in Alk3\(^{fl/fl}\) and Alk3\(^{fl/fl}\); Alb-Cre mice, 72 hours after injection with Ad.GFP or Ad.IL-6 (Figure 2). Hepatic hepcidin mRNA levels did not differ in Alk2\(^{fl/fl}\) and Alk2\(^{fl/fl}\); Alb-Cre mice injected with Ad.GFP. However, the ratio of serum iron levels to hepatic hepcidin mRNA levels was markedly higher in the latter, consistent with the previously reported concept that hepatic Alk2 deficiency impairs the responsiveness of hepcidin gene expression to serum iron levels.\(^{23}\) Injection with Ad.IL-6 induced hepatic hepcidin gene expression similarly in both Alk2\(^{fl/fl}\) and Alk2\(^{fl/fl}\); Alb-Cre mice (2.6- to 2.8-fold, respectively; Figure 2A). The observation that serum iron levels and transferrin saturations remained higher in Alk2\(^{fl/fl}\); Alb-Cre mice than in Alk2\(^{fl/fl}\) mice even after Ad.IL-6 injection is most likely attributable to the sum of the impact of Alk2 on basal iron metabolism and the Alk2-independent ability of IL-6 to induce hepcidin gene expression.

Hepatic hepcidin mRNA levels were markedly less in Ad.GFP-injected Alk2\(^{fl/fl}\); Alb-Cre mice than in Ad.GFP-injected Alk2\(^{fl/fl}\) mice, consistent with the previously reported effect of Alk3 deficiency on basal hepatic hepcidin expression. Ad.IL-6 injection induced hepatic hepcidin gene expression in Alk3\(^{fl/fl}\) mice but did not increase hepcidin mRNA levels in the livers of Alk3\(^{fl/fl}\); Alb-Cre mice (Figure 2B). These results suggest that Alk3, but not Alk2, is required for the induction of hepcidin gene expression by IL-6.

We previously reported that Id-1, a BMP target gene, was also induced by IL-6 in a BMP signaling–dependent manner.\(^{18,24}\) To examine the importance of Alk2 and Alk3 in the ability of IL-6 to induce Id-1 gene expression, we measured hepatic Id-1 mRNA levels in Alk2\(^{fl/fl}\) and Alk2\(^{fl/fl}\); Alb-Cre, as well as in Alk3\(^{fl/fl}\) and Alk3\(^{fl/fl}\); Alb-Cre mice, that were injected with either Ad.GFP or Ad.IL-6. Hepatic Id-1 mRNA levels did not differ in Ad.GFP-injected Alk2\(^{fl/fl}\) and Alk2\(^{fl/fl}\); Alb-Cre mice, Sustained exposure to IL-6 induced hepatic Id-1 gene expression in both Alk2\(^{fl/fl}\) and Alk2\(^{fl/fl}\); Alb-Cre mice (Figure 2C). In contrast, hepatic Id-1 mRNA levels were less in Alk3\(^{fl/fl}\); Alb-Cre mice injected with Ad.GFP than in Alk3\(^{fl/fl}\) mice injected with Ad.GFP. Prolonged exposure to IL-6 induced hepatic Id-1 gene expression in Alk3\(^{fl/fl}\) mice but not in Alk3\(^{fl/fl}\); Alb-Cre mice (Figure 2D). These data indicate that induction of hepatic Id-1 gene expression by IL-6 is dependent on Alk3.

Inhibition of BMP signaling does not affect the induction of STAT3 phosphorylation and HO-1 gene expression by IL-6

Having observed that IL-6 was unable to induce hepcidin and Id-1 gene expression in mice with hepatocyte-specific Alk3 deficiency, we investigated the possibility that BMP signaling was required for activation of STAT3 by IL-6 using both cultured cells and genetically-modified mice. HepG2 cells were pretreated with and without LDN-193189, an inhibitor of BMP type I receptor signaling, and then were exposed to IL-6 or BMP6. In a previous study, we reported that LDN-193189 could prevent the induction of hepcidin gene expression in HepG2 cells incubated with IL-6.\(^{18}\) In the absence of LDN-193189, incubation of HepG2 cells with IL-6 induced phosphorylation of
induced HO-1 gene expression similarly in both Alk3fl/fl and Alk3fl/fl; Alb-Cre mice (Figure 4D). Taken together, these results indicate that deficiency of Alk2 or Alk3 does not interfere with the ability of IL-6 to induce STAT3 signaling.

Impact of BMP type I receptor deletion on the ability of recombinant mIL-6 to induce hepatic hepcidin gene expression in mice

To confirm our observations in mice treated with Ad.IL-6, we challenged mice with mIL-6 and measured hepatic hepcidin mRNA levels. A time course study revealed that injection of mIL-6 induced hepatic hepcidin mRNA levels in wild-type mice after 4 hours (supplemental Figure 2A). We did not observe a decrease in serum iron levels in mice challenged with mIL-6 4 hours earlier, likely because there was insufficient time for elevated hepatic hepcidin production to reduce ferroportin levels and sequester iron intracellularly (supplemental Figure 2B).

