Deficiency of Interleukin-1 Receptor Antagonist Deteriorates Fatty Liver and Cholesterol Metabolism in Hypercholesterolemic Mice*

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Kiikou Isoda‡§¶, Shojiro Sawada‡§¶, Makoto Ayaori‡, Taizo Matsuki‡, Reiko Horai‡, Yutaka Kagaat*, Koji Miyazaki‡, Masatoshi Kusuhara‡, Mitsuyo Okazaki‡‡, Osamu Matsubara**, Yoichiro Iwakura†, and Fumitaka Ohsuzu‡

From ‡Internal Medicine I and the **Second Department of Pathology, National Defense Medical College, Tokorozawa 359-8513, the †Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, and the ‡‡Laboratory of Chemistry, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Chiba 272-0827, Japan

Although the anti-inflammatory effect of interleukin-1 (IL-1) receptor antagonist (IL-1Ra) has been described, the contribution of this cytokine to cholesterol metabolism remains unclear. Our aim was to ascertain whether deficiency of IL-1Ra deteriorates cholesterol metabolism upon consumption of an atherogenic diet. IL-1Ra-deficient mice (IL-1Ra−/−) showed severe fatty liver and portal fibrosis containing many inflammatory cells following 20 weeks of an atherogenic diet when compared with wild type (WT) mice. Expectedly, the levels of total cholesterol in IL-1Ra−/− mice were significantly increased, and the start of lipid accumulation in liver was observed earlier when compared with WT mice. Real-time PCR analysis revealed that IL-1Ra−/− mice failed to induce mRNA expression of cholesterol 7α-hydroxylase, which is the rate-limiting enzyme in bile acid synthesis, with concurrent up-regulation of small heterodimer partner 1 mRNA expression. Indeed, IL-1Ra−/− mice showed markedly decreased bile acid excretion, which is elevated in WT mice to maintain cholesterol level under atherogenic diet feeding. Therefore, we conclude that the lack of IL-1Ra deteriorates cholesterol homeostasis under atherogenic diet-induced inflammation.

When compared with other organs, the liver has one of the largest populations of macrophages, which are key components of the innate immune system. Resident hepatic macrophages, i.e. Kupffer cells, are derived from circulating monocytes that arise from bone marrow progenitors. Once localized within the liver, these cells differentiate to perform specialized functions, including phagocytosis. Kupffer cells also generate various products, including cytokines. These factors regulate not only the phenotypes of the Kupffer cells that produce them but also the phenotypes of neighboring cells, such as hepatocytes (1). Many recent studies suggest that several proinflammatory cytokines produced by activated Kupffer cells might be involved in the onset of liver disease, including alcoholic and nonalcoholic fatty liver disease (NAFLD) (2–4). For instance, elevated circulating levels of tumor necrosis factor-α, IL-1β, and IL-6 have been observed in human patients (5) and animal models of both NAFLD (4) and alcohol-induced liver injury (6).

In contrast to proinflammatory cytokines, anti-inflammatory cytokines are considered to have hepatoprotective effects (5). IL-1 receptor antagonist (IL-1Ra) is one of the negative regulators to IL-1 signaling by binding and blocking the functional receptor (IL-1 receptor type-I) without activation (7). IL-1Ra plays an anti-inflammatory role in acute and chronic inflammation (8). IL-1Ra is also produced by hepatocytes as well as macrophages/monocytes as an acute phase protein in vivo (9). Furthermore, we recently reported that IL-1Ra-deficient (IL-1Ra−/−) mice showed decreased weight gain when consuming the same amount of food as wild-type mice and that body lipid accumulation remained impaired even when they were fed a high fat diet (10). However, the function of IL-1Ra in NAFLD is still not yet well understood.

Atherogenic diet has been widely used to study atherogenesis in animal models. Early atherogenic diets contained high concentrations of cholesterol (5%) and fat (30%) either supplemented with cholic acid (2%) (11) or fed in combination with irradiation treatments (12). These early diets produced high mortality and were subsequently modified to reduce the concentrations of cholesterol (1.25%), fat (15%), and cholate (0.5%) (13). Although this modified atherogenic diet produces an atherogenic lipoprotein profile and fatty streak lesions (14), it also induces inflammatory gene expression in the liver (15). Furthermore, it has been reported that a fat-enriched diet induced NAFLD in animal models (4).

