Interleukin-1 plays a major role in vascular inflammation and atherosclerosis in male apolipoprotein E-knockout mice[☆]

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Abstract

Objective: To examine the role of the balance between interleukin (IL)-1 and IL-1 receptor antagonist (IL-1Ra) in atherosclerosis and vascular inflammation.
Methods: Transgenic (Tg) mice overexpressing either secreted IL-1Ra or intracellular IL-1Ra1 as well as IL-1Ra-deficient mice (IL-1Ra −/−) were crossed with apolipoprotein E-deficient mice (ApoE −/−).
Results: In males fed a cholesterol-rich diet for 10 weeks, average atherosclerotic lesion area within aortic roots was significantly decreased in ApoE −/− secreted IL-1Ra Tg (−47%) and ApoE −/− intracellular IL-1Ra1 Tg (−40%) mice as compared to ApoE −/− non-Tg controls. The extent of sudanophilic lesions was reduced within the thoraco-abdominal aorta in ApoE −/− secreted IL-1Ra (−53%) and ApoE −/− intracellular IL-1Ra1 (−67%) Tg mice. In parallel experiments, we observed early mortality and illness among double deficient mice, whereas ApoE −/− IL-1Ra +/+ and ApoE +/+ IL-1Ra −/− mice were apparently healthy. After 7 weeks of diet, ApoE −/− IL-1Ra −/− mice exhibited massive aortic inflammation with destruction of the vascular architecture, but no signs of atherosclerosis. ApoE −/− IL-1Ra +/+ had atherosclerosis and a moderate inflammatory reaction, whereas ApoE +/+ IL-1Ra −/− mice were free of vascular lesions. Macrophages were present in large amounts within inflammatory lesions in the adventitia of ApoE −/− IL-1Ra −/− mice.
Conclusion: Our results demonstrate that the IL-1/IL-1Ra ratio plays a critical role in the pathogenic mechanisms leading to vascular inflammation and atherosclerosis in ApoE −/− mice.
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Keywords: Atherosclerosis; Cytokines; Inflammation

1. Introduction

Interleukin (IL)-1 is a prototypic pro-inflammatory cytokine that induces the production of cytokines and chemokines and increases the expression of adhesion molecules on endothelial cells, thus leading to the recruitment of inflammatory cells. In addition, IL-1 contributes to the development of tissue damage by stimulating cell proliferation and the release of matrix metalloproteases [1]. IL-1Ra is a natural inhibitor of IL-1 that binds to IL-1 receptors but does not induce any cellular responses. The

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administration of recombinant IL-1Ra prevents inflammatory responses and ameliorates the course of different experimental models of inflammatory diseases [2]. In addition, administration of recombinant IL-1Ra is approved in the treatment of rheumatoid arthritis and has recently been shown to ameliorate other inflammatory diseases as well [3,4]. Several reports suggest that the balance between endogenous IL-1 and its antagonist plays a major role in the modulation of inflammatory responses [5]. Mice lacking IL-1Ra (IL-1Ra −/−) are highly susceptible to lipopolysaccharide-induced lethality [6]. In the collagen-induced arthritis model, these mice exhibit early arthritis onset and more severe joint destruction [7]. Moreover, IL-1Ra −/− mice develop various type of spontaneous inflammatory diseases according to their genetic background [8,9].

IL-1Ra is produced as four different isoforms from the same gene by the use of different first exons, alternative mRNA splicing, or alternative translation initiation. One isoform is secreted (sIL-1Ra), whereas the three others lack a complete leader peptide sequence and remain intracellular (icIL-1Ra1, 2, 3). The biological role of intracellular isoforms is still unclear [2].

Atherogenesis is a complex process in which endothelial cell and smooth muscle cell activation appears to play a central role [10]. Inflammation is now recognized as a major contributor through effects on lipoprotein metabolism and activation of endothelial cells [11]. Monocytes/macrophages stand out as playing a particular relevant role, as demonstrated by the use of mice deficient in monocyte colony-stimulating factor or neutralizing antibodies against monocyte colony-stimulating factor [12,13]. Important progress has been made in the understanding of the role of inflammation in atherogenesis by the use of strains of mice prone to develop atherosclerosis [14]. Knockout mice having targeted inhibition on apolipoprotein E (ApoE −/−) or low density lipoprotein receptor (LDLR −/−) genes exhibit massive elevation of circulating cholesterol levels after cholesterol-rich diet and develop atherosclerotic lesions throughout the aorta [15,16]. The pathogenic role of IL-1 was recently investigated in these two strains of mice. Subcutaneous administration of recombinant human sIL-1Ra in ApoE −/− mice fed with a cholesterol-rich diet decreased the size of atherosclerotic lesions [17]. LDLR −/− mice crossed with transgenic mice expressing high levels of murine sIL-1Ra were also partially protected as compared to their non-transgenic controls. In contrast, LDLR +/+ IL-1Ra −/− mice had a tendency to develop foam cell lesions after a diet rich in cholesterol and cholate [18]. Taken together, these results indicate that IL-1 contributes to the mechanisms of atherogenesis in mice. Immunostaining for IL-1Ra revealed that endothelial cells express IL-1Ra in mice fed with a cholesterol/cholate-rich diet [18]. However, the differential expression of IL-1Ra isoforms has not been investigated and the potential role of endogenous IL-1Ra in atherogenesis is still unclear.

