Hepcidin Regulation by BMP Signaling in Macrophages Is Lipopolysaccharide Dependent

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation
Wu, Xinggang, Lai-Ming Yung, Wai-Hang Cheng, Paul B. Yu, Jodie L. Babitt, Herbert Y. Lin, and Yin Xia. 2012. Hepcidin regulation by BMP signaling in macrophages is lipopolysaccharide dependent. PLoS ONE 7(9): e44622.

Published Version
doi:10.1371/journal.pone.0044622

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:10498784

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Hepcidin Regulation by BMP Signaling in Macrophages Is Lipopolysaccharide Dependent

Xinggang Wu¹, Lai-Ming Yung⁴, Wai-Hang Cheng¹, Paul B. Yu⁴, Jodie L. Babitt⁵, Herbert Y. Lin⁵, Yin Xia¹,²,³

Introduction

Hepcidin, a small cationic peptide, was first identified based on its antimicrobial and iron release from macrophages. Hepcidin is synthesized mainly in the liver, where hepcidin is regulated by iron loading, inflammation and hypoxia. Recently, we have demonstrated that bone morphogenetic protein (BMP)-hemojuvelin (HJV)-SMAD signaling is central for hepcidin regulation in hepatocytes. Hepcidin is also expressed by macrophages. Studies have shown that hepcidin expression by macrophages increases following bacterial infection, and that hepcidin decreases iron release from macrophages in an autocrine and/or paracrine manner. Although previous studies have shown that lipopolysaccharide (LPS) can induce hepcidin expression in macrophages, whether hepcidin is also regulated by BMPs in macrophages is still unknown. Therefore, we examined the effects of BMP signaling on hepcidin expression in RAW 264.7 and J774 macrophage cell lines, and in primary peritoneal macrophages. We found that BMP4 or BMP6 alone did not have any effect on hepcidin expression in macrophages although they stimulated Smad1/5/8 phosphorylation and Id1 expression. In the presence of LPS, however, BMP4 and BMP6 were able to stimulate hepcidin expression in macrophages, and this stimulation was abolished by the NF-κB inhibitor Ro1069920. These results suggest that hepcidin expression is regulated differently in macrophages than in hepatocytes, and that BMPs regulate hepcidin expression in macrophages in a LPS-NF-κB dependent manner.

Abstract

Hepcidin is an antimicrobial peptide, which also negatively regulates iron in circulation by controlling iron absorption from dietary sources and iron release from macrophages. Hepcidin is synthesized mainly in the liver, where hepcidin is regulated by iron loading, inflammation and hypoxia. Recently, we have demonstrated that bone morphogenetic protein (BMP)-hemojuvelin (HJV)-SMAD signaling is central for hepcidin regulation in hepatocytes. Hepcidin is also expressed by macrophages. Studies have shown that hepcidin expression by macrophages increases following bacterial infection, and that hepcidin decreases iron release from macrophages in an autocrine and/or paracrine manner. Although previous studies have shown that lipopolysaccharide (LPS) can induce hepcidin expression in macrophages, whether hepcidin is also regulated by BMPs in macrophages is still unknown. Therefore, we examined the effects of BMP signaling on hepcidin expression in RAW 264.7 and J774 macrophage cell lines, and in primary peritoneal macrophages. We found that BMP4 or BMP6 alone did not have any effect on hepcidin expression in macrophages although they stimulated Smad1/5/8 phosphorylation and Id1 expression. In the presence of LPS, however, BMP4 and BMP6 were able to stimulate hepcidin expression in macrophages, and this stimulation was abolished by the NF-κB inhibitor Ro1069920. These results suggest that hepcidin expression is regulated differently in macrophages than in hepatocytes, and that BMPs regulate hepcidin expression in macrophages in a LPS-NF-κB dependent manner.

