

The Effect of Interleukin-1 Receptor Antagonist on Arteries and Cholesterol Metabolism

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This review summarizes both the structure and function of IL-1 receptor antagonist (IL-1Ra), and relates our new findings, particularly those obtained in IL-1Ra-deficient mice (IL-1Ra^{-/-}), to the role of IL-1Ra in arterial diseases and cholesterol metabolism. IL-1Ra^{-/-} mice show an increase in neointima-formation after arterial injury. Heterozygosity in the IL-1Ra gene against the apolipoprotein E-deficient background revealed a role for IL-1 in promoting atherogenic cell signaling and that the larger lesions of IL-1Ra^{-/-} mice are enriched in macrophages and depleted of smooth muscle cells. Furthermore, IL-1Ra^{-/-} mice developed severe fatty livers and hypercholesterolemia following 20 weeks on a atherogenic diet compared to WT mice. Taken together, these results suggest that IL-1Ra plays important roles in restenosis after angioplasty, the development of atherosclerosis, and the metabolism of cholesterol *in vivo*. *J Atheroscler Thromb*, 2006; 13: 21–30.

Key words: Interleukin-1 receptor antagonist, Inflammation, Atherogenesis, Cholesterol metabolism

Introduction

Interleukin (IL)-1 is a physiologically active factor produced and secreted by a variety of cells including those responsible for controlling immunity. Furthermore, it plays an important role in immune reactions, cell damage, and cell-proliferation (1, 2). IL-1 consists of two molecules, IL-1 α and IL-1 β , both of which exert similar but not completely overlapping biological functions mediated through the IL-1 type I receptor (IL-1RI). Another IL-1 receptor, the type II receptor (IL-1RII), has also been identified, but this receptor is not considered to be involved in signal transduction, but is rather thought to play a regulatory role as a “decoy”. In addition, another member of the IL-1 gene family, IL-1 receptor antagonist (IL-1Ra), binds to IL-1 receptors without exerting agonistic activity. IL-1Ra as well as IL-1RII and the secretory forms of

IL-1RI and IL-1RII are considered to negatively regulate IL-1 signaling (3).

The balance between IL-1 and IL-1Ra has significant effects on host responses to inflammation and infection (4, 5). In the immune system, IL-1 has many systemic effects in the protection of the body, being involved in fever, the response to stress, and the metabolism of insulin, lipid and bone (6, 7). Notably, in vascular homeostasis, IL-1 is considered one of the most potent proinflammatory cytokines acting on endothelial cells (ECs) and smooth muscle cells (SMCs) (8). IL-1 is produced from these cells as well as macrophages (M ϕ s) and hepatocytes (9, 10). IL-1 induces the expression of surface leukocyte adhesion molecules in ECs, proliferation of SMCs, and secretion of other cytokines and chemokines from ECs, SMCs, and M ϕ s (11, 12). These effects of IL-1 are strongly implicated in cardiovascular diseases (13–15). IL-1Ra, one of the negative regulators of IL-1 signaling, plays a role as an anti-inflammatory cytokine, similar to IL-10 and TGF- β , in acute- and chronic- inflammation of the vascular wall (16, 17). IL-1Ra is also produced by ECs and SMCs as well as M ϕ s for maintaining vascular homeostasis (18, 19). This re-

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view, focuses on the effects of IL-1Ra on atherogenesis and cholesterol metabolism.