It is now well recognized that inflammation or cytokines increase serum lipid levels (16, 17). This increase in serum lipid levels can be considered part of the acute phase response that results in marked changes in the levels of a large number of circulating protein primarily due to alterations in the liver (18). However, the role of IL-1Ra on lipid metabolism remains poorly understood.

To address the question more directly whether deficiency of IL-1RAs promotes development of NAFLD and changes lipid metabolism, we employed IL-1Ra−/− mice we had previously gen...

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¶ Both authors contributed equally to this work.

* To whom correspondence should be addressed: Internal Medicine I, National Defense Medical College, 5-2, Namiki, Tokorozawa, Saitama, 359-8513, Japan. Tel.: 81-42-995-1597; Fax: 81-42-996-5200; E-mail: isoda@me.ndmc.ac.jp.

The abbreviations used are: NAFLD, nonalcoholic fatty liver disease; ACAT2, Acyl-CoA cholesterol acyltransferase 2; ALT, alanine aminotransferase; Ct, threshold cycle number; CYP7A1, cholesterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; IL-1, interleukin-1; IL-1Ra, IL-1 receptor antagonist; IL-1Ra−/−, IL-1Ra-deficient; LXR-1, liver receptor homolog-1; SHP, small heterodimer partner 1; SR-BI, scavenger receptor class B type I; WT, wild type; HPLC, high pressure liquid chromatography; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; HMG, human menopausal gonadotropin; JNK, c-Jun N-terminal kinase.

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Experimental Procedures

Animals and Experimental Procedure—The creation of IL-1Ra−/− mice used in this study has been described previously (19). In these mutant mice, the genes for all four isoforms of the IL-1Ra were disrupted. These mice were backcrossed to C57BL/6J strain mice for eight generations. IL-1Ra−/− mice and corresponding WT mice, the genes for all four isoforms of the IL-1Ra were disrupted. These mice were switched to a high fat/cholesterol and cholate diet containing 15% total fat (8% cocoa butter), 1.25% cholesterol, and 0.5% sodium cholate (Clea Japan, Akita, Japan) according to the procedure as described by Usui et al. (21).

Liver Size and Serum Transaminase—Liver size was determined by morphological and histological studies (data not shown). We next tested the effect of an atherogenic diet (chow supplemented with 15% fat, 1.25% cholesterol, and 0.5% sodium cholate). In contrast to little changes in WT mice, there

Table I

| PCR primers | Forward | Reverse |
|-------------|---------|---------|
| ACAT2       | 5′-GCCCGAGTTCTACAAAGCAG-3′ | 5′-GTGCTGAGTTGCTGAGAATG-3′ |
| HMG-CoA reductase | 5′-TTCTTACGGCTGAGACATAC-3′ | 5′-GTTCTCAGCTTACGGAGCT-3′ |
| 18S rRNA    | 5′-AGCTAGGAGTTGGAAGCTAC-3′ | 5′-CCGGCTCAGGAAAACACTG-3′ |
| CYP7A1      | 5′-GGTTGCTCCAGAGAAGCTCAAG-3′ | 5′-CCTACCATCTTCTCCACAGAT-3′ |
| LXRα        | 5′-GTCATCGAGGCACGCTATG-3′ | 5′-CAGACTCACACTGTGACCTG-3′ |
| SREBP2      | 5′-CGCTGGAGCTCCAGGACTG-3′ | 5′-TCACTGGTCTGAGGTGACA-3′ |
| LDL receptor | 5′-AGCTTGTGAGTGGAGAACC-3′ | 5′-ACCGCCAGCTCCAAGAGTCA-3′ |
| SHP         | 5′-ATTCCTCCCGAGAAGCTCAAG-3′ | 5′-GGACCAAGATGCAAGTTC-3′ |
| FXR         | 5′-GCCTGTTCTCTCTAATCACA-3′ | 5′-GCAAGTCTGATCCTGAGG-3′ |
| FXR         | 5′-CTTTACCTTCATCCGCTCTGAGA-3′ | 5′-CATATGTGTGTTGCAGCCTCT-3′ |
| ABCA1       | 5′-CAGAGGCAAAAAGCGACTC-3′ | 5′-GAGTCTGATGAGGTCTCC-3′ |
| SR-B1       | 5′-TTCCAGGAGCTCCAGAA-3′ | 5′-AGGTTCTCCAGAGCCATG-3′ |
| IL-1Ra      | 5′-CTTATCTCTTCATCCTGAGA-3′ | 5′-GCCAGTGTGACCGTGAACATGA-3′ |
| IL-1β       | 5′-GAGTCTGATGAGGTCTCC-3′ | 5′-GCCAGTGTGACCGTGAACATGA-3′ |
| CD68        | 5′-GCCAGTGTGACCGTGAACATGA-3′ | 5′-GCCAGTGTGACCGTGAACATGA-3′ |
| TGF β       | 5′-AGCTGGTCTGAGGTGACA-3′ | 5′-GCCAGTGTGACCGTGAACATGA-3′ |