Citation: Wu X, Yung L-M, Cheng W-H, Yu PB, Babitt JL, et al. (2012) Hepcidin Regulation by BMP Signaling in Macrophages Is Lipopolysaccharide Dependent. PLoS ONE 7(9): e44622. doi:10.1371/journal.pone.0044622

Received March 26, 2012; Accepted August 6, 2012; Published September 13, 2012

Copyright: © 2012 Wu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: YX is supported in part by the startup funding offered by the Chinese University of Hong Kong and Hong Kong Research Grant Council (RGC) 2140759. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: Xia.Yin@cuhk.edu.hk
the procedures were performed in accordance with Animal
Smad1/5/8 levels or hepcidin mRNA levels were measured. All
with 0.1% BSA as indicated. Cells were harvested and phospho-
BMP4 or BMP6 and/or LPS in FCS-free DMEM supplemented
supplemented with 1% FCS overnight and then incubated with
macrophage were allowed to attach overnight and fed with fresh
Interestingly, we found that macrophages did not increase hepcidin
expression in response to BMP stimulation, unless BMP signaling
regulation in hepatocytes, we investigated whether or not BMP
signaling regulates hepcidin expression in macrophages. Surpris-
ingly, we found that macrophages did not increase hepcidin
expression in response to BMP stimulation, unless BMP signaling
was accompanied by co-stimulation with LPS.

Materials and Methods

Chemical and biochemical reagents

BMP4 and BMP6 were purchased from R & D Systems
(Minneapolis, MN). Lipopolysaccharides (LPS) from Escherichia
coli 0127:B8 was purchased from Sigma-Aldrich (Saint Louis,
MO). Phospho-Smad1/5/8, Smad1, phospho-NF-kB p65 and
NF-kB p65 antibodies were purchased from Cell Signaling
Technology (Beverly, MA). β-actin antibodies were purchased
from Sigma-Aldrich. NF-kB inhibitor Ro1069920 was purchased
from Santa Cruz Biotechnology, Inc (Santa Cruz, CA).

Cell Culture

RAW264.7 and J774 macrophage cell lines (ATCC) were
incubated in Dulbecco’s modified Eagle’s medium (DMEM)
supplemented with 10% heat-inactivated fetal bovine serum
(S invitrogen), 2 mM L-glutamine, and antibiotics. Cells were
starved with serum-free DMEM supplemented with 0.1% bovine
serum albumin before they were incubated with BMP4, BMP6,
LPS or a combination of BMP ligands and LPS as indicated. Cells
were lysed for real-time PCR quantification of mRNA transcripts
or for Western blotting analyses for phosphorylated Smad1/5/8
and phosphorylated NF-kB p65 levels.

Mature resident peritoneal macrophages were harvested and
cultured as previously described [25]. Briefly, C57Bl/6J mice were
sacrificed by cervical dislocation. Under sterile conditions, midline
incision was made and abdominal skin was retraced gently with
forceps. 10 mL sterile PBS was injected slowly into the abdominal
cavity. Peritoneal fluids were withdrawn slowly. Approximately 6–
8 mL of fluid were recovered from each mouse. Lavage fluids from
several mice were pooled and cells were collected by centrifugation
at 400 g for 10 min. Cell were counted and cultured in DMEM
supplement with 10% FCS at a density of 6 x 10^5 cells/mL. Primary
macrophage were allowed to attach overnight and fed with fresh
medium to culture for another day. Cells were starved in DMEM
supplemented with 1% FCS overnight and then incubated with
BMP4 or BMP6 and/or LPS in FCS-free DMEM supplemented
with 0.1% BSA as indicated. Cells were harvested and phospho-
Smaad1/5/8 levels or hepcidin mRNA levels were measured. All
the procedures were performed in accordance with Animal

Regulation of Hepcidin by BMP in Macrophages

Western blotting

RAW264.7 cells or peritoneal primary macrophages were lysed in
TBS (50 mM Tris-HCl, 150 mM NaCl, and 1% Triton X-100
[pH 7.4]) containing protease inhibitor mixture (Pierce) and
phosphatase inhibitor mixture (Pierce) for 30 min on ice. After
centrifugation for 10 min at 4°C, the supernatant was assayed for
protein concentration by colorimetric assay (BCA kit; Pierce). A
total of 20–40 μg of protein was separated by SDS-PAGE and
transferred to polyvinylidene difluoride membranes. Membranes
were probed with anti-phospho-Smad1/5/8 (1:1000) or anti–
phospho-NF-kB p65 (1:1000). Membranes were stripped in 0.2 M
glycine (pH 2.5) and 0.5% Tween 20 for 10 min, and reprobed
with anti-Smad1 (1:1000), anti-NF-kB p65 (1:1000) or anti-β-actin
(1:10,000) antibodies.

Data analysis

Results from real time PCR analyses are expressed as the means ±
S.D. of three replicates. Differences were assessed by Student’s t
with P<0.05 used to indicate significance.