2. Materials and methods

2.1. Mice and genotyping

Transgenic (Tg) mice overexpressing either human sIL-1Ra or icIL-1Ra1 under the control of a strong and ubiquitous promoter were bred into a DBA/1 background, as described [19]. These mice were crossed with ApoE −/− mice bred into the C57BL/6J background (Jackson Laboratory, Bar Harbor, ME). Offspring (ApoE +/− IL-1Ra Tg or non-Tg) were intercrossed to generate ApoE −/− sIL-1Ra Tg, ApoE −/− icIL-1Ra1 Tg, and ApoE −/− non-Tg mice of identical mixed background (50% C57BL/6J and 50% DBA/1). In parallel, IL-1Ra −/− mice (kindly given by the laboratory of Dr. Hirsch, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY) on a DBA/1 background were crossed with ApoE −/− mice on a C57BL/6J background. Mice from the first generation (ApoE +/− IL-1Ra +/−) were intercrossed in order to obtain ApoE −/− IL-1Ra +/+, ApoE +/+ IL-1Ra −/−, and ApoE −/− IL-1Ra −/− mice in the same mixed background (50% C57BL/6J and 50% DBA/1). Genotyping was performed by PCR analysis of DNA extracted from tail biopsies, as previously described [6,12,19]. All comparisons were made to littermate controls of identical mixed genetic background (50% C57BL/6J and 50% DBA/1). All animal studies were approved by institutional (University of Geneva School of Medicine) as well as local veterinary authorities, and conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Experimental design

Only males were used in the following experiments to avoid the influence of gender on atherosclerosis. ApoE −/− sIL-1Ra Tg mice (n=6), ApoE −/− icIL-1Ra1 Tg mice (n=9), and their non-transgenic ApoE −/− controls (n=6 and n=10, respectively) were used at 10 weeks of age and fed with a high-cholesterol diet (1.25% cholesterol, 0% cholate; Research Diets, New Brunswick, NJ) for 10 weeks. In a separate experiment, 10-week-old ApoE −/− IL-1Ra +/+ (n=8), ApoE +/+ IL-1Ra −/− (n=9), and ApoE −/− IL-1Ra −/− mice (n=6) were fed with a cholesterol-rich diet for 7 weeks. After diet, mice were sacrificed, blood was taken, and aortas were subsequently perfused with 0.9% NaCl. Aortas were separated into 3 parts, of which the roots and arches were snap-frozen in OCT compound and the abdominal parts were fixed in 2% paraformaldehyde.

2.3. Analysis of atherosclerotic lesions

The extent of atherosclerosis was assessed in aortic roots and thoracoabdominal aortas according to standardized methods [20]. Quantification was performed by computer image analysis using Zeiss KS400 Software. Briefly, we

calculated, for each aortic root, an average of lesion area from 6 sections at 50 μm distant from each other by measuring the area of lipid deposition (stained with Sudan IV) below the aortic valves. The thoracoabdominal aortas were stained with Sudan IV and then opened longitudinally to the iliac bifurcation and pinned out on black wax surface using 0.2-mm steel pins. The percentage of lipid deposition was calculated within the total surface area (15 mm from the iliac bifurcation to the thoracic section of the aorta) using computer analysis.

The composition of the atherosclerotic lesions was analysed on cryosections from aortic roots and arches, fixed in acetone, air-dried, and stained with hematoxylin–eosin or immunolabeled with an anti-mouse Mac-1 monoclonal antibody 1:50 (Pharmingen, Heidelberg, Germany). Sections were incubated with the primary antibody diluted in PBS supplemented with 10% of the species respective normal goat serum, followed by the respective biotinylated secondary antibody and avidin–biotin complex (Vector Laboratories, Burlingame, CA). The reaction was visualized by using alkaline phosphatase substrate kit (Vectastain ABC Systems, Vector Red Substrate kit, Vector Laboratories). Levamisole solution (Vector Laboratories) was added to the substrate solution in order to inhibit endogenous alkaline phosphatase. Sections were then counterstained with Mayer's hematoxylin solution (Sigma Fluka Chemie AG, Buchs, Switzerland). In some experiments, macrophages were identified in sections from aortic roots with a rat monoclonal anti-CD68 antibody (Serotec, Dusseldorf, Germany) followed by FITC goat anti-rat antibodies (Jackson Immunoresearch Laboratories, Maine). Sections were then counterstained with Evans blue (red signal) and with DAPI (blue nuclear staining). Van-Gieson Miller staining was performed on 5 μm cryostat sections from aortic roots. Nuclei were counterstained with Harris hematoxylin solution (Sigma).