Structure of Human IL-1 Ra

IL-1Ra was originally discovered as an inhibitor of IL-1 in the urine of patients with fever (20). A cDNA encoding the secreted form of the molecule was identified in a human monocyte library (21). Secretory IL-1Ra (sIL-1Ra) is synthesized as a 177-amino acid protein requiring the cleavage of a 25-amino acid leader sequence prior to secretion as a variably glycosylated 152-amino acid protein. A second cDNA coding for an intracellular form of IL-1Ra (icIL-1Ra) was cloned from a different human monocyte library (22). These two isoforms of IL-1Ra are created by alternative splicing yielding different first exons (23). The internal splice acceptor site for icIL-1Ra was located within the first exon for sIL-1Ra, near the 3' end of the sequence coding for the signal peptide. icIL-1Ra does not have a functional leader sequence and remains in the cytoplasm. The sIL-1Ra protein is produced by many cells that can synthesize IL-1. icIL-1Ra is found constitutively in keratinocytes and other epithelial cells but is also a delayed product of stimulated M ϕ s (24, 25). Neutrophils contain only sIL-1Ra mRNA, whereas fibroblasts are capable of producing the mRNA and protein for both IL-1Ra isoforms, when appropriately stimulated (26).

Function of IL-1 Ra

In spite of extensive studies on IL-1 over the past two decades, the important roles that this cytokine may play in normal biology are unclear (27, 28). Furthermore, it remains unknown whether the function of IL-1Ra is limited to regulating the agonistic effects of extracellular IL-1 in normal biologic processes or in pathophysiological conditions. Studies on the functional consequences of overexpression or absence of expression of IL-1Ra in transgenic or knockout mice, respectively, may clarify some possible roles of this cytokine in normal biology. This review relates our new findings (obtained from IL-1Ra-deficient mice (IL-1Ra^{-/-})) about the effects of IL-1Ra on arteries and cholesterol metabolism.

IL-1Ra and neointima formation after injury

Neointimal hyperplasia is characterized by the activation, migration, and proliferation of SMCs and is associated with inflammatory mediators such as cytokines. IL-1 β is a chemoattractant and mitogen for SMCs (28) that is overexpressed at sites of the active proliferation and migration of this cell type subsequent to injury (29). Furthermore, a recent report demonstrated that IL-1RI gene-deficient mice tended to develop less neointima than wild-type mice (30). In sum, these previous studies suggested

that IL-1 might promote neointimal formation. However, it remained uncertain whether IL-1Ra, the endogenous inhibitor of this central cytokine, could significantly suppress this response in the vasculature. Using IL-1Ra^{-/-} mice (on the C57BL/6J background) and wild-type (IL-1Ra^{+/+}) mice, we investigated neointimal formation 3 weeks after femoral artery injury induced with an external vascular cuff. The mean intimal thickness and the intima/media ratio of IL-1Ra^{-/-} mice increased by 249% and 257%, respectively, compared with IL-1Ra^{+/+} mice (Fig. 1A) (31). Control immunostaining for IL-1 β and the endogenous inhibitor in the endothelium and inflammatory cells of adventitia in IL-1Ra^{+/+} mice but not IL-1Ra^{-/-} mice (Fig. 1B) (31). These results suggest that IL-1Ra protein prevents inflammation of both the intima and adventitia after cuff injury. Indeed, IL-1Ra^{-/-} mice showed an increase in the proliferating cell nuclear antigen (PCNA) index of the intima and adventitia after injury. Within the adventitia, proliferating monocytes and macrophages comprised the majority of PCNA-positive cells. Recent studies have shown that adventitial passive fibroblasts can become active myofibroblasts under conditions of adventitial inflammation (32, 33). On the other hand, SMCs were the predominant proliferating cell type in the intima (31). IL-1 itself is a mitogen for SMCs (28), and furthermore, a recent study showed that vascular intima formation after mechanical injury mainly involves inflammatory cells that originate from the bone marrow (34). Our study demonstrated definitively that a deficiency of endogenous IL-1Ra promotes neointimal formation, revealing a crucial role for this protein in hyperplastic responses of the vasculature. Our results may be compatible with the report that p80 IL-1 type I receptor knockout mice tended to develop a smaller (7-fold) neointimal area induced by low shear stress compared to wild-type controls (30). This report demonstrated that IL-1 modulates low shear stress-induced neointimal formation, thus providing a direct proinflammatory cytokine signaling link between biomechanical forces to a vessel wall and the remodeling response of the artery. They also concluded that specific anti-IL-1 therapy may lessen neointimal formation.