To determine the impact of the BMP type I receptors Alk2 and Alk3 on the ability of recombinant IL-6 to modulate hepatic hepcidin mRNA levels, mice with and without hepatocyte-specific deficiency of Alk2 or Alk3 were challenged with mIL-6. Four hours after injection, blood was withdrawn for measurement of serum iron levels, and livers were harvested for measurement of hepcidin, Alk2, and Alk3 mRNA levels, as well as liver iron content (LIC). Spleens were also harvested for measurement of splenic iron content (SIC). Challenge with mIL-6 induced hepatic hepcidin mRNA levels similarly in Alk2fl/fl and Alk3fl/fl; Alb-Cre mice (Figure 5A). As anticipated, hepatic Alk2 mRNA levels were markedly reduced in Alk2fl/fl; Alb-Cre mice (Figure 5B). Serum iron levels were increased in mice with hepatocyte-specific Alk2 deficiency. Injection with mIL-6 did not change serum iron levels at 4 hours in Alk2fl/fl and Alk3fl/fl; Alb-Cre mice (supplemental Figure 3A). LIC was higher in Alk2fl/fl; Alb-Cre mice than in Alk3fl/fl mice. Injection with mIL-6 did not change LIC after 4 hours in Alk2fl/fl; Alk3fl/fl; Alb-Cre mice (supplemental Figure 3A). SIC did not differ in Alk2fl/fl or Alk3fl/fl; Alb-Cre mice treated with mIL-6 or vehicle (supplemental Figure 3B). Consistent with our findings using Ad.IL-6, these results suggest that the ability of mIL-6 to induce hepatic hepcidin gene expression does not require Alk2.

Figure 3. Inhibition of BMP signaling is independent of STAT3 phosphorylation in HepG2 cells. Hepatocarcinoma cells (HepG2) were treated with and without LDN-193189 (100 nM). Thirty minutes later, cells were stimulated without or with BMP6 (5 ng/mL) or IL-6 (100 ng/mL) for an additional 30 minutes and harvested. Levels of phosphorylated STAT3 (P-STAT3), total STAT3, and GAPDH were determined in injection with Ad.GFP or Ad.IL-6. (B) Hepatic levels of IL-6/STAT3 target gene,15 in the livers of STAT3 by IL-6 in vivo, we measured HO-1 mRNA levels, a known Alk2 fl/fl

Figure 4A-B). To further examine the impact of BMP signaling on activation of STAT3 by IL-6 in vivo, we measured HO-1 mRNA levels, a known IL-6/STAT3 target gene,15 in the livers of Alk2fl/fl, Alb-Cre; Alk3fl/fl, Alb-Cre mice injected with Ad.IL-6 or Ad.GFP. Hepatic HO-1 mRNA levels did not differ in Ad.GFP-injected Alk2fl/fl and Alk2fl/fl; Alb-Cre mice. Prolonged exposure to IL-6 increased HO-1 mRNA levels similarly in both Alk2fl/fl and Alk2fl/fl; Alb-Cre mice (Figure 4C). HO-1 mRNA levels did not differ in Ad.GFP-injected Alk3fl/fl and Alk3fl/fl; Alb-Cre mice, and stimulation with IL-6
Discussion

Studies in animal models and in patients have revealed an important role for IL-6–induced hepcidin gene expression in the pathogenesis of ACD.\textsuperscript{12,27-29} Inhibition of BMP signaling has been shown to reduce hepcidin induction by IL-6\textsuperscript{18,23,24} and to prevent the development of...
However, the BMP type I receptor involved in the induction of hepatic hepcidin gene expression by IL-6 was unknown. We report that hepatocyte-specific Alk2 deficiency does not prevent the ability of IL-6 to decrease serum iron concentrations or increase hepatic hepcidin gene expression. In contrast, hepatocyte-specific Alk3 deficiency markedly impairs the ability of IL-6 to decrease serum iron concentrations and increase hepcidin mRNA levels.

As previously reported, we observed that hepatocyte-specific deficiency of Alk2 and Alk3 in mice led to mild and severe iron overload, respectively. The presence of Alk3 is the primary determinant of basal hepatic hepcidin mRNA levels, as well as serum and hepatic iron concentrations. In contrast, both Alk2 and Alk3 are required for BMP2 and iron to induce hepatic hepcidin gene expression (in primary hepatocytes and in vivo, respectively). In the current report, we used adenovirus-mediated gene transfer to direct hepatic expression of human IL-6 for prolonged periods. We observed that Ad-IL-6 induced hepcidin gene expression and reduced serum iron levels in mice with hepatocyte-specific Alk2 deficiency but not in mice with hepatocyte-specific Alk3 deficiency. These results suggest that the Alk3-dependent mechanisms by which IL-6 induces hepcidin gene expression differ from the Alk2- and Alk3-dependent mechanisms required for regulation of hepcidin gene expression by BMP2 and iron.