2.4. Determination of plasma levels of total cholesterol, serum amyloid A (SAA), human IL-1Ra, mouse IL-1β, and IL-1Ra

Mouse plasma cholesterol concentrations were determined before and after cholesterol-rich diet in the different mouse strains, as described elsewhere [21]. The levels of SAA were determined using a direct ELISA, as previously described [22]. The plasma concentrations of human IL-1Ra and mouse IL-1β were measured using commercial Duo Set ELISA Systems (R&D Systems, Abington, GB). The circulating concentrations of mouse IL-1Ra were determined by ELISA, as previously described [23].

2.5. Determination of sIL-1Ra and icIL-1Ra1 mRNA levels

Aortic roots were dissected from 10-week-old wild-type and ApoE −/− mice fed with usual diet and ApoE −/− fed with cholesterol-rich diet for 10 weeks. Total RNA was extracted using the Trizol® reagent according to the manufacturer's instructions (Invitrogen, Basel, Switzerland), and quantified by spectrometry.

After DNase I digestion, RNA samples (3 μg) were reverse-transcribed using AMV-RT (Promega, Wallisellen, Switzerland) and random hexamers (Promega). PCR for sIL-1Ra (40 cycles), icIL-1Ra1 (45 cycles) and β-actin (28 cycles) were performed with primers described in previous studies [23,24]. Amplifications were carried out under the following conditions: denaturation for 3 min at 94 °C followed by cycles of 30 s denaturation at 94 °C, 30 s annealing at 55 °C and 45 s elongation at 72 °C. PCR products were visualized by ethidium bromide/2% agarose gels.

2.6. Statistical analysis

Values are expressed as mean±S.E.M. The Student's t test of unpaired observations was used to determine differences of body weight and total cholesterol levels before and after diet in the same group, and differences of atherosclerotic lesion areas, lipid staining areas, plasma IL-1Ra levels and SAA levels between the different strains of mice. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Overexpression of sIL-1Ra or icIL-1Ra1 reduces the development of atherosclerotic lesions in ApoE −/− mice

To examine the relative effects of IL-1Ra isoforms on the development of atherosclerosis, transgenic mice overexpressing either human sIL-1Ra or icIL-1Ra1 were crossed with ApoE −/− mice. Four different groups were created, including ApoE −/− sIL-1Ra Tg mice, ApoE −/− icIL-1Ra1 Tg mice, and their respective control ApoE −/− littermates. Circulating levels of human IL-1Ra were elevated in ApoE −/− sIL-1Ra Tg mice (15.8±3.16 μg/mL) and to a lesser extent, in ApoE −/− icIL-1Ra1 Tg mice (0.63±0.094 μg/mL). In contrast, human IL-1Ra was undetectable in the serum of non-transgenic mice (Table 1). At 10 weeks of age on normal chow diet, the average weights and total plasma cholesterol levels were not significantly different in transgenic mice and their respective littermate controls (Table 1). Atherosclerosis was induced experimentally by feeding the mice with a cholesterol-rich diet. The body weight and total plasma cholesterol levels increased significantly after 10 weeks of diet; however, there were no significant differences between transgenic mice and their controls (Table 1).

After 10 weeks of cholesterol-rich diet, the mice were sacrificed and the extent of atherosclerosis was evaluated. Aortic root atherosclerotic lesion areas in ApoE −/− sIL-1Ra Tg mice (0.159±0.014 mm²) were significantly decreased by 47% as compared to ApoE −/− control mice

Table 1
Characteristics of ApoE −/− sIL-1Ra Tg, ApoE −/− icIL-1Ra1 Tg, and ApoE −/− non-Tg mice

	ApoE −/− (n=6)	ApoE −/− sIL-1Ra Tg (n=6)	ApoE −/− (n=10)	ApoE −/− icIL-1Ra1 Tg (n=9)
IL-1Ra (μg/mL)	ND	15.8±3.16	ND	0.63±0.09
Body weight (g)				
Before diet	30.83±0.74	27.08±0.92	20.5±0.5	20.94±1.66
After diet	35.48±2*	31.86±1.23*	27.1±1.12*	25.35±0.96*
Total cholesterol (g/L)				
Before diet	7.05±0.8	7.7±0.54	5.4±0.4	5.28±0.35
After diet	17.67±1.61*	18.67±1.59*	10.9±0.8*	9.72±0.49*
SAA (μg/mL)	5.47±2.2	ND†	2.24±0.18	0.18±0.09†

Mice were fed with a cholesterol-rich diet for 10 weeks. Values represent the mean±S.E.M. ND: not detectable, n: number of mice per group. *$P<0.05$, significantly different before and after diet. †$P<0.05$, significantly different between transgenic and non-transgenic mice.