Results

BMP signaling does not stimulate hepcidin expression in macrophages

It is well documented that BMPs stimulate hepcidin expression
in hepatocytes. To examine whether or not BMP signaling plays a
role in hepcidin expression in macrophages, we incubated
RAW264.7 macrophages with BMP4. Surprisingly, hepcidin
mRNA levels were not stimulated by BMP4 with concentrations
of up to 200 ng/ml (Fig. 1A), while expression of Id1 mRNA, a
well-known target gene of the BMP pathway, was increased by
BMP4 in a dose-dependent manner (Fig. 1B). A time course of 0 to
8 h for BMP4 treatment (50 ng/ml) also demonstrated no change
in hepcidin expression (Fig. 1C), despite an increase in Id1
expression and phosphorylated Smaad1/5/8 levels 1 h after BMP4
treatment (Fig. 1D &1E). Similar results were seen with J774
macrophages (data not shown). In addition, BMP4 did not alter
hepcidin expression in primary peritoneal macrophages isolated

Measurement of Gene Expression

Real-time quantification of mRNA transcripts was performed
using an AB 7600 real-time system (Applied Biosystems). First-
strand cDNA was amplified with previously described mouse
hepcidin and Id1 primers (11), and detected using SYBR® Green
PCR Master Mix (Applied Biosystems) according to the manu-
facturer’s instructions. In parallel, Rpl19 (ribosomal protein-like
19) transcripts were amplified and detected in a similar manner to
serve as an internal control. Standard curves were generated from
accurately determined dilutions of plasmid cDNAs or purified
PCR fragments as templates. Results are expressed as a ratio of
the gene of interest to Rpl19.

To examine the effect of HJV overexpression on hepcidin
expression in RAW264.7 macrophages, cells were transfected with
Flag-HJV cDNA in 12-well plates using Lipofectamine 2000
(Invitrogen). Approximately 24 h after transfection, the media was
replaced with serum-free DMEM medium supplemented with
0.1% BSA. 16 h later, the cells were harvested for measurement of
hepcidin mRNA expression.

PLOS ONE | www.plosone.org 2 September 2012 | Volume 7 | Issue 9 | e44622

[22]; [23]. In contrast to hepatocytes, hepcidin expression in
macrophages is not induced by iron loading in mice [17].
Interestingly, expression of hepcidin in myeloid cells is increased
under stimulation of bacterial pathogens and LPS, and this
stimulation is dependent on TLR4 and NF-κB activities [18]; [22];
[23]. The autocrine and paracrine effects of hepcidin secreted by
monocytic lineages is to decrease iron efflux and promote iron
retention in these cells [19]; [21]. In fact, hepcidin-mediated iron
loading in macrophages and monocytes appears to increase their
inflammatory potential both in vitro and in vivo [24], consistent with
its other functions in promoting host defense. Considering 90–
95% of the serum iron comes from recycling of iron from damaged
erythrocytes by macrophages, the autocrine/paracrine action of
hepcidin in macrophages may be an important mechanism in
innate immune defense by which the host reduces the availability
of iron from pathogens, while simultaneously augmenting the
activity of innate immune effector cells.

Based on the pivotal role of BMP signaling in hepcidin
regulation in hepatocytes, we investigated whether or not BMP
signaling regulates hepcidin expression in macrophages. Surpris-
ingly, we found that macrophages did not increase hepcidin
expression in response to BMP stimulation, unless BMP signaling
was accompanied by co-stimulation with LPS.
from mice, while it induced phosphorylated Smad1/5/8 levels (the left and right panels, Fig. 1F).

BMP6 appears to be a key endogenous BMP ligand for hepcidin expression in the liver [11]; [15]. Therefore, we also examined whether BMP6 could regulate hepcidin expression in macrophages. BMP6 failed to stimulate hepcidin expression in RAW264.7 macrophages (Fig. 2A), while it dramatically increased Id1 expression (Fig. 2B) and stimulated phosphorylated Smad1/5/8 levels (Fig. 2C). Similarly, BMP6 did not have any effect on hepcidin expression in primary peritoneal macrophages (Fig. 2D), while it stimulated Smad1/5/8 phosphorylation (the right panel, Fig. 1F). In addition, BMP6 did not affect hepcidin expression in J774 macrophages either (data not shown).