Analysis of Fecal Bile Acid—Stools were collected from each animal over 72 h immediately prior to study, dried, weighed, and ground in a mechanical blender. Aliquots were taken for the measurement of total bile acid content by an enzymatic method as described (24). The daily stool output (g/day/100 g of body weight) and fecal bile acid content (µmol/g of stool) were used to calculate the rate of bile acid excretion (µmol/day/100 g of body weight).

Tissue Preparation and Histology—Livers were harvested, fixed overnight in 4% paraformaldehyde for knock-out, were studied at different times. Mice were maintained on a 12-h light/12-h dark cycle and fed a normal rodent diet containing 4.6% crude fat with less than 0.02% cholesterol (Clea Japan, Inc, Tokyo, Japan). Eight-week-old mice were switched to a high fat/cholesterol and cholate diet containing 15% total fat (8% cocoa butter), 1.25% cholesterol, and 0.5% sodium cholate (Clea Japan), hereafter referred to as the atherogenic diet. IL-1Ra−/− mice and WT mice were characterized immediately before and after consumption of the atherogenic diet for 1, 4, 8, and 20 weeks. The studies were carried out according to the protocols approved by the National Defense Medical College Board for Studies in Experimental Animals.

Chemical Analysis of Serum and Tissue—On the day of analysis, food was removed from the cages in the morning, and the mice were fasted for 7 h. Blood was drawn from mice by cardiac puncture under light methoxyflurane anesthesia. Animals were sacrificed by cervical dislocation, and livers were immediately collected and weighed, and tissue samples were divided; some were fixed in 4% paraformaldehyde, and others were frozen in liquid nitrogen and stored at −80 °C. Blood was transferred into tubes, and serum was collected by centrifugation. Serum alanine aminotransferase (ALT) determination was performed using a commercially available assay kit according to the manufacturer’s instructions (Sigma diagnostic kit; Sigma). Plasma analysis of albumin and bilirubin levels was performed using a Paramax RX automated analyzer (Dade International). The plasma total cholesterol, HDL cholesterol, and triglyceride levels were measured by enzymatic assays as described previously (20). Furthermore, plasma lipoproteins were analyzed by an on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides by HPLC at Skylight Biotech Inc. (Akita, Japan) according to the procedure as described by Usui et al. (21). 200 µl of 20× saline-diluted sera was injected into two tandem connected TSK gel LipropakXL columns (300 × 7.8-mm; Tosoh), and cholesterol and triglyceride contents in lipoproteins separated by size were determined by using enzymatic reagents specially prepared by Kyowa Medex (Tokyo, Japan). Total cholesterol and triglyceride concentrations (in mg/dL) were calculated using cholesterol and triglyceride standards prepared by the methods of Folch et al. (23). The extract was dissolved in 2-propanol and subsequently analyzed for total cholesterol, free cholesterol, and triglycerides using a commercially available reagent (TC kit, FC kit, and TG kit, Wako, Japan).

