Interleukin-18–deficient mice develop dyslipidemia resulting in nonalcoholic fatty liver disease and steatohepatitis

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We investigated potential pathophysiological relationships between interleukin 18 (IL-18) and dyslipidemia, nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). Compared with *Il18*$^{+/+}$ mice, IL-18 knockout (*Il18*$^{-/-}$) mice developed hypercholesterolemia and hyper-high-density-lipoprotein-cholesterolemia as well as hypertriglyceridemia as they aged, and these disorders occurred before the manifestation of obesity and might cause secondary NASH. The analyses of molecular mechanisms involved in the onset of dyslipidemia, NAFLD, and NASH in *Il18*$^{-/-}$ mice identified a number of genes associated with these metabolic diseases. In addition, molecules related to circadian rhythm might affect these extracted genes. The intravenous administration of recombinant IL-18 significantly improved dyslipidemia, inhibited the body weight gain of *Il18*$^{+/+}$ mice, and prevented the onset of NASH. The expression of genes related to these dysfunctions was also affected by recombinant IL-18 administration. In conclusion, this study demonstrated the critical function of IL-18 in lipid metabolism and these findings might contribute to the progress of novel treatments for NAFLD or NASH. (Translational Research 2016;173:101–114)

Abbreviations: *Apoa4* = apolipoprotein A-IV; BMAL1 = brain and muscle Arnt-like protein; *Camk2b* = calcium/calmodulin-dependent protein kinase II beta; *Ccnd1* = cyclin D1; CLOCK = circadian locomotor output cycles kaput; *G6pc* = glucose-6-phosphatase, catalytic; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; *Gnrh1* = gonadotropin-releasing hormone 1; HDL = high-density-lipoprotein; H-cho = HDL cholesterol; *Htr2b* = 5-hydroxytryptamine (serotonin) receptor 2B; IL-18 = interleukin 18; *Il18* = interleukin 18; *Il18*$^{-/-}$ = IL-18 knockout; IPA = Ingenuity Pathway Analysis; *Mfsd2a* = major facilitator superfamily domain containing 2A; NAFLD = nonalcoholic fatty liver disease; NAS = NAFLD activity score; NASH = nonalcoholic steatohepatitis; PER2 = period circadian clock 2; qRT-PCR = quantitative reverse transcription polymerase chain reaction; T-cho = total cholesterol; TG = triglyceride; rIL-18 = recombinant IL-18;

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Sorl1 = sortilin-related receptor, L(DLR class) A repeats-containing; *Steap2* = six transmembrane epithelial antigen of prostate 2; *Syvn1* = synovial apoptosis inhibitor 1, synoviolin; *Usp2* = ubiquitin-specific peptidase 2

AT A GLANCE COMMENTARY

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Background

Interleukin-18 (IL-18) was identified as an interferon-γ–inducing proinflammatory factor. However, we examined IL-18 from a different perspective and added novel information regarding the physiological functions of IL-18.

Translational Significance

Interleukin-18–deficient ($Il18^{-/-}$) mice showed IL-18 was causally associated with dyslipidemia resulting in nonalcoholic fatty liver diseases and steatohepatitis. In addition, the administration of recombinant IL-18 dramatically improved dyslipidemia, inhibited body weight gain in wild-type mice, and prevented the progression of nonalcoholic steatohepatitis in $Il18^{-/-}$ mice. Therefore, our study suggests that IL-18 might contribute to a novel treatment option to correct the energy unbalance and suppress nonalcoholic fatty liver disease and nonalcoholic steatohepatitis.

INTRODUCTION

The prevalence of obesity and/or dyslipidemia is increasing dramatically, resulting in the elevated morbidity of metabolic syndromes.[1] Obesity is closely related to changes in the serum concentration of important lipid biomarkers, as well as dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and the development of nonalcoholic steatohepatitis (NASH).[1-3] Despite a large number of patients experiencing these conditions, the contributing factors to dyslipidemia, NAFLD, or NASH, and their effective treatment remain to be elucidated.

The cytokine interleukin-18 (IL-18) was originally identified as an interferon-γ–inducing proinflammatory factor; however, there is increasing evidence to support its nonimmunologic effects on physiological functions.[4,5] IL-18 is produced as an inactive 24-kDa precursor and is processed by inflammasomes to an active 18-kDa mature form.[6-9] Previous studies reported that mice deficient for IL-18 developed hyperphagia, obesity, and insulin resistance.[10] Moreover, IL-18–deficient mice with NASH caused by a methionine-choline–deficient diet presented with a higher severity of NASH compared with wild-type mice.[11] In human studies, the serum concentration of IL-18 was significantly higher in patients with type II diabetes mellitus and with metabolic syndrome than in healthy controls.[12,13]

A previous study demonstrated that IL-18-knockout ($Il18^{-/-}$) mice had a remarkably increased body weight accompanying dyslipidemia[10]; however, this pathophysiological mechanism and relationship between dyslipidemia, obesity, NAFLD, and NASH remains uncertain. Therefore, this study investigated whether IL-18 promoted the efficient use of energy through lipid metabolism. Furthermore, we examined whether IL-18–deficient mice developed dyslipidemia in youth, obesity and steatosis in the liver during their growth phase, and finally developed NAFLD and NASH. To verify these hypotheses, we analyzed the role of IL-18 on lipid metabolism in the liver by determining the existence of obesity and steatosis in the liver by histopathologic observation and by measuring the serum concentration of several lipids and deviation of liver enzymes in $Il18^{-/-}$ mice during growth. Furthermore, we analyzed the molecular mechanisms affected during these disease processes, assessed the lipid-normalizing influence of recombinant IL-18 (rIL-18) by short- and long-term administration, and evaluated the normalization of body weight of $Il18^{-/-}$ mice to prevent obesity, NAFLD, and NASH.

MATERIALS AND METHODS

Animals. $Il18^{-/-}$ male mice were generated on the C57Bl/6 mice background as previously described.[14] Littermate C57Bl/6 $Il18^{+/+}$ male mice were used as controls. They were housed in groups of 3–5 in polycarbonate cages placed in a colony room maintained at a constant temperature (22 ± 1°C) and humidity (50%–60%), under a 12-h light/dark cycle (lights on at 8 AM) with free access to standard food (MF; Oriental Yeast Co, Ltd, Tokyo, Japan) and water. All mice were sacrificed at 10 AM in the morning. Animal experiments were conducted according to the "Guide for Care and Use of Laboratory Animals'" published by the National Institutes of Health and approved by the by the Animal Care Committee of Hyogo College of Medicine (#28041 and #14-020). $Il18^{+/+}$ and $Il18^{-/-}$ mice were used, and the body weight of each was measured at the following time points: ages 6, 12, 24, 36, and 48 weeks with n = 6–30. From a separate experiment, samples for