IL-1Ra and atherogenesis

Atherogenesis is a complex process in which the activation of ECs and SMCs appears to be a central theme (8). IL-1 is produced by these cells as well as M ϕ s and hepatocytes (9, 10). Furthermore, stimulation and activation of ECs and SMCs by IL-1 causes a wide range of inflammatory processes within the atheroma, such as the enhanced expression of leukocyte adhesion molecules (9, 12), clotting factors and inhibitors of fibrinolysis (11), and chemokines (28), as well as increased proliferation of SMCs (8, 15), suggesting a central role for IL-1 in the development of atherosclerosis. The activity of IL-1 is

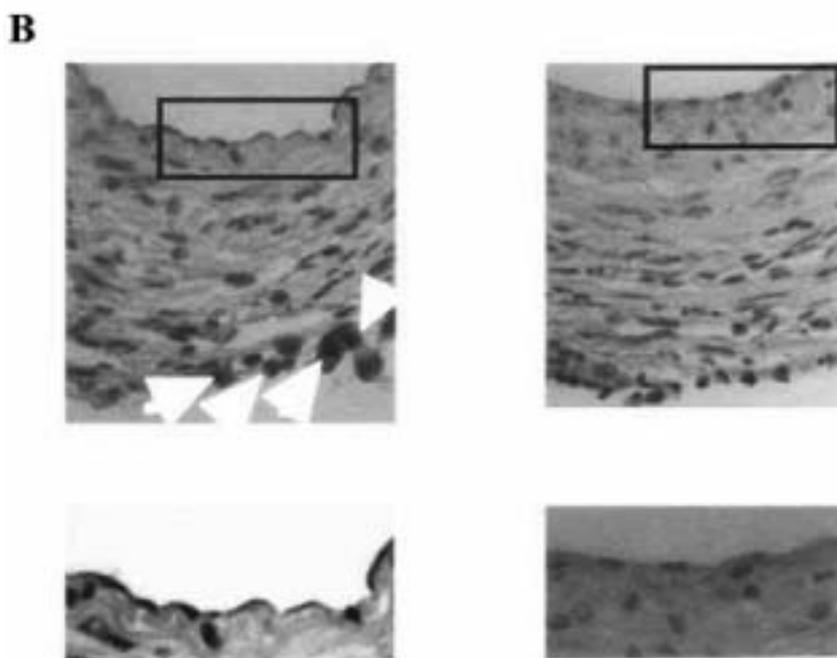
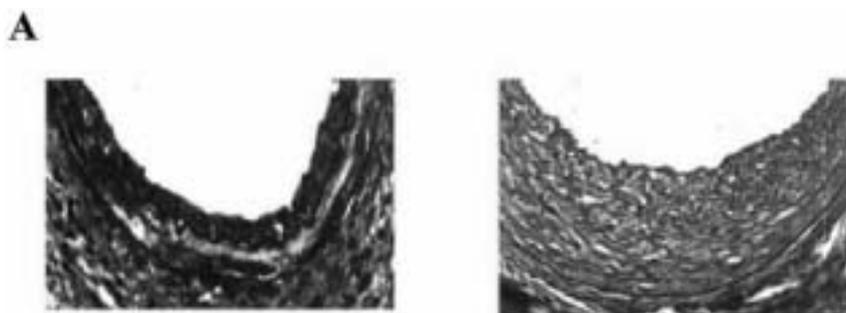