RESULTS

Liver Size and Serum Transaminase—Analysis of livers of IL-1Ra−/− mice fed a standard laboratory rodent diet indicated that these mice appeared identical to those of WT mice as determined by morphological and histological studies (data not shown). We next tested the effect of an atherogenic diet (chow supplemented with 15% fat, 1.25% cholesterol, and 0.5% sodium cholate). In contrast to little changes in WT mice, there

erated (19). The present study aimed to definitively test the hypothesis that deficiency of IL-1Ra promotes NAFLD and alters lipid metabolism employing IL-1Ra−/− and wild type (WT) mice on atherogenic diet.
were dramatic morphological changes in the livers of IL-1Ra\(^{-/-}\) mice fed the same diet (Fig. 1). Following 20 weeks of atherogenic diet, there was a prominent color and size change in the livers of IL-1Ra\(^{-/-}\) mice versus WT mice (Fig. 1a). Four weeks after the start of the atherogenic diet, the liver weight to body weight ratios in the IL-1Ra\(^{-/-}\) mice tended to become larger (but not significant) than those of WT (8.6 \pm 0.9\%, n = 5 versus 6.0 \pm 0.6\%, n = 5; p = NS; Fig. 1b). Following 8 weeks of the atherogenic diet, this parameter increased by 166\% in IL-1Ra\(^{-/-}\) mice when compared with WT mice (15.7 \pm 2.6\%, n = 5 versus 5.9 \pm 0.5\%, n = 5; p < 0.05). Following 20 weeks of the atherogenic diet, the parameter increased 3-fold when compared with before atherogenic diet feeding in IL-1Ra\(^{-/-}\) mice; however, the atherogenic diet caused only a small increase in liver weight to body weight ratios in WT mice (17.6 \pm 2.4\%, n = 5 versus 6.7 \pm 0.6\%, n = 5; p < 0.01). In conclusion, continued atherogenic diet caused significantly greater enlargement of livers in IL-1Ra\(^{-/-}\) mice than in WT mice.

As markers of liver injury, serum ALT, bilirubin, and albumin were monitored throughout the study. Before atherogenic diet, the serum level of ALT in both IL-1Ra\(^{-/-}\) and WT mice was 40 units/liters. Following 20 weeks of atherogenic diet, the serum levels of ALT and bilirubin increased by 93 and 875\%, respectively, in IL-1Ra\(^{-/-}\) mice when compared with WT mice (Fig. 1c). In contrast, the serum level of albumin decreased by 28\% when compared with WT mice (Fig. 1c). We detected moderate ascites in all IL-1Ra\(^{-/-}\) mice but not in WT mice 20 weeks after the start of atherogenic diet. These data suggest that the atherogenic diet induces a more severe liver injury in IL-1Ra\(^{-/-}\) mice than in WT mice.

**Histopathological Changes**—Histological examination of livers from both mice demonstrated a time-dependent increase of the number and size of intracellular vacuoles, characteristics of lipid deposits (Fig. 2). These changes, however, were prominent in IL-1Ra\(^{-/-}\) but not WT livers. Furthermore, following 20 weeks of the atherogenic diet, the morphology of the IL-1Ra\(^{-/-}\) livers had been substantially altered, including extensive portal fibrosis and collagen deposition in a pericellular distribution in the lobule (Fig. 2b). Moreover, both lobular and portal inflammation was detected in IL-1Ra\(^{-/-}\) liver (Fig. 2b).

Oil red O staining of livers from both types of mice revealed that the deposition of neutral lipid increased in a time-dependent manner (Fig. 3). However, the start of lipid deposition in IL-1Ra\(^{-/-}\) livers occurred earlier when compared with the WT livers. Indeed, we could detect lipid deposits in IL-1Ra\(^{-/-}\) liver after 1 week of atherogenic diet, with no deposits in WT liver. Furthermore, as expected from the gross morphology, neutral