$(0.3\pm0.029$ mm^2) (Figs. 1a and b, 2a). Similarly, aortic root lesion areas in ApoE −/− icIL-1Ra1 Tg mice $(0.161\pm0.013$ mm^2) were significantly reduced by 40% as compared to their littermate controls $(0.267\pm0.028$ mm^2) (Figs. 1c and d, 2b). Atherosclerotic lesion development was also examined in preparations of the descending aorta stained with Sudan
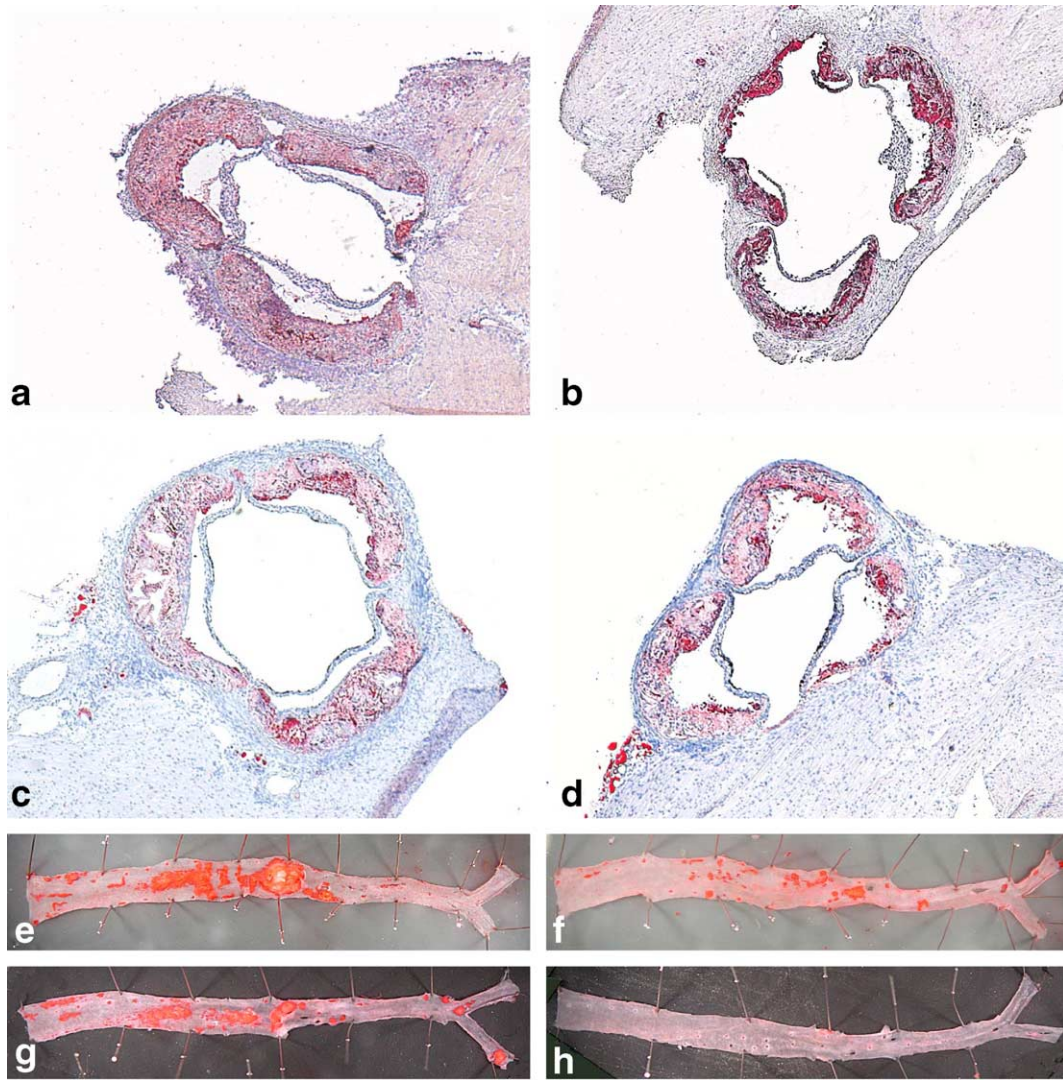