HJV is a co-receptor for BMP signaling that increases BMP signaling and hepcidin expression in hepatocytes. Our previous study showed that expression of endogenous HJV in RAW264.7 cells is low [26]. Overexpression of HJV did not alter hepcidin expression in RAW264.7 cells (Fig. 3A), although it was sufficient to potentiate Id1 expression (Fig. 3B), suggesting that the lack of a

---

**Figure 1. Effects of BMP4 on hepcidin expression in macrophages.** (A, B, C and D) Effects of BMP4 on hepcidin and Id-1 expression in RAW264.7 macrophages. Cells were incubated overnight with increasing amounts of BMP4 (A & B) or with 50 ng/ml of BMP4 for increasing periods of time (C &D) in serum free DMEM supplemented with 0.1% BSA. Cells were collected for measurements of hepcidin (A & C), Id1 (B & D) and RPL19 mRNA levels. (E) Effects of BMP4 on phosphorylation levels of Smad1/5/8 in RAW264.7 macrophages. Cells were incubated with BMP4 for 1 h before cells were lysed for Western blotting for phospho-Smad1/5/8 and Smad1 levels. (F) Left panel: Effects of BMP4 on hepcidin expression in primary mouse peritoneal macrophages. Cells were incubated for 8 h with increasing amounts of BMP4 before cells were collected for quantification of hepcidin and RPL19 mRNA levels. Right panel: Effects of BMP4 and BMP6 on phosphorylation levels of Smad1/5/8 in primary mouse peritoneal macrophages. Cells were incubated with BMP4 or BMP6 for 1 h before cells were lysed for Western blotting for phospho-Smad1/5/8 and Smad1 levels. **, P<0.01; ***, P<0.001.

doi:10.1371/journal.pone.0044622.g001
response to BMP stimulation was not attributable to decreased expression of this co-receptor in RAW264.7 cells. These results suggest that, unlike hepatocytes, macrophages do not respond to BMP stimulation with an increase in hepcidin expression. This unresponsiveness cannot be attributed to defects in BMP signaling in macrophages because Smad1/5/8 phosphorylation and Id1 expression were induced by treatment with BMP4 or BMP6, and Id1 expression was induced by overexpression of HJV.

BMP4 and BMP6 stimulate hepcidin expression in macrophages in the presence of LPS

Hepcidin in macrophages is stimulated by bacterial pathogens and LPS [18]. Consistent with these observations, LPS significantly increased hepcidin expression in RAW264.7 cells in a time-dependent manner (Fig. 4). To examine whether BMP can influence hepcidin expression in macrophages activated by LPS, we incubated RAW264.7 cells (Fig. 5A & B) or primary peritoneal macrophages (Fig. 5C & D) with varying concentrations of BMP4 or BMP6 in the presence of LPS. In the presence of LPS co-stimulation, BMP4 and BMP6 were able to stimulate hepcidin expression in both the RAW264.7 macrophage cell line and primary peritoneal macrophages. These results suggest that additional intracellular signals are required for BMP regulation of hepcidin expression in macrophages.

The stimulation of hepcidin expression by BMP signaling in the presence of LPS is dependent on the NF-κB pathway

A previous study showed that LPS-induced hepcidin expression is mediated by the NF-κB signaling pathway in human leukocytes [23]. To examine whether the NF-κB is also involved in hepcidin expression induced by LPS and BMP4 in macrophages, we used the NF-κB inhibitor Ro1069920 [27]. As shown in Fig. 6A and 6B, this inhibitor reduced phosphorylation levels of NF-κB p65 induced by LPS, but did not affect phosphorylation levels of Smad1/5/8 induced by BMP4 in RAW264.7 macrophages. While LPS stimulated hepcidin expression more than 3-fold above basal levels (Fig. 6C, bar 2), the addition of BMP4 further increased hepcidin mRNA levels (Fig. 6C, bar 3). Incubation with Ro1069920 reduced hepcidin levels stimulated by LPS alone (Fig. 6C, bar 4) or by BMP4 in combination with LPS (Fig. 6C, bar 5) to basal levels. Similarly, BMP6 in combination of LPS increased hepcidin expression over LPS alone (compare bars 2 and 3, Fig. 6D). In the presence of Ro1069920, hepcidin levels were dramatically inhibited and they were no longer different between cells treated with LPS alone and with BMP6 plus LPS (compare bars 4 and 5, Fig. 6D). Taken together, these results suggest that the potentiation of LPS-induced hepcidin expression by BMP signaling in macrophages is dependent on NF-κB co-signaling.
Discussion