It is of note that in Alk3-deficient mice, sustained exposure to IL-6 reduced serum transferrin saturations even though serum iron levels did not change. We observed that total iron binding capacity tended to be greater after Ad-IL-6 injection than after Ad.GFP injection in all 4 mouse genotypes. It seems plausible that the impact of IL-6 on serum transferrin saturation represents the combination of an Alk3-dependent effect on iron metabolism and an Alk3-independent effect on transferrin levels.

Iron and BMP ligands are known to activate BMP-responsive SMAD signaling, whereas IL-6 activates STAT3 signaling. The hepcidin gene promoter contains both STAT3-binding and SMAD-binding sites. We previously reported that induction of hepcidin gene expression by IL-6 in HepG2 cells required BMP signaling. We considered the possibility that BMP signaling is required for STAT3-signaling in response to IL-6. We observed that the ability of IL-6 to induce STAT3 phosphorylation was not altered in HepG2 cells treated with a small molecule inhibitor of BMP type I receptor kinase activity or in mice with hepatocyte-specific Alk2 or Alk3 deficiency. Moreover, induction of HO-1 gene expression by IL-6/STAT3 signaling, an event reported to occur predominantly in hepatocytes, was not altered by deficiency of either Alk2 or Alk3. Taken together, these findings suggest that BMP/Alk3 signaling is required for the ability of IL-6 to induce hepatic hepcidin gene expression, but that reduced BMP signaling does not alter IL-6 signaling via STAT3 phosphorylation.

As a complementary approach that avoided nonspecific effects of adenovirus infection, we measured hepatic hepcidin mRNA levels in mice that received recombinant mIL-6 as a bolus injection. Consistent with our observations using Ad-IL-6, we observed that mIL-6 injection induced hepatic hepcidin gene expression in Alk2-/-, Alk2fl/fl, Alb-Cre, and Alk3-/- mice. In contrast, injection of mIL-6 was insufficient to induce hepatic hepcidin gene expression in Alk3-/-, Alb-Cre mice. These observations confirm that the ability of IL-6 to induce hepatic hepcidin gene expression requires Alk3. The observation that Alk3 is required for the induction of hepcidin gene expression by IL-6 is consistent with the findings of Wang and colleagues, who reported that deficiency of SMAD4 abrogated the ability of IL-6 to induce hepatic hepcidin gene expression. Our findings are also consistent with those of Verga Falzacappa and colleagues, who reported that binding of phosphorylated STAT3 to the hepcidin gene promoter without activation of the BMP-responsive SMAD binding element is insufficient to induce hepcidin gene transcription, and that mutation of the BMP response element decreases the ability of IL-6 to induce hepcidin gene transcription.

Increased hepcidin levels participate in the development of ACD. Inhibition of BMP signaling by small molecules, such as LDN-193189, decreases hepcidin gene expression and prevents the development of ACD in animal models. However, the BMP inhibitors used to treat ACD in animal models can act on multiple BMP type I receptors. It is known that different BMP type I receptors have distinct tissue distribution patterns and different physiological roles. Our current findings suggest that BMP type I receptor-specific inhibitors represent an attractive strategy for the treatment of ACD. The observation that Alk3 plays a critical role in the induction of hepcidin expression by IL-6 suggests that targeting Alk3 selectively might effectively reduce hepcidin gene expression in settings associated with ACD. A therapeutic agent that specifically targets Alk3 might avoid deleterious effects associated with inhibiting Alk1 (eg, reduced endothelial cell survival and angiogenesis) or Alk2 (eg, increased bone mineral density caused by decreased osteoclast activity). In conclusion, we report that induction of hepcidin gene expression by IL-6 requires the presence of the BMP type I receptor, Alk3. Hepatocyte-specific deficiency of Alk3, but not Alk2, markedly impairs the ability of IL-6 to decrease serum iron concentrations. The BMP signaling mechanisms required to induce hepcidin gene expression in response to IL-6 (Alk3-dependent) appear to differ from those required to respond to increased iron levels (dependent on both Alk2 and Alk3). Our findings suggest that Alk3 represents a potential target for the treatment of ACD.

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**Authorship**


Conflict-of-interest disclosure: The Massachusetts General Hospital has filed patents related to the use of small molecule inhibitors of BMP signaling to modulate iron metabolism, and P.B.Y., R.T.P., and...
K.D.B. may be eligible to receive royalties. The remaining authors declare no competing financial interests.

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References


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