Fig. 1. Representative photographs of atherosclerotic lesions in the aortic roots and thoracoabdominal aortas. ApoE −/− sIL-1Ra Tg mice (b, f), their littermate ApoE −/− controls (a, e), ApoE −/− icIL-1Ra1 Tg mice (d, h), and their littermate ApoE −/− controls (c, g), were fed a cholesterol-rich diet for 10 weeks.
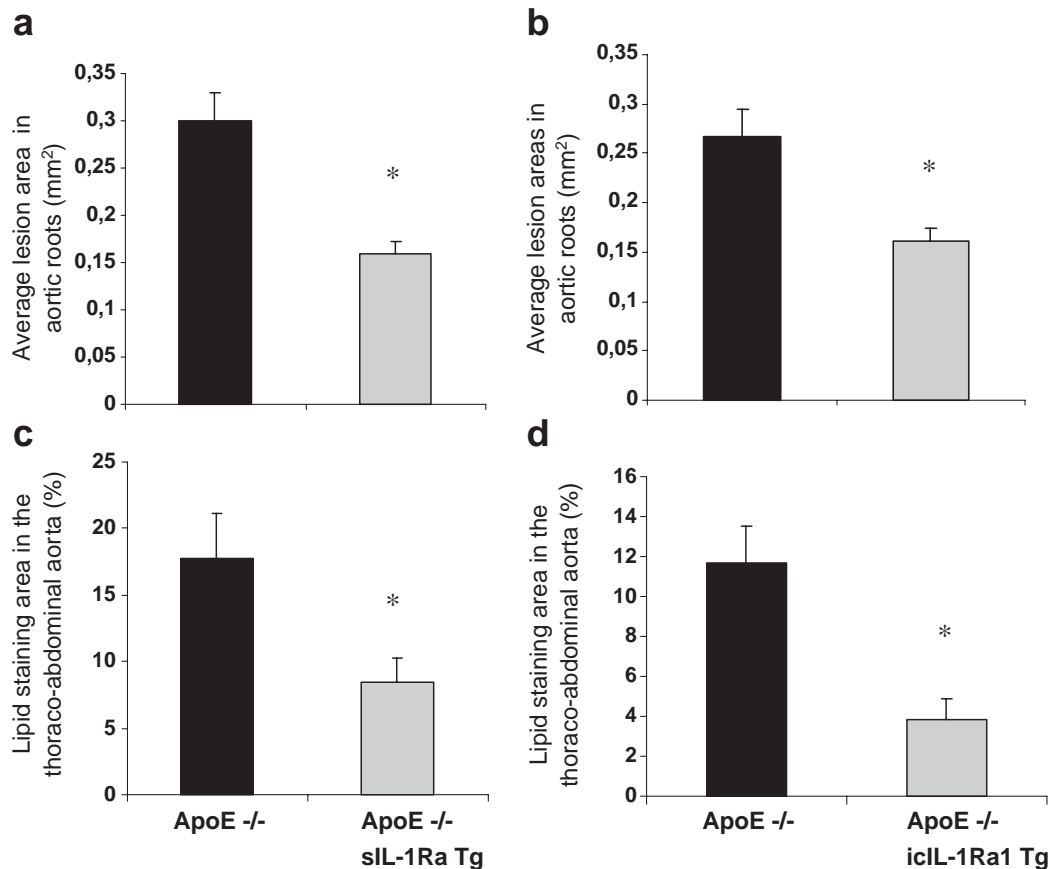
Fig. 2. Quantitative analysis of atherosclerotic lesion areas in aortic roots and thoracoabdominal aortas. After 10 weeks of cholesterol-rich diet, the extent of atherosclerotic lesions was analyzed by computer-assisted image quantification within aortic roots (a, b) and thoracoabdominal aortas (c, d). Data represent mean values±S.E.M. of ApoE −/− sIL-1Ra Tg mice (n=6), their littermate ApoE −/− controls (n=6) (a and c), ApoE −/− icIL-1Ra1 Tg mice (n=9), and their littermate ApoE −/− controls (n=10) (b and d).

IV (Figs. 1e through h, 2c and d). The formation of sudanophilic lipid-rich lesions in abdominal aortas of ApoE −/− sIL-1Ra Tg mice (8.46±1.85 %) or ApoE −/− icIL-1Ra1 Tg mice (3.82±1.04%) decreased significantly by 53% and 67% as compared to their littermate control groups (17.7±3.4% and 11.7±1.83%, respectively). We then examined the formation of atherosclerotic lesions in aortic arch sections stained with hematoxylin–eosin. Atherosclerotic lesions typically developed at lesion-prone sites, such as the lesser curvature of the aortic arch and the outflow tracts of the brachiocephalic, common carotid, and subclavian arteries. Atherosclerotic lesions at the lesser curvature and brachiocephalic arteries were less extensive in ApoE −/− sIL-1Ra Tg mice (Fig. 3b) as compared to ApoE −/− control mice (Fig. 3a). ApoE −/− icIL-1Ra1 Tg mice (Fig. 3d) had very little signs of plaque formation as compared to their littermate controls (Fig. 3c).

SAA is an acute-phase protein produced by the liver in response to pro-inflammatory cytokines, including IL-6 and IL-1. Plasma levels of SAA were undetectable in the serum of ApoE −/− sIL-1Ra Tg mice, whereas detectable levels were measured in the serum of control ApoE −/− mice (5.47±2.2 μg/mL) (Table 1). Similarly, circulating levels of

SAA were significantly lower in ApoE −/− icIL-1Ra1 Tg as compared to ApoE −/− control mice (0.18±0.09 μg/mL versus 2.24±0.18 μg/mL, P<0.05) (Table 1).