Hepcidin enhances the host immune defense by its antimicrobial and antifungal activities, and by its negative regulation of serum iron levels. Hepcidin is mainly produced by the liver, but it is also expressed in myeloid cells including macrophages and monocytes. Infections stimulate hepcidin expression in both hepatocytes and myeloid cells, leading to an increase in hepcidin concentrations in circulation and in urine. Although the contribution of myeloid cells to the circulating hepcidin relative to that of hepatocytes during infections is difficult to determine, emerging evidence suggests that hepcidin produced from macrophages and monocytes under the stimulation of bacterial pathogens or LPS inhibits the growth of bacteria while inducing iron retention in these cells. Depletion of iron from monocytic lineage impairs LPS-induced expression of inflammatory cytokines including interferon β, whereas iron retention appears to potentiate these cytokine responses [24]. Therefore, the paracrine/autocrine actions of hepcidin may play an important role in the innate immunity mediated by macrophages and monocytes.

A number of differences in the regulatory mechanisms of hepcidin expression have been identified for myeloid cells and hepatocytes. For example, hepcidin expression in macrophages in the spleen is not regulated by serum iron levels, while hepatocytes increase hepcidin expression in response to iron loading. In leukocytes [23] and lymphocytes [28], TNF-α is potent in inducing hepcidin expression. In hepatocytes, however, IL-6 and IL-1 stimulate hepcidin expression while TNF-α does not have such an effect [4]; [6]; [29]; [30]; [31]. In the present study, we have identified another difference between hepatocytes and macrophages, i.e. unlike hepatocytes, macrophages did not increase hepcidin expression in response to BMP signaling under basal conditions. In our experiments, the exposure of RAW264.7 or J774 macrophages to two distinct BMP ligands (BMP4 and BMP6) at varying doses and duration, the transfection of RAW264.7 with HJV, a BMP co-receptor and potent hepcidin inducer in hepatocytes, or the stimulation of primary peritoneal macrophages with BMP4 and BMP6 all failed to induce hepcidin expression.

The mechanism underlying the failure of BMP signaling to stimulate hepcidin expression in macrophages remains unknown. Phosphorylation of Smad1/5/8 and Id1 expression was stimulated by BMP4 or BMP6 in macrophages. This suggests that the BMP signaling pathway is not defective. Whether there are any transcription factors that are important for hepcidin transcription but are not present or not activated in macrophages, and whether there are any epigenetic differences in the hepcidin promoter between hepatocytes and macrophages remain to be addressed. A recent study showed that the binding motif of HNF4α, a liver specific transcription factor, in the hepcidin promoter is critical for the HJV and BMP response of hepcidin in hepatocytes [32]. However, we failed to rescue the BMP response of hepcidin expression by forced expression of HNF4α in RAW264.7 macrophages (data not shown).

STAT3 is a key transcription factor involved in regulation of hepcidin in hepatocytes in inflammatory conditions [30]; [31]. However, in neutrophils or leukocytes, inhibition of STAT3 did not have any effect on LPS-induced hepcidin expression, while inhibition of NF-κB activity abolished the stimulation of hepcidin expression by LPS. Therefore, hepcidin expression induced by LPS in neutrophils or leukocytes depends on the NF-κB pathway [23]. In fact, there is a NF-κB binding site in the proximal region of the mouse hepcidin promoter, and mutation of the NF-κB site completely abolished hepcidin promoter activity in RAW264.7 macrophages.

Figure 3. Effects of HJV on hepcidin expression in RAW264.7 macrophages. Cells were transfected with HJV cDNA (4 ng/ml). 48 hours after transfection, the cells were collected for real time RT-PCR analyses to qualify hepcidin (A) and Id1 (B). *, P<0.05. doi:10.1371/journal.pone.0044622.g003