Fig.1. Lack of IL-1Ra promotes neointimal formation after injury

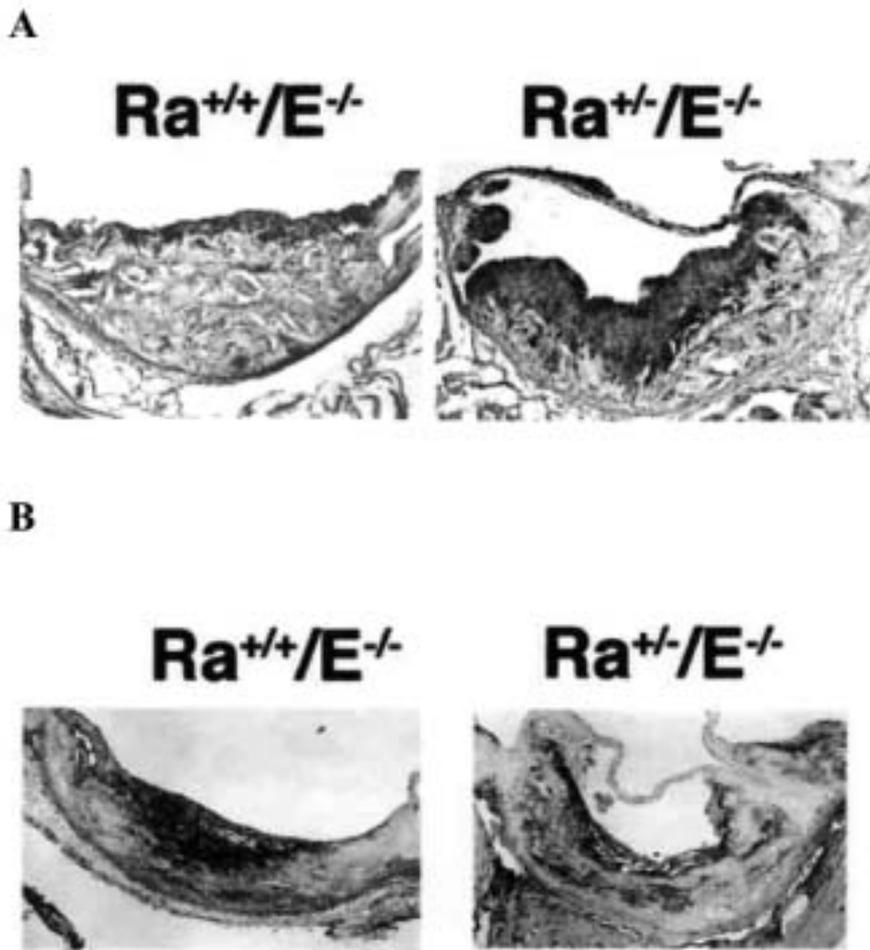
A. Histology of cuffed femoral arteries (day 21) of IL-1Ra^{+/+} (left) and IL-1Ra^{-/-} (right) mice. Intimal thickness was significantly increased in IL-1Ra^{-/-} mice compared with IL-1Ra^{+/+} mice. Sections were stained with Masson's trichrome. Original magnification X 50.

B. Representative photomicrographs depict immunohistochemical staining for IL-1Ra in cuffed femoral arteries of IL-1Ra^{+/+} (left) and IL-1Ra^{-/-} (right) mice (day 7). Boxed areas are enlarged at the bottom of the panels. IL-1Ra protein is present in both the endothelium and some inflammatory cells (arrows) in IL-1Ra^{+/+} mice. Original magnification X 100 (upper panels) and X150 (bottom panels).

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counter-regulated by its endogenous inhibitor IL-1Ra (15, 27) and a previous report showed that IL-1Ra is expressed in ECs and atherosclerotic lesions (18). Treatment with recombinant IL-1Ra proved an effective therapy for atherosclerosis in apoE^{-/-} mice (35). Furthermore, low density lipoprotein receptor-deficient (LDLR^{-/-}) mice crossed with transgenic mice expressing high levels of murine sIL-1Ra were also partially protected compared to their non-transgenic controls. In contrast, LDLR^{-/-}/IL-1Ra^{-/-} mice had a tendency to develop foam cell lesions on a diet rich in cholesterol and cholate (36). Moreover, in humans IL-1Ra gene polymorphism is significantly associated with coronary artery disease (37). These findings suggest that endogenous IL-1Ra may suppress atherosclerosis. To directly answer the question of whether a deficiency of IL-1Ra promotes the development of ath-