3.2. IL-1Ra deficiency promotes uncontrolled vascular inflammation in ApoE −/− mice

To further test the role of endogenous IL-1Ra and IL-1β in the pathogenesis of atherosclerosis and vascular inflammation, we first measured the presence of IL-1Ra and IL-1β in the circulation of ApoE −/− mice. The levels of both cytokines remained undetectable before and after cholesterol-rich diet. As shown in Fig. 4, sIL-1Ra mRNA was present in all the tissue samples from wild-type and ApoE −/− mice fed with normal chow diet, whereas icIL-1Ra1 mRNA was only weakly expressed in some of these mice. In ApoE −/− mice fed with cholesterol-rich diet for 10 weeks, sIL-1Ra mRNA levels were elevated and icIL-1Ra1 increased in only two out of four mice.

To examine the role of endogenous IL-1Ra in the regulation of atherosclerosis, ApoE −/− mice were crossed with IL-1Ra −/− mice to obtain ApoE −/− IL-1Ra +/+, ApoE +/+ IL-1Ra −/−, and ApoE −/− IL-1Ra −/− mice.
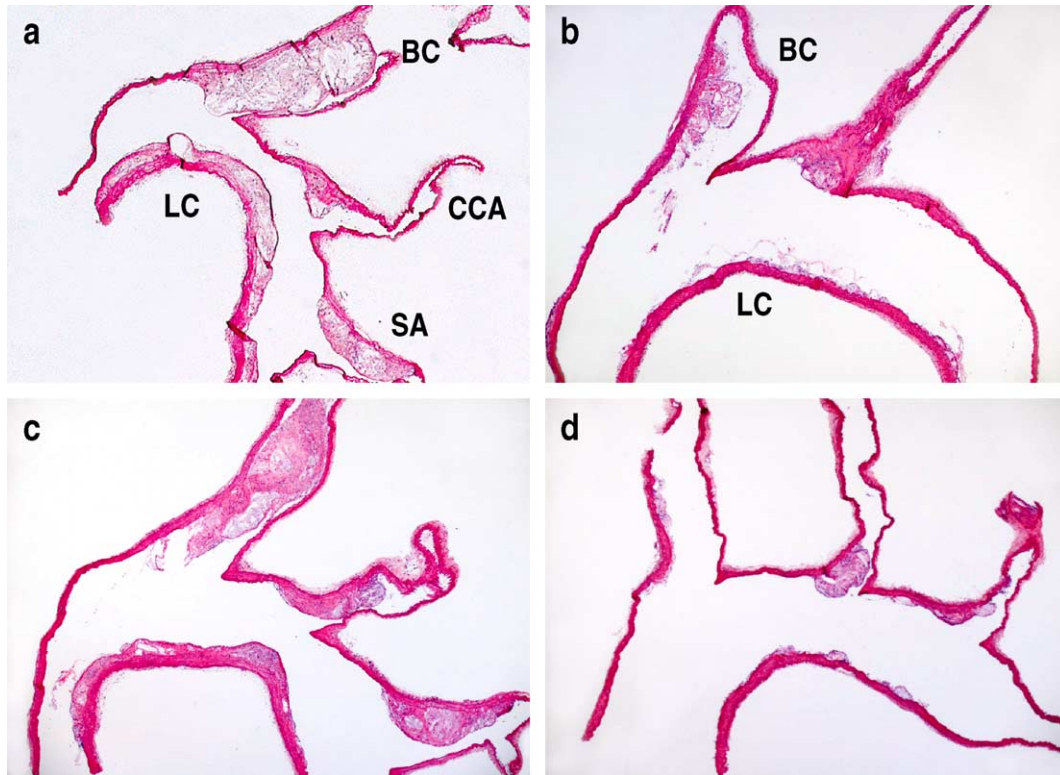
Fig. 3. Atherosclerotic lesions in the aortic arches. Representative photographs of hematoxylin–eosin stained aortic arch sections from ApoE −/− sIL-1Ra Tg mice (b), their littermate ApoE −/− controls (a), ApoE −/− icIL-1Ra1 Tg mice (d), and their littermate ApoE −/− controls (c) fed with cholesterol-rich diet for 10 weeks (a through d, original magnification ×25). LC, lesser curvature; BC, brachiocephalic artery; CCA, common carotid artery; SA, subclavian artery.