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**Fig. 1. Effects of atherogenic diet on liver morphology, liver size, and serum ALT, bilirubin, and albumin.** a. Macroscopic appearance of livers from IL-1Ra\(^{-/-}\) (Ra\(^{-/-}\)) and WT mice fed an atherogenic diet for 20 weeks. b. Liver mass relative to the total body mass of IL-1Ra\(^{-/-}\) and WT mice fed an atherogenic diet for 0, 1, 4, 8, or 20 weeks. *, p < 0.05; **, p < 0.01 for IL-1Ra\(^{-/-}\) mice versus WT mice. c. The upper panel shows the serum ALT levels of IL-1Ra\(^{-/-}\) and WT mice fed chow supplemented with the atherogenic diet for 0, 1, 4, 8, or 20 weeks. The middle panel shows serum bilirubin level, and the lower panel shows serum albumin level. The measurements of all these markers were performed on plasma pooled from groups of five male mice.
lipid accumulation was markedly more pronounced in the IL-1Ra−/− than the WT liver following 20 weeks of atherogenic diet.

**Alteration of Lipid Homeostasis in IL-1Ra−/− Mice**—Following 4 weeks of the atherogenic diet, levels of total plasma cholesterol did not differ between IL-1Ra−/− and WT mice (224.9 ± 12.4 mg/dl versus 207.1 ± 20.5 mg/dl; n = 5; p = NS; Fig. 4a). However, after 8 weeks of the atherogenic diet, the levels of total cholesterol in IL-1Ra−/− mice increased by 198% when compared with WT mice (610 ± 224 mg/dl, versus 205 ± 12 mg/dl; n = 5; p < 0.05). After 20 weeks of atherogenic diet, moreover, the total cholesterol levels increased significantly in IL-1Ra−/− mice when compared with WT mice (942 ± 160 mg/dl versus 240 ± 13 mg/dl, n = 5; p < 0.01).

High resolution HPLC analysis of plasma lipoproteins following 4 weeks of the atherogenic diet revealed no significant differences in the levels of chylomicron-, VLDL-, LDL-, and HDL-cholesterol between the IL-1Ra−/− and WT mice (Fig. 4, b and c). However, after 20 weeks of atherogenic diet, HPLC analysis revealed markedly increased cholesterol levels in the VLDL and LDL fractions in the IL-1Ra−/− mice when compared with the WT mice. The cholesterol levels in the VLDL and LDL fractions were 699 ± 128 mg/dl (n = 5, p < 0.01) and 192 ± 36 mg/dl (n = 5, p < 0.01), respectively, in IL-1Ra−/− mice when compared with 136 ± 7 mg/dl (n = 5) and 50 ± 4 mg/dl (n = 5), respectively, in the WT mice. In contrast, levels of HDL-cholesterol were significantly lower in IL-1Ra−/− mice (31 ± 10 mg/dl, n = 5) when compared with those in WT mice (54 ± 3 mg/dl, n = 5; p < 0.05; Fig. 4, b and c).

The hepatic cholesterol levels of both mice showed a time-dependent increase (Fig. 5). However, these changes were more prominent in the liver of IL-1Ra−/− than in WT mice, and the difference between these mice increased with prolonged diet. In agreement with the enlarged liver and distinct color change, hepatic cholesterol was significantly increased in IL-1Ra−/− mice after 20 weeks of the atherogenic diet regimen. No significant differences were observed for hepatic triglyceride levels between the IL-1Ra−/− and WT mice. The major component of hepatic cholesterol in both genotypes was cholesterol ester.

**Alteration of mRNA Expressions in IL-1Ra−/− Mice**—Analyses of mRNA expression of genes involved in cytokine, monocyte activation, and the regulation of cholesterol, fatty acid, and bile acid metabolism were performed to identify pathways potentially responsible for the observed alterations in IL-1Ra−/− mice. Real-time PCR employed mRNA extracted from livers of both mice (Fig. 7). Prior to the start of the atherogenic diet (0 week), expression levels of cholesterol 7α-hydroxylase (CYP7A1) in IL-1Ra−/− mice were 30% lower than those in WT mice (p < 0.05; Fig. 7a). After 1 and 4 weeks of atherogenic diet, CYP7A1 expression decreased in both mice; however, transcript levels in IL-1Ra−/− mice were markedly lower (or absent). These results suggest that bile acid biosynthesis in IL-1Ra−/− mice might be attenuated as early as 1 week after the start of the atherogenic diet regimen. Although a previous study showed that lipopolysaccharide suppresses both CYP7A1 and sterol 27-hydroxylase (CYP27A1) (25), expression levels of CYP27A1 in both IL-1Ra−/− and WT mice decreased after 1 week of the atherogenic diet, and there was no significant difference between these two groups (Fig. 7c). Although atherogenic diet increased small heterodimer partner 1 (SHP) mRNA in both mice, expression was 1.8-fold higher (p < 0.01) after 1 week and 3-fold higher (p < 0.001) after 4 weeks of the atherogenic diet in IL-1Ra−/− when compared with WT mice (Fig. 7b).

