Expression of Rgmc, the murine ortholog of hemojuvelin gene, is modulated by development and inflammation, but not by iron status or erythropoietin

Jan Krijt, Martin Vokurka, Ko-Tung Chang, and Emanuel Nečas

Mutations of hepcidin (HAMP) and hemojuvelin (HJV) genes have been recently demonstrated to result in juvenile hemochromatosis. Expression of HAMP is regulated by iron status or infection, whereas regulation of HJV is yet unknown. Using quantitative real-time polymerase chain reaction, we compared expression of Hamp and Rgmc (the murine ortholog of HJV) in livers of mice treated with iron, erythropoietin, or lipopolysaccharide (LPS), as well as during fetal and postnatal development. Iron overload increased Hamp expression without effect on Rgmc mRNA. Erythropoietin decreased Hamp mRNA, but Rgmc expression was unchanged. Hamp mRNA level decreased after birth by 4 orders of magnitude, without significant changes in Rgmc expression. Administration of LPS elevated Hamp mRNA levels, while markedly decreasing hepatic Rgmc mRNA levels (to ~5% after 6 hours). The responses of Hamp and Rgmc were quite different and suggested that human HJV expression could be modulated by inflammation.

(Blood. 2004;104:4308-4310)

© 2004 by The American Society of Hematology

Study design

All animal experiments were approved by the Animal Care Committee of the First Faculty of Medicine. Male C57BL/6N mice (Charles River, Sulzfeld, Germany) were treated with lipopolysaccharide (LPS, serotype 0111:B4, 1 mg/kg intraperitoneally; Sigma Aldrich, Prague, Czech Republic) and humanely killed by cervical dislocation after 90 minutes or 6 hours. Iron overload (600 mg/kg) was induced by a single subcutaneous injection of iron polysyalminolate (Ferrum Lek; Lek, Ljubljana, Slovenia); mice were humanely killed 1 week or 3 weeks after application. Erythropoietin (EPREX 10 000, Cilag AG, Schaffhausen, Switzerland) was administered at 50 U/mouse for 4 days, and mice were killed on day 5.

Liver RNA was extracted using RNABlue (Top-Bio, Prague, Czech Republic), treated with DNase I (Gibco, Life Technologies, Gaithersburg, MD), and 1 μg total RNA was reverse transcribed by the RevertAid First-Strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania).

Levels of Hamp and Rgmc mRNA were determined by real-time polymerase chain reaction (PCR) on a Roche LightCycler instrument, using LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics, Mannheim, Germany). Primer sequences were: β-actin forward 5′-GAACATGGAGAAGATCTGGCA-3′, reverse 5′-GACATTCTATCGGATGTCACACG-3′; Hamp forward 5′-CTGACGAGCACCACACTATCTC-3′, Hamp reverse 5′-TGCTCTTCTGATGTTCGTCACC-3′; Rgmc forward 5′-GCTACGGAGATACGATCACTGA-3′, Rgmc reverse 5′-CAAGTAATATCCTGTCACGTCG-3′. β-actin mRNA was used as the internal control. Expression was calculated by the 2−ΔΔCt method.

Orthologs of the HJV gene have been identified in zebrafish, mice, and rats; the mouse HJV ortholog Rgmc is, like HJV, expressed mainly in skeletal muscle, heart, and liver.7 The aim of the present study was to examine whether experimental conditions known to influence hepatic Hamp expression in mice will also change hepatic Rgmc mRNA levels and to compare possible similarities or discrepancies in the regulation of these 2 genes.

Introduction

During the past few years, a number of new genes participating in iron metabolism have been identified. Mutations in 2 genes, hepcidin (HAMP)7 and hemojuvelin (HJV)2,3 have shown to result in juvenile hemochromatosis. Hepcidin, a small peptide synthesized predominantly in hepatocytes, is emerging as an important regulator of iron homeostasis, which inhibits iron absorption from the intestine and iron release from macrophages. Hepcidin expression is controlled by iron status and erythropoietic activity, as well as by inflammatory stimuli; inappropriate expression of hepcidin probably plays a role in the pathophysiology of hereditary hemochromatosis and anemia of inflammation.5 On the other hand, the function and regulation of hemojuvelin are at present unknown. Prior to identification of the HJV gene, it was speculated that its product could function in the hepcidin signaling pathway, possibly as a hepcidin receptor,5 whereas a current concept proposes that hemojuvelin could modulate hepcidin expression.2,6

Orthologs of the HJV gene have been identified in zebrafish, mice, and rats; the mouse HJV ortholog Rgmc is, like HJV, expressed mainly in skeletal muscle, heart, and liver.7 The aim of the present study was to examine whether experimental conditions known to influence hepatic Hamp expression in mice will also change hepatic Rgmc mRNA levels and to compare possible similarities or discrepancies in the regulation of these 2 genes.