Figure 4. Effects of LPS on hepcidin expression in RAW264.7 macrophages. Cells were incubated with LPS (10 ng/ml) in serum free DMEM supplemented with 0.1% BSA, and were collected at the indicated times for real time RT-PCR analyses to qualify hepcidin and RPL19 mRNA levels. *, P<0.05; **, P<0.01; ***, P<0.001. doi:10.1371/journal.pone.0044622.g004
cells under the stimulation of *M. tuberculosis*, suggesting a critical role of NF-κB for hepcidin promoter activity [22]. In the present study, we found that, in the presence of LPS co-stimulation, BMP4 stimulated hepcidin expression in RAW264.7 and primary peritoneal macrophages. Interestingly, the potentiation of LPS-induced hepcidin by BMP4 or BMP6 was completely abrogated by inhibition of NF-κB activity in RAW264.7 macrophages. These results suggest that the action of LPS on hepcidin expression is also dependent on NF-κB activity in macrophages, and that NF-κB pathway plays a key role in determining the BMP response of hepcidin expression in macrophages. A previous study showed that inhibition of NF-κB signaling pathway completely blocked the induction of iNOS expression by BMP6 in RAW264.7 cells [33]. These results suggest that the crosstalk between the NF-κB

Figure 5. Effects of BMP4 on hepcidin expression in macrophages in the presence of LPS. (A and B) RAW264.7 cells were incubated with increasing amounts of BMP4 (A) or BMP6 (B) in the presence of LPS (10 ng/ml) for 8 h. Cells were collected for real time RT-PCR analyses to qualify hepcidin and RPL19 mRNA levels. (C and D) Primary mouse peritoneal macrophages were incubated with BMP4 (C) or BMP6 (D) in the presence of LPS (10 ng/ml) for 8 h. Cells were collected for real time RT-PCR analyses to qualify hepcidin and RPL19 mRNA levels. (B) *, P<0.05; **, P<0.01. doi:10.1371/journal.pone.0044622.g005
pathway and the Smad pathway may be important for macrophage functions.

The mechanisms responsible for the effects of BMPs on hepcidin expression in the presence of LPS co-stimulation were not examined in the present study. Previous studies have shown that LPS induces SOCS3 expression, and SOCS3 inhibits hepcidin expression as a negative feedback. Studies have also shown that TGF-β1 inhibits SOCS3 expression [34]. It is tempting to speculate that BMP signaling may also inhibit SOCS3 expression, thus potentiating LPS action in stimulating hepcidin expression.

Augmented BMP signaling via the enhanced expression of BMP ligands, Smad effectors or their downstream activation, has been frequently observed to be a hallmark of infection, inflammation and local tissue injury [33]; [36]; [37]; [38]. Just as it is possible that the activation of pathogen associated molecular pattern (PAMP) signaling pathways via molecules such as LPS might adaptively recruit innate immune responses in synergy with tissue damage signals marked by BMP ligand signaling, it is also possible that the absence of BMP signals may limit inflammation when it is inappropriate. We speculate further that this novel mechanism of cooperative signaling between BMP and LPS in regulating hepcidin in monocytic lineages might be exploited therapeutically to enhance immunity or dampen inflammation via pharmacologic BMP signaling modulation.

Acknowledgments

The authors thank Mr. Yueshui Zhao, Dr. Xiaoling Li, Prof. Hui Zhao, Prof. Chao Wan and Prof. Ya Ke for helpful discussions.

Author Contributions

Conceived and designed the experiments: YX. Performed the experiments: XW LMY WHC. Analyzed the data: XW LMY WHC YX. Wrote the paper: YX XW PBY JLB HYL.

References

1. Krause A, Neitz S, Magert H-J, Schulz A, Forsmann W-G, et al. (2000) LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FERS Letters 460: 147–150. doi:10.1016/S0014-5793(00)01920-7.

2. Park CH, Valore EV, Waring AJ, Ganz T (2001) Hepcidin, a Urinary Antimicrobial Peptide Synthesized in the Liver. Journal of Biological Chemistry 276: 7806–7810. doi:10.1074/jbc.M008922200.
22. Sose FB, Alvarez GR, Gross RP, Satoskar AR, Schlesinger LS, et al. (2009) Role of STAT1, NF-κB, and C/EBPβ in the macrophage transcriptional regulation of hepcidin by mycobacterial infection and IFN-γ. Journal of Leukocyte Biology 86: 1247–1258. doi:10.1189/jlb.1207119.

23. Wu S, Zhang K, Le C, Wang H, Cheng B, et al. (2011) Nuclear factor-κB mediated lipopolysaccharide-induced mRNA expression of hepcidin in human peripheral blood leukocytes. Innate Immun 18: 318–324. Available: http://www.ncbi.nlm.nih.gov/pubmed/21615415. Accessed 12 Dec 6.