Furthermore, expression of liver receptor homolog-1 (LRH-1) increased in both mice after 4 weeks of atherogenic diet; however, a 1.8-fold higher (p < 0.05) increase was observed in IL-1Ra−/− mice when compared with WT mice (Fig. 7c). Moreover, after 1 week of the atherogenic diet, mRNA expression of Acyl-CoA cholesterol acyltransferase 2 (ACAT2) was only mar-
originally if at all increased in the IL-1Ra−/− mice, whereas a 2-fold increased level was observed after 4 weeks of the atherogenic diet (*p < 0.05; Fig. 7a). In contrast, no differences were observed in hepatic expression of LDL receptor, HMG-CoA reductase, SREBP2, nuclear liver X-receptor, and farnesoid X-receptor between IL-1Ra−/− and WT mice. Furthermore, hepatic expression of the HDL-cholesterol regulatory genes ATP binding cassette-A1 (ABCA1) and scavenger receptor class B type I (SR-BI) did not differ between IL-1Ra−/− and WT mice.

IL-1Ra, as expected, was absent in IL-1Ra−/− mice, whereas the expression level of IL-1β in IL-1Ra−/− mice was significantly elevated even before atherogenic diet (p < 0.05) and more than 10-fold (p < 0.001) up-regulated following 4 weeks of the atherogenic diet (Fig. 7d). Moreover, 4 weeks of atherogenic diet increased transforming growth factor-β (4.5-fold, p < 0.01) and CD68 (7-fold, p < 0.01) in the IL-1Ra−/− mice when compared with WT mice.

**DISCUSSION**

The present study demonstrates that IL-1Ra−/− mice develop severe NAFLD and portal fibrosis with many inflammatory cells following 20 weeks of the atherogenic diet when compared with WT mice. We also found that mRNA levels of IL-1β and transforming growth factor-β were significantly elevated in livers of IL-1Ra−/− mice. These findings are in accord with several previous studies implicating cytokine imbalances in murine models of NAFLD (4) and fibrosis (26, 27). These findings suggest that cytokines, such as IL-1β, may play an important role in the pathogenesis of NAFLD and hepatic fibrosis. In our IL-1Ra−/− mice, excessive IL-1 signaling may induce inflammation in the liver and, thus, the IL-1 system might play a role in the development of NAFLD and fibrosis. The atherogenic diet we used in this study appears associated with these dramatic changes in the livers of IL-1Ra−/− mice, corroborating a recent study showing that high cholesterol levels induce expression of several genes.
Levels of cholesterol (ester). Levels of cholesterol (upper panel), triglycerides (middle panel), and cholesterol ester (bottom panel) content in hepatic lipid extracts of IL-1Ra−/− (Ra−/) and WT mice fed an atherogenic diet for 0, 1, 4, 8, or 20 weeks were measured. Levels were determined enzymatically in hepatic lipid extracts. All values are expressed as mean ± S.E. *, p < 0.05; **, p < 0.01 for IL-1Ra−/− mice versus WT mice.

involved in acute inflammation and that cholate induces expression of genes involved in extracellular matrix deposition in hepatic fibrosis (28).

Expectedly, the levels of total cholesterol in IL-1Ra−/− mice were significantly higher, and the start of lipid deposition in livers of IL-1Ra−/− mice was observed earlier than in WT mice. These results suggest that deficiency of IL-1Ra yields abnormal lipid metabolism upon feeding on atherogenic diet. The primary route of cholesterol elimination from the body is via bile, based on both direct canalicular excretion of biliary cholesterol as well as conversion of hepatic cholesterol to bile acids (29). In our study, WT but not IL-1Ra−/− mice demonstrated increased bile acid excretion to maintain physiological cholesterol levels, suggesting that impairment of bile acid excretion yields significant cholesterol accumulation in response to atherogenic diet in IL-1Ra−/− mice.

