Hepcidin is a liver-made peptide proposed to be a central regulator of intestinal iron absorption and iron recycling by macrophages. In animal models, hepcidin is induced by inflammation and iron loading, but its regulation in humans has not been studied. We report that urinary excretion of hepcidin was greatly increased in patients with iron overload, infections, or inflammatory diseases. Hepcidin excretion correlated well with serum ferritin levels, which are regulated by similar pathologic stimuli. In vitro iron loading of primary human hepatocytes, however, unexpectedly down-regulated hepcidin mRNA, suggesting that in vivo regulation of hepcidin expression by iron stores involves complex indirect effects. Hepcidin mRNA was dramatically induced by interleukin-6 (IL-6) in vitro, but not by IL-1 or tumor necrosis factor α (TNF-α), demonstrating that human hepcidin is a type II acute-phase reactant. The linkage of hepcidin induction to inflammation in humans supports its proposed role as a key mediator of anemia of inflammation. (Blood. 2003;101:2461-2463)

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BLOOD, 1 APRIL 2003 • VOLUME 101, NUMBER 7

2461
at 550 g for 30 minutes in 46% isoosmotic Percoll (Sigma) gradient. Monocyte purity was 80% by Wright stain. Monocytes were cultured at 1 × 10⁶/mL in Iscove modified Dulbecco medium supplemented with 10% fetal bovine serum and 20% autologous serum. The Mo-LPS conditioned medium (CM) was prepared by incubating monocytes with LPS for 4 days and collecting the cell-free supernatant.

RNA isolation and Northern blot analysis

Hepatocyte RNA was prepared using TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (15 µg per lane) was separated on a 1% agarose formaldehyde gel, transferred, and ultraviolet (UV) light–crosslinked. Hepcidin probe (173–base pair [bp]) was generated by polymerase chain reaction (PCR). A 218-bp probe for ferritin heavy chain was generated from hepatocyte RNA with the following primers: forward, 5'-CTGTCGATGTCTTACTTCTTGGACC-3' and reverse, 5'-TCCAAATGTAATGCACACTCC-3'. Hybridization was performed at 42°C in Ultrasylhe (Ambion, Austin, TX) with random-primer, 32P-labeled cDNA probes for hepcidin, ferritin, and glyceraldehyde-3-phosphate dehydrogenase (G3PD).

Results and discussion

Patients with anemia of inflammation, diagnosed by elevated serum ferritin and compatible clinical history, had elevated urinary hepcidin excretion compared with healthy subjects and patients with iron deficiency anemia or well-controlled hereditary hemochromatosis (Figure 1A). Patients with transfusion-induced iron overload (2 sickle-cell anemia, 1 myelodysplasia) identified by compatible clinical history, Fe/TIB2 higher than 50% and ferritin levels higher than 300 ng/mL, also showed greatly increased urinary hepcidin. Urinary hepcidin excretion correlated with serum ferritin levels (R² = 0.69, Figure 1B). In a patient with epididymitis and sepsis (Figure 1C), Western blotting showed very high urinary hepcidin excretion on day 1 (1.5 mg/d), which gradually decreased over a period of days to undetectable levels (day 25) as the infection resolved with treatment. Because of its small size (approximately 2 kDa) and disulfide crosslinking, hepcidin is expected to be filtered into the urine, where it apparently escapes tubular proteolysis and recycling. Unless circulating hepcidin is variably degraded by as yet unknown metabolic pathways, its urinary excretion should closely reflect production rates. This is the first direct evidence that infection and inflammation induce hepcidin production in humans.

We next explored the molecular basis of hepcidin regulation in primary human hepatocytes. These were incubated either in serum-free medium, or with 10% fetal calf serum (FCS), with iron loading or inflammatory stimuli (Figure 2A). Surprisingly, iron loading of hepatocytes resulted in a 50% decrease in hepcidin mRNA. When higher concentrations of iron were used (up to 10 mM, not shown), hepcidin mRNA decreased even more. In contrast, ferritin H expression increased with higher doses of iron (not shown). Since hepcidin expression was induced in mice fed or injected with iron, our data raise the possibility that other iron-sensing cells signal to hepatocytes to induce the production of hepcidin during iron overload. An indirect link between iron and hepcidin induction in hepatocytes is also supported by studies in a mouse model of anemia where hepcidin mRNA was down-regulated in the liver, despite normal or even increased hepatic iron.

Treatment of hepatocytes with Mo-LPS CM increased hepcidin mRNA up to 25-fold, but LPS alone caused only a small (2- to 3-fold) increase (Figure 2B). Monocytes/macrophages exposed to LPS secrete cytokines that mediate acute-phase response. Of the 2 major patterns of acute-phase response in hepatocytes, 11,12 type I response is induced by IL-1–like cytokines (IL-1α, IL-1β, TNF-α, and TNF-β) and increases the production of serum amyloid A, C-reactive protein, and complement C3; whereas type II response is induced by IL-6–like cytokines and results in increased synthesis of fibrinogen, haptoglobin, and α1-antitrypsin. Hepcidin was induced within 8 hours by IL-6 (25-fold) and Mo-LPS CM, but not by IL-1α or TNF-α, indicating that induction of hepcidin is a type II acute-phase response. After 24 hours of cytokine treatment, hepcidin mRNA was also induced by IL-1α, but this was probably an indirect effect of IL-1 via induction of IL-6 synthesis in hepatocytes. Also, addition of IL-1 receptor antagonist to LPS-conditioned monocyte medium did not reduce hepcidin expression, supporting the finding that IL-1 is not an important direct mediator of hepcidin induction.

Ferritin H mRNA showed a weak type I pattern of induction in hepatocytes: IL-1α and TNF-α, but not IL-6, increased ferritin mRNA, and addition of IL-1Ra to Mo-LPS CM decreased ferritin levels.
mRNA. Although measurements of urinary hepcidin and serum ferritin correlate in patients with different iron disorders, and although both are shown to be regulated by iron and inflammation, the respective molecular pathways regulating their expression appear to be distinct.

Hepcidin is thus a type II acute-phase protein that provides a molecular link between inflammation, resulting anemia, and the regulation of iron metabolism. Additional studies will be necessary to explain the mechanism of hepcidin effect on iron transport and the more complex indirect effects that appear to mediate the regulation of hepcidin production by iron stores.

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References