erosclerotic lesions and/or can modulate the phenotype of atheroma, we employed IL-Ra^{-/-} mice. Using apoE^{-/-} mice as an animal model of atherosclerosis, we established three genotypes (IL-1Ra^{+/+}/apoE^{-/-}, IL-1Ra^{+/-}/apoE^{-/-}, and IL-1Ra^{-/-}/apoE^{-/-} mice) by cross-breeding. This study focused on the comparison of atherosclerotic lesions and IL-1Ra^{+/+}/apoE^{-/-} and IL-1Ra^{+/-}/apoE^{-/-} mice, because of the significantly leaner phenotype in IL-1Ra^{-/-}/apoE^{-/-} mice. Interestingly, the size of the atherosclerotic lesion after 16 weeks was significantly increased (30%) in IL-1Ra^{+/-}/apoE^{-/-} mice compared to IL-1Ra^{+/+}/apoE^{-/-} mice (38). Following 32 weeks, the differences in lesion size between these mice failed to achieve statistical significance (38). However, immunostaining demonstrated an 86% increase in the MOMA-2-stained area in IL-1Ra^{+/-}/apoE^{-/-} mice (Fig. 2A). In addition, α -actin stain-



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Fig.2. Deficiency of IL-1Ra modulates plaque composition in ApoE^{-/-} mice.

A. Representative photomicrographs of sections of advanced atherosclerotic plaques (immunohistochemical staining for MOMA-2) from the aortic sinus of IL-1Ra^{+/+}/apoE^{-/-} (Ra^{+/+}/E^{-/-}) (left) and IL-1Ra^{+/-}/apoE^{-/-} (Ra^{+/-}/E^{-/-}) (right) mice at 32 weeks of age. IL-1Ra^{+/-}/apoE^{-/-} mice showed markedly increased numbers of macrophages in lesions compared with IL-1Ra^{+/+}/apoE^{-/-} mice. Original magnification X100.

B. Representative photomicrographs of sections of advanced atherosclerotic plaques (immunohistochemical staining for α -smooth muscle cell actin) from the aortic sinus of IL-1Ra^{+/+}/apoE^{-/-} (Ra^{+/+}/E^{-/-}) (left) and IL-1Ra^{+/-}/apoE^{-/-} (Ra^{+/-}/E^{-/-}) mice (right) at 32 weeks of age. SMC numbers in the lesions in IL-1Ra^{+/-}/apoE^{-/-} mice were significantly decreased compared with those in IL-1Ra^{+/+}/apoE^{-/-} mice. Original magnification X100.

ing in these lesions was significantly decreased (~15%) compared to that in IL-1Ra^{+/+}/apoE^{-/-} mice (Fig. 2B) (38). Our real-time polymerase chain reaction (RT-PCR) analysis revealed that deletion of IL-1Ra increases the mRNA expression of the adhesion molecules vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 in the aorta, and enhances mRNA levels of monocyte chemoattractant protein (MCP)-1 (38). These changes may contribute to the enhanced accumulation of M ϕ s in advanced plaques. Interestingly, IL-1 β ^{-/-}/apoE^{-/-} mice showed opposite results, thus demonstrating that the size of atherosclerotic lesions at the aortic sinus in IL-1 β ^{-/-}/apoE^{-/-} mice at 12 and 24 weeks of age showed a significant decrease of 30% compared with that in IL-1 β ^{+/+}/apoE^{-/-} mice, and the mRNA levels of VCAM-1 and MCP-1 in the IL-1 β ^{-/-}/apoE^{-/-} aorta were significantly reduced compared with those in the IL-

1 β ^{+/+}/apoE^{-/-} mice (39). They suggested that IL-1 β exerts an atherogenic action by enhancing the expression of VCAM-1 and MCP-1 in the aorta. This report may support our results. Taken together, these findings suggest an important role for IL-1Ra in suppressing the development of lesions early during atherogenesis and furthermore, implicate it in the modulation of plaque composition.

