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

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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.

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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 polysodiumtate (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'-GACTGGAGAAGATCTGGCA-3', reverse 5'-GGTCTTTACGGATGT-CACAG-3'; Hamp forward 5'-CTGAGCAGCACCACTATCTC-3'; Hamp reverse 5'-TGGCTCTAGGCTATGTTTGGC-3'; Rgmc forward 5'-CCCA-GATCCCCGTGTGACTATGA-3'; Rgmc reverse 5'-CAGGAAGATTGTCACC-CTCAG-3'. Rgmc primers were designed to amplify a sequence from exons 3 and 4 of Rgmc DNA. Because 5 possible splice variants have been reported for human HJV, data from representative experiments were verified with 2 alternative primer pairs targeting sequences from exon 1 and exon 4, respectively.

Target mRNA amounts were normalized to β-actin mRNA, and calculated as described previously, assuming exact doubling of amplified cDNA in each PCR cycle. Results are expressed as the relative amount of target mRNA in comparison to β-actin mRNA (Figure 1), or as percent of β-actin–normalized target mRNA in experimental groups versus control groups (Table 1). Statistical analyses were performed using the 2-tailed t test.
Results and discussion

Real-time PCR allowed detection of Hamp and Rgmc mRNAs in adults 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 data2 for human HJV (results not shown).

Hepcidin expression increases during iron overload9 and decreases following erythropoietin administration.10 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.2 Because Hamp expression displays significant changes during both prenatal and postnatal periods,11 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.12,13 Hepcidin was originally described as an antimicrobial peptide,14 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.2,12 Human hepcidin has therefore been characterized as an acute-phase protein,12 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.9,13,15 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.16-18 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,18,19 which encodes the iron exporter ferroportin1. 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 hemojuvelin,2,20 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.

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References

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