24. Wang L, Harrington L, Trebicka E, Shi EY, Kagan JC, et al. (2009) Selective modulation of TLR4-activated inflammatory responses by altered iron homeostasis in mice. J Clin Invest 119: 3322–3328. doi:10.1172/JCI39939.

25. Lee GT, Kwon SJ, Lee JH, Jeon SS, Kang KT, et al. (2010) Induction of interleukin-6 expression by bone morphogenic protein-6 in macrophages requires both SMAD and p38 signaling pathways. J Biol Chem 285: 39404–39408. doi:10.1074/jbc.M110.103705.

26. Xia Y, Cortez-Retamozo V, Niederkofer V, Salie R, Chen S, et al. (2011) Druegen (regulative guidance molecule b) inhibits IL-6 expression in macrophages. J Immunol 186: 1369–1376. doi:10.4049/jimmunol.1002047.

27. Swiney DC, Xu Y-Z, Scarafia LE, Lee I, Mak AY, et al. (2002) A small molecule ubiquitination inhibitor blocks NF-kappa B-dependent cytokine expression in cells and rats. J Biol Chem 277: 23573–23581. doi:10.1074/jbc.M200942200.

28. Pinto JP, Dias V, Zoller H, Porto G, Carmon H, et al. (2010) Hepcidin messenger RNA expression in human lymphocytes. Immunology 130: 217–230. doi:10.1111/j.1365-2567.2009.03226.x.

29. Song S-NJ, Tornosogu N, Kawabata I, Ishikawa T, Nishikawa T, et al. (2010) Down-regulation of hepcidin resulting from long-term treatment with an anti-IL-6 receptor antibody (tocilizumab) improves anemia of inflammation in multicentric Castleman disease. Blood 116: 3627–3634. doi:10.1182/blood-2010-03-271791.

30. Wrighting DM, Andrews NC (2006) Interleukin-6 induces hepcidin expression through STAT3. Blood 106: 3204–3209. doi:10.1182/blood-2006-06-027651.

31. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, et al. (2007) STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood 109: 335–336. doi:10.1182/blood-2006-07-039869.

32. Guo SX, Li J, Peng H, Lee P, Beutler E (2005) Selective interleukin-6 expression by bone morphogenetic protein-6 in macrophages requires both SMAD and p38 signaling pathways. J Biol Chem 280: 39408–39413. doi:10.1074/jbc.M110.103705.

33. Kwon SJ, Lee GT, Lee JH, Kim WJ, Kim IY (2009) Bone morphogenetic proteins 2, 4, and 9 stimulate murine hepcidin 1 expression independently of Hfe, transferrin receptor 2 (Tfr2), and IL-6. Proceedings of the National Academy of Sciences of the United States of America 106: 1906–1910. doi:10.1073/pnas.0900981106.

34. Steinbicker AU, Bartmaks TB, Lohmeyer LK, Leyton P, Faasse SA, et al. (2009) BMP-6 is a key endogenous regulator of hepcidin expression and iron metabolism. Nat Genet 41: 482–487. doi:10.1038/ng.335.

35. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, et al. (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet 41: 478–481. doi:10.1038/ng.320.

36. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, et al. (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet 41: 478–481. doi:10.1038/ng.320.

37. Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, et al. (2008) BMP type I receptor inhibition reduces heterotopic ossification. Nat Med 14: 643–649. doi:10.1038/nm.1766.

38. Fukuda T, Kohda M, Kanomata K, Nojima J, Nakamura A, et al. (2009) Regulation of Distinct Critically Important Bone Morphogenetic Protein-specific Response Elements in the Id1 Promoter. Journal of Biological Chemistry 284: 4893–4891. doi:10.1074/jbc.M110122000.

39. Tsuchiya K, Kwon SJ, Lee J, McFarland M, Hentze MW, et al. (2009) STAT1, NF-κB, and C/EBPβ in the macrophage transcriptional regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. Blood Mol Dis 35: 47–56. doi:10.1016/j.bcmd.2005.04.006.

40. Pesyronaux C, Zinkernagel AS, Datta V, Lahti X, Johnson RS, et al. (2006) TLR3-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood 107: 3727–3732. doi:10.1182/blood-2005-06-2259.