IL-1Ra and cholesterol metabolism during chronic inflammation

Infection and inflammation induce an acute-phase response (APR) (40), leading to multiple alterations in lipid and lipoprotein metabolism (41). Serum triglyceride (TG) levels are increased by multiple cytokines, including IL-1, IL-2, IL-6 and tumor necrosis factor (42–48), because of increased secretion of very low density lipoprotein

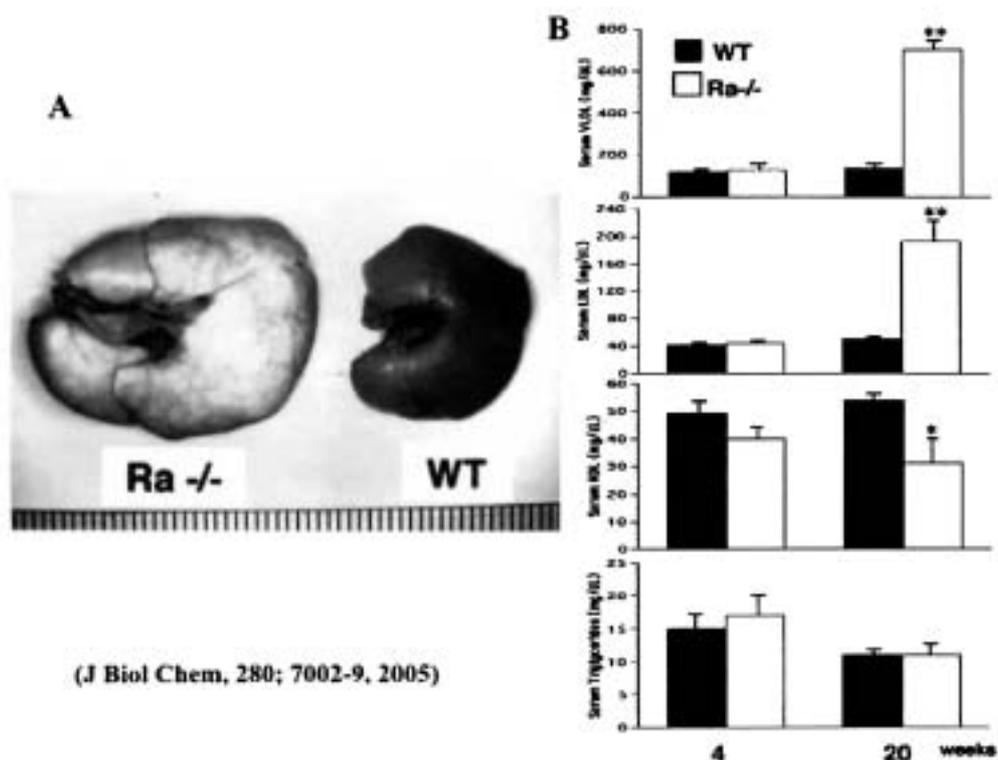


Fig.3. IL-1Ra^{-/-} mice showed severe fatty liver and hypercholesterolemia following 20 weeks on an atherogenic diet when compared with IL-1Ra^{+/+} mice.

A. Macroscopic appearance of livers from IL-1Ra^{-/-} (Ra^{-/-}) and IL-1Ra^{+/+} (WT) mice fed an atherogenic diet for 20 weeks. There was a prominent change in color and size in the liver of IL-1Ra^{-/-} versus IL-1Ra^{+/+} mice.

B. Plasma levels of VLDL (upper), LDL (middle upper), HDL (middle lower) and triglycerides (lower) in IL-1Ra^{-/-} (Ra^{-/-}) and wild-type (WT) mice. All values are expressed as the mean + SEM. * $p < 0.05$, ** $p < 0.01$ for Ra^{-/-} mice versus WT mice.