From the Institute of Pathological Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic.


Supported by grant VZ 111100003 from the Ministry of Education of the Czech Republic. J.K. and M.V. contributed equally to this study.

Reprints: Jan Krijt, Institute of Pathological Physiology, First Faculty of Medicine, Charles University, U nemocnice 5, 128 53 Prague, Czech Republic; e-mail: jki@uf1.cuni.cz.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology
Results and discussion

Real-time PCR allowed detection of Hamp and Rgmc mRNAs in adult as well as in fetal liver samples, with the amount of Hamp mRNA exceeding Rgmc mRNA in adult liver by more than 1 order of magnitude. Tissue-specific expression of Rgmc agreed with published data for human HJV (results not shown).

Hepcidin expression increases during iron overload and decreases following erythropoietin administration. Subcutaneous injection of a single dose of iron (600 mg/kg) increased the amount of Hamp mRNA more than 4-fold when measured 1 week or 3 weeks after treatment; however, the amount of hepatic Rgmc mRNA was not significantly changed (Table 1). Administration of erythropoietin for 4 days decreased Hamp mRNA levels to less than 5% of control values, again without a statistically significant effect on hepatic Rgmc mRNA levels. These results indicate that, in contrast to Hamp mRNA, Rgmc mRNA content is not influenced by iron overload or increased erythropoiesis.

It has been previously shown that HJV is expressed in fetal liver. Because Hamp expression displays significant changes during both prenatal and postnatal periods, we examined whether the expression pattern of Hamp and Rgmc would be similar. Although both Hamp and Rgmc mRNAs increased during embryonic liver development, a striking difference was noted in the postnatal expression of the 2 genes (Figure 1). Hamp mRNA dropped by 4 orders of magnitude after birth and remained low until weaning, whereas Rgmc mRNA levels decreased only to about 30% at postnatal day 3 and reached adult levels at day 8. These results show that the 2 genes are regulated differently during the postnatal period.

In addition to iron homeostasis, expression of hepcidin is also regulated by inflammatory cytokines. Hepcidin was originally described as an antimicrobial peptide, and the link between hepcidin and the immune response has been further strengthened by the observations that urinary hepcidin levels rise by 2 orders of magnitude in patients with infections. Human hepcidin has therefore been characterized as an acute-phase protein, whose induction is probably responsible for the changes in iron homeostasis during anemia of inflammation. Accordingly, an increase of hepatic Hamp mRNA has been documented in experimental animals treated with LPS. As shown in Table 1, a single injection of LPS slightly increased hepatic Hamp mRNA levels, measured 6 hours after LPS administration, while decreasing hepatic Rgmc mRNA levels by more than 1 order of magnitude. Thus, the response of Hamp and Rgmc to inflammatory stimuli appears to be fundamentally different.

The link between iron metabolism and inflammation has been well established, with expression of many of the proteins involved in iron metabolism responding to infection or LPS treatment. LPS treatment decreases plasma iron concentrations and generally down-regulates iron export from the cells. In this respect, it is interesting to note that the response of Rgmc to LPS resembles the response of the Slc40a1 gene, which encodes the iron exporter ferroportin. Both hepatic Rgmc and Slc40a1 mRNAs show a similar decrease following administration of LPS to mice, with only slight changes at 90 minutes and substantial down-regulation 6 hours after LPS administration.

In conclusion, this study shows that, despite the postulated functional link between hepcidin and hemojulvin, murine Hamp and Rgmc genes respond differently to changes in iron status or inflammation. Although the results are based on mRNA quantification only, and as such do not reflect possible posttranscriptional regulation, they nevertheless indicate that whereas Hamp mRNA sensitively reacts to iron overload or increased erythropoiesis, hepatic Rgmc mRNA content is not significantly changed. In addition, hepatic Hamp and Rgmc mRNA levels respond in an opposite manner to bacterial LPS challenge. The decrease of hepatic Rgmc mRNA level following LPS treatment suggests that human HJV expression could be down-regulated during inflammation.

Acknowledgments

The technical assistance of Dana Duricová and Lydie Tauchenová is gratefully acknowledged.

References


4. Nicolas G, Chauvet C, Viattle L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and...
Expression of *Rgmc*, the murine ortholog of hemojuvelin gene, is modulated by development and inflammation, but not by iron status or erythropoietin

Jan Krijt, Martin Vokurka, Ko-Tung Chang and Emanuel Necas