At 10 weeks of age on a normal chow diet, plasma total cholesterol levels in ApoE −/− IL-1Ra +/+ and ApoE −/− IL-1Ra −/− were comparable (5.9±0.6 g/L and 4.88±0.546 g/L, respectively) (Table 2). In contrast, the levels of plasma cholesterol in ApoE +/+ IL-1Ra −/− mice (1.22±0.085 g/L) were significantly lower than in ApoE deficient mice (Table 2), but in the same range as wild-type mice (data not shown). At 10 weeks of age, males were fed with cholesterol-rich diet to induce atherosclerosis. Whereas, ApoE −/− IL-1Ra +/+ and ApoE +/+ IL-1Ra −/− mice were apparently healthy, double knockout mice showed signs of severe illness. The death rate was elevated in ApoE −/− IL-1Ra −/− mice (6/12), and thus all the mice were sacrificed after 7 weeks of diet. The evolution of body
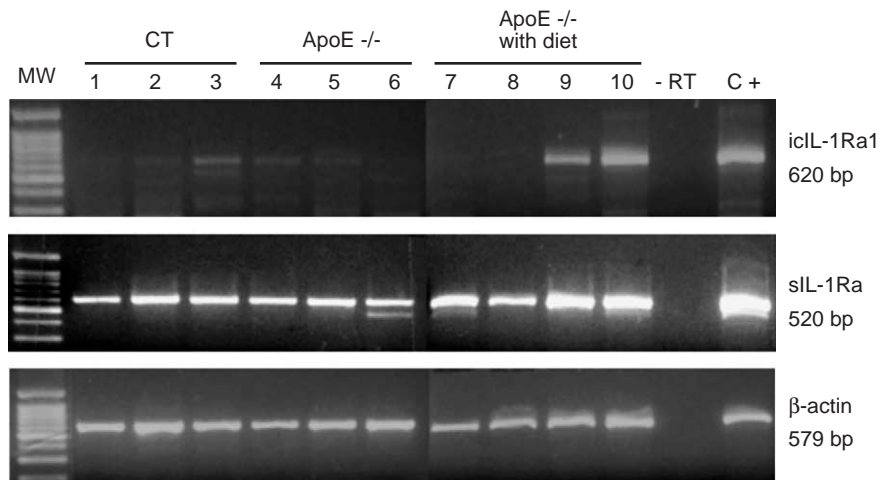

Fig. 4. The presence of IL-1Ra mRNA isoforms in aortic roots with and without atherosclerotic lesions. Aortic roots were dissected from wild-type (lanes 1 to 3) and ApoE −/− mice (lanes 4 to 6) fed with chow diet and ApoE −/− mice fed for 10 weeks with cholesterol-rich diet (lanes 7 to 10). Total RNA was prepared and examined by RT-PCR by using specific primers for icIL-1Ra1, sIL-1Ra, and β-actin mRNA. The PCR products were analyzed by ethidium bromide/agarose gel electrophoresis. Total RNA treated without RT (−RT, lane 11) and thymus total RNA from mice treated with lipopolysaccharide (C+, lane 12) were used as negative and positive controls, respectively. MW represents molecular weight markers.

Table 2
Characteristics of ApoE −/− and/or IL-1Ra −/− mice

	ApoE −/− IL-1Ra +/+ (n=8)	ApoE +/+ IL-1Ra −/− (n=9)	ApoE −/− IL-1Ra −/− (n=6)
Body weight (g)			
Before diet	28.7±0.39	19.5±0.559[†]	17.3±1.045[†]
After diet	35.7±2.567*	22.9±0.976*,[†]	19.6±0.33[†]
Total cholesterol (g/L)			
Before diet	5.9±0.6	1.22±0.085[†]	4.88±0.546
After diet	9.4±0.69*	1.05±0.119[†]	5.475±0.86[†]

Mice were fed with a cholesterol-rich diet for 7 weeks. Values represent the mean±S.E.M., *n*: number of mice per group. *$P<0.05$, significantly different before and after diet. [†]$P<0.05$, significantly different from ApoE −/− IL-1Ra +/+ mice.

weight and plasma cholesterol levels was significantly different in IL-1Ra deficient mice than in IL-1Ra +/+ mice. The mean body weight was significantly lower in ApoE +/+

IL-1Ra −/− and ApoE −/− IL-1Ra −/− mice than in ApoE −/− IL-1Ra +/+ mice (Table 2). Plasma cholesterol levels were markedly increased in ApoE −/− IL-1Ra +/+ (9.4±0.69 g/L), but remained unchanged in ApoE −/− IL-1Ra −/− and ApoE +/+ IL-1Ra −/− mice (5.475±0.86 g/L and 1.05±0.119 g/L, respectively) (Table 2). The levels of IL-1β remained below the detection limit of the ELISA in the three groups of mice.