(VLDL) as a result of adipose tissue lipolysis (49–51). With more severe inflammation, the clearance of VLDL decreases secondary to decreased lipoprotein lipase and apolipoprotein E in VLDL (52–54). LPS and cytokines reduce total serum cholesterol levels in primates, whereas in rodents they increase cholesterol levels by stimulating de novo cholesterol synthesis (55, 56), decreasing lipoprotein clearance (57), limiting the conversion of cholesterol to bile acids (58, 59), and decreasing the secretion of cholesterol into the bile (60–63). Many of the changes in lipoproteins during inflammation help to protect the host from harmful effects of the stimuli. However, if prolonged, these changes in the structure and function of lipoproteins will contribute to atherogenesis. Of note, inflammatory cytokines are increased and play a pathogenic role in a variety of very common disorders, such as diabetes, obesity, metabolic syndrome, and athero-

sclerosis (64–68). Many of these disorders display abnormalities in lipid metabolism that are similar to those that occur during infection and inflammation. However, the effect of chronic inflammation on lipid metabolism has been unclear. Furthermore, there is no report that shows the role of IL-1Ra in the metabolism of cholesterol under chronic inflammatory conditions.

To elucidate the role of IL-1Ra, we fed an atherogenic diet (with cholate) to both IL-1Ra^{-/-} and IL-1Ra^{+/+} mice. IL-1Ra^{-/-} mice developed severe fatty liver after 20 weeks compared to IL-1Ra^{+/+} mice (Fig. 3A) (69). Histological examination revealed an increase in the number and size of intracellular vacuoles, portal fibrosis, and collagen deposition as well as lobular and portal inflammation in livers of IL-1Ra^{-/-} mice. Expectedly, the plasma lipid profile became more proatherogenic with increased total cholesterol levels (942 ± 160 mg/dl versus 240 ± 13 mg/

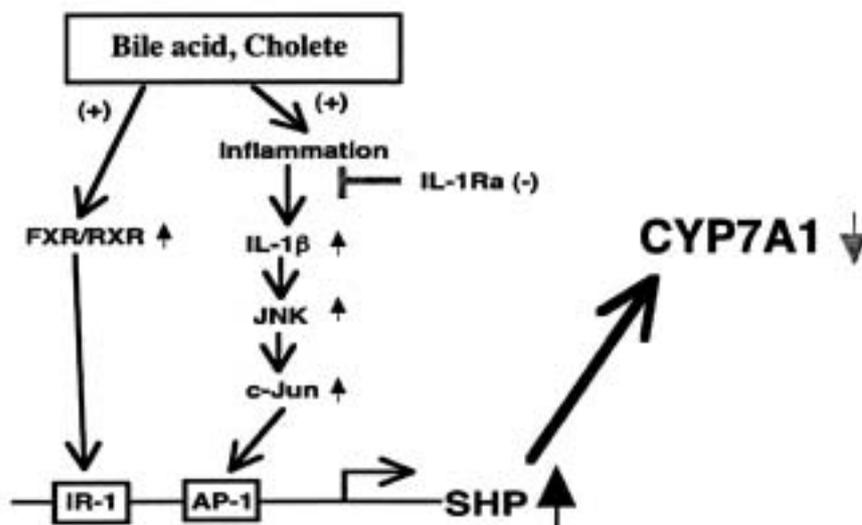


Fig.4. Model for up-regulation of SHP gene expression in the absence of IL-1Ra. Bile acids activate FXR and induce inflammation. The lack of IL-1Ra could not reduce inflammation and might induce the production of a large amount of IL-1. Then IL-1 activated the JNK signaling cascade. Activated FXR and c-Jun enhanced SHP transcription by binding to the IR-1 and AP-1 elements in the SHP promoter. Elevated SHP protein levels in turn repressed CYP7A1 transcription and the down-regulation of CYP7A1 expression increased amounts of bile acids in hepatocytes. Finally this loop formed a vicious circle in the metabolism of bile acids. See text for details.