In our study, a 2-fold increase in mRNA level of ACAT2 was observed in the IL-1Ra−/− mice when compared with WT mice after 4 weeks. ACAT2 is thought to function in intestinal cholesterol absorption and transport into chylomicrons as well as in providing cholesteryl esters for VLDL assembly in the liver. Indeed, ACAT2-deficient mice have reduced cholesterol absorption and are resistant to diet-induced hypercholesterolemia by a high fat, high cholesterol diet (30). Thus, the increased mRNA level of ACAT2 in IL-1Ra−/− mice might contribute to the hypercholesterolemia and the accumulation of lipids in the liver.

Another interesting finding in this study is that the deficiency of IL-1Ra enhances both SHP and LRH-1 mRNA expression and decreases CYP7A1 transcript in mice fed an atherogenic diet. The flux of bile acids is tightly controlled by nuclear receptors. When hepatic cholesterol levels are high, oxysterols accumulate and activate nuclear liver X-receptors, which stimulate transcription of CYP7A1 (31, 32), eventually resulting in increased bile acid synthesis and subsequent excretion of cholesterol. When bile acid levels are high, bile acid synthesis is inhibited through a regulatory cascade based on farnesoid X-receptor, SHP, and LRH-1 (33, 34). Since SHP is a particularly potent inhibitor of LRH-1 function and LRH-1 is essential for CYP7A1 expression, SHP induction results in decreased CYP7A1 expression, as confirmed in vivo using SHP null mice (35, 36). In the present study, levels of LRH-1 in IL-1Ra−/− mice were lower when compared with WT mice at basal level. The reduction of LRH-1 might be caused by chronic inflammation due to the lack of IL-1Ra, in accord with a recent report showing independently reduced LRH-1 expression during inflammation (37). The decrease of LRH-1, in turn, might suppress expression of CYP7A1 at the basal level. However, levels of LRH-1 in IL-1Ra−/− mice significantly increased when compared with WT mice at 4 weeks. This change might be induced by the cholestasis of IL-1Ra−/− mice since Bohan et al. (38) reported that cytokine-dependent up-regulation of LRH-1 was detected after bile duct ligation in the rat.

Several previous reports demonstrated that administration of cholic acid in mice induced SHP gene expression (33, 34) and that SHP reduces CYP7A1 expression (38). Increased bile acids in the liver could, in turn, induce inflammation, and the lack of IL-1Ra, an anti-inflammatory cytokine, might deteriorate inflammation in IL-1Ra−/− liver. Furthermore, a large amount of cytokines induced by severe inflammation in IL-1Ra−/− mice could also play an important role in the up-regulation of SHP. Cytokine-dependent signaling leads to the activation of c-Jun N-terminal kinase (JNK) and other mitogen-activated protein kinases (39, 40). Recently, Gupta et al. (41) showed that c-Jun activated by cytokines induces SHP promoter activity and mutations in the AP-1 binding
site abolished bile acid responsiveness of the rat SHP pro-
moter. Thus, they suggested that activation of the JNK/c-Jun
pathway is needed for the induction of SHP by bile acids.
Furthermore, Miyake et al. (42) demonstrated that bile acid
induction of cytokine expression (such as tumor necrosis fac-
tor-α and IL-1) by macrophages correlates with repression
of hepatic CYP7A1, further supporting our findings. Thus,
atherogenic diet-induced inflammation under both high IL-1
levels and deficiency of IL-1Ra caused up-regulation of SHP
and, in turn, down-regulation of CYP7A1. The suppression
of CYP7A1 accumulates more cholesterol to a large extent.
We conclude that the significant increase in SHP expression in
IL-1Ra−/− mice is an indirect effect of IL-1Ra deletion, but
IL-1Ra plays an important role in maintaining the choles-
terol homeostasis under cholic acid-induced inflammation.