dl, $n = 5$; $p < 0.01$), higher apoB-containing lipoprotein levels (699 ± 126 mg/dl versus 192 ± 36 mg/dl; $p < 0.01$), and decreased HDL levels (31 ± 10 mg/dl versus 54 ± 3 mg/dl; $p < 0.05$) in IL-1Ra^{-/-} mice compared to IL-1Ra^{+/+} mice after 20 weeks on the atherogenic diet (Fig. 3B). Real-time PCR analysis revealed that the decrease in the IL-1Ra mRNA level was accompanied by an increase in the levels of IL-1 β ($P < 0.001$), TGF- β ($P < 0.01$) and CD68 mRNA ($P < 0.001$) in the IL-1Ra^{-/-} liver compared to the WT liver. Furthermore, IL-1Ra^{-/-} mice failed to express mRNA of cholesterol 7 α -hydroxylase (CYP7A1) ($p < 0.05$), the rate-limiting enzyme in bile acid synthesis, with upregulation of small heterodimer partner 1 (SHP) mRNA expression ($p < 0.001$) following 4 weeks on the atherogenic diet (69). Indeed, IL-1Ra^{-/-} mice showed markedly decreased bile acid excretion, which is promoted in WT mice to maintain cholesterol levels while feeding on an atherogenic diet. Our results show that both bile acid and high cytokine levels in IL-1Ra^{-/-} mice reduced the mRNA expression of CYP7A1 with an upregulation of SHP mRNA expression. We summarize the mechanism of these changes in Fig. 4. Several previous reports demonstrated that administration of cholic acid in mice induced SHP gene expression (70, 71) and SHP reduces CYP7A1 expression (72). Increased concentrations of bile acids in the liver could, in turn, induce inflammation and the lack of IL-1Ra, an anti-inflammatory cytokine, might

worsen the inflammation in IL-1Ra^{-/-} liver. Furthermore, large amounts of cytokines produced in response to severe inflammation in IL-1Ra^{-/-} mice could also play an important role in the up-regulation of SHP expression. Cytokine-dependent signaling leads to the activation of c-Jun N-terminal kinase (JNK) and other mitogen-activated protein kinases (73, 74). Recently, Gupta *et al.* showed that c-Jun activated by cytokines induces SHP-1 promoter activity and mutations in the AP-1 binding site abolished bile acid responsiveness of the rat SHP promoter (75). 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.* demonstrated that bile acid-induced expression of cytokines (such as TNF- α and IL-1) by macrophages correlates with repression of hepatic CYP7A1 (76), further supporting our findings. Thus, atherogenic diet-induced inflammation with both a high IL-1 level and deficiency of IL-1Ra caused an up-regulation of SHP expression and, in turn, down-regulation of CYP7A1. The suppression of CYP7A1 causes more cholesterol to accumulate in IL-1Ra^{-/-} mice. We conclude that the significant increase in SHP expression in IL-1Ra^{-/-} liver is an indirect effect of loss of IL-1Ra, but IL-1Ra plays an important role in maintaining cholesterol homeostasis under conditions of cholic acid-induced inflammation.

Conclusion

During the last five years, transgenic and gene knock-out studies in murine models of vascular disease have established IL-1 and IL-1Ra as pivotal players in the regulation of vascular cell functions and cholesterol metabolism. Although genetic differences between mouse and man preclude a direct translation of these findings to human disease, these studies have identified several pathways whose perturbation has the potential to significantly shift the balance between disease progression and retardation. An important goal of future studies will be more-detailed investigations of the particular genes and pro- and anti-inflammatory pathways regulated by different cytokines in atherogenesis and cholesterol metabolism. This challenge could lead to promising novel therapeutic targets for anti-inflammatory therapies, potentially even harnessing some of the sophisticated regulatory systems designed to normally limit the inflammatory response.

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