Cholesterol conversion to bile acids occurs via two different
pathways: the classic and the alternative pathway. The classic
pathway begins with the rate-limiting enzyme CYP7A1 (43, 44).
After 1 and 4 weeks of the atherogenic diet, the expression of
CYP7A1 mRNA decreased in both types of mice. However, the
levels in IL-1Ra−/− mice were markedly lower than those in the
WT mice, suggesting that the function of bile acid biosynthesis in
IL-1Ra−/− mice was significantly attenuated following only 1
week of atherogenic diet. Our findings are in accord with a
previous study that demonstrated lipopolysaccharide and cyto-
kines resulted in a marked and very rapid decrease in CYP7A1
activity and mRNA levels (45). In contrast, differences in hepatic
expression of LDL receptor and HMG-CoA reductase were not
observed between IL-1Ra−/− and WT mice. All these data sug-
gest that hypercholesterolemia and accumulation of lipids in
IL-1Ra−/− mice is mainly caused by attenuation of cholesterol
excretion from the liver by SHP-induced CYP7A1 suppression.
However, neither the suppression of LDL receptor nor the up-
regulation of HMG-CoA reductase implicated phenotype.

The decrease in HDL-cholesterol levels in IL-1Ra−/− mice is
also in accord with the changes known to be caused by changes
of ABCA1 and SR-BI in the acute phase response (17). How-
ever, no differences were observed between IL-1Ra−/− and WT
mice in hepatic expression of ABCA1 and SR-BI, suggesting
that neither ABCA1 nor SR-BI contributes to the decrease of
HDL-cholesterol in IL-1Ra−/− mice. We think other factors,
such as secretory phospholipase A2, endothelial lipase, and
lecithin cholesterol acyltransferase, may affect the decrease of
HDL-cholesterol in IL-1Ra−/− mice. Indeed, secretory phospho-
lipase A2 and endothelial lipase, which hydrolyze phospholip-
ids in HDL-cholesterol, are induced and lecithin cholesterol
acyltransferase, which esterifies HDL-cholesterol, is reduced
during inflammation (46–48).

Recently, Devlin et al. (49) showed that IL-1Ra knock-out
C57BL mice fed a cholesterol/cholate diet for 3 months had a
3-fold decrease in non-HDL cholesterol when compared with
WT littermate controls. However, this study did not detect
differences in bilirubin levels between IL-1Ra knock-out and
WT mice, whereas ALT levels were reduced. Their results

FIG. 7. Expression of genes involved in lipid metabolism (a), transcription factors (b and c), and inflammatory-related genes (d) in the livers of IL-1Ra−/− (Ra−/−) and wild type mice. The mRNA levels of the indicated genes in the livers of IL-1Ra−/− and WT mice were quantified using real-time PCR with SYBR-green detection after 0, 1, and 4 weeks of atherogenic diet. Degree of change in gene expression is based on the day 0 baseline expression level of WT mice. All values are expressed as mean ± S.E., *, p < 0.05; **, p < 0.01, and ***, p < 0.001 for IL-1Ra−/− mice versus WT mice. LXRα, nuclear liver X-receptor α; FXR, farnesoid X-receptor; TGF, transforming growth factor.
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Differ from our results, and the study did not demonstrate why IL-1Ra knock-out mice are protected from cholate-induced inflammation. Furthermore, their work did not analyze the mechanisms responsible for the change of cholesterol metabolism. In our study, cholesterol/cholate diet induced inflammation in the liver as reported previously (15), and the inflammation increased serum lipid levels in our mice. We think these results are in agreement with previous reports that showed that inflammation can change lipid metabolism (16, 17). Moreover, we uncovered the mechanisms of why the deficiency of IL-1Ra deteriorated cholesterol metabolism. Thus, we demonstrated for the first time that IL-1Ra plays an important role in the prevention of both fatty liver and hypercholesterolemia under inflammatory conditions.

In conclusion, deficiency of IL-1Ra deteriorated fatty liver development and cholesterol metabolism under atherogenic diet. Our results show that high cytokine levels in IL-1Ra−/− mice reduced mRNA expression of CYP7A1 with concurrent up-regulation of SHP mRNA expression. We conclude that IL-1Ra plays an important role in maintaining the cholesterol homeostasis under inflammatory conditions induced by cholic acid.

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