After 7 weeks of cholesterol-rich diet, we observed the presence of abundant white tissue surrounding the aortic arches in double deficient mice (Fig. 5b) but not in ApoE −/− IL-1Ra +/+ (Fig. 5a) and ApoE +/+ IL-1Ra −/− mice (data not shown). Staining with Sudan IV revealed the presence of atherosclerotic lesions in aortic arches of ApoE −/− IL-1Ra +/+ mice (Fig. 5c), whereas ApoE −/− IL-1Ra −/− (Fig. 5d) and ApoE +/+ IL-1Ra −/− mice (data not shown) did not develop intimal thickening. Most interestingly, histological examination of aortic arches
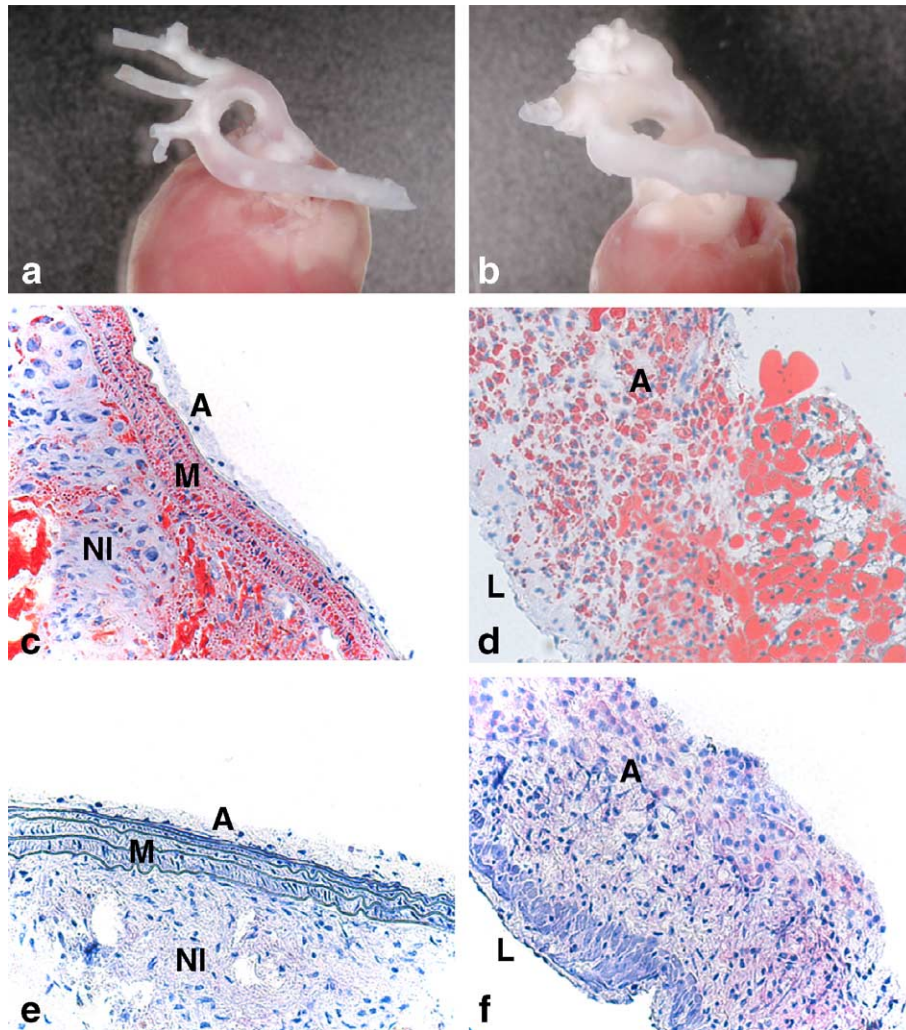

Fig. 5. Macroscopic and histological examination of hearts and aortic arches. ApoE −/− IL-1Ra +/+ (a, c, e) and ApoE −/− IL-1Ra −/− (b, d, f) mice were fed with cholesterol-rich diet for 7 weeks. Mice were sacrificed and heart and aortic arches were taken for macroscopic and histological examination. The results show representative photographs of heart and aortic arches (a and b), representative photographs of 5 μm sections of aortic arches stained in red with Sudan IV for lipid deposition (original magnification ×200) (c and d), and representative photographs of immunostaining for Mac-1 (macrophages) in the brachiocephalic arteries of aortic arches (original magnification ×200) (e and f). A, adventitia; L, lumen; M, media; NI, neointima.

showed the presence of massive infiltration of inflamma-
tory cells full of lipids and staining positive for macro-
phages in the aortic adventitia of ApoE −/− IL-1Ra −/−
mice (Fig. 5d and f). Of note, a milder form of
inflammatory infiltrate in aortic roots was also present in
the adventitia of ApoE −/− IL-1Ra +/+ mice (Fig. 6a and

d) but not in ApoE +/+ IL-Ra −/− mice (Fig. 6b and e).
Van-Gieson Miller stained sections showed that the elastic
lamina within the media of aortic roots and coronary
arteries was destroyed to a large extent in double deficient
mice (Fig. 6c, f, and i), whereas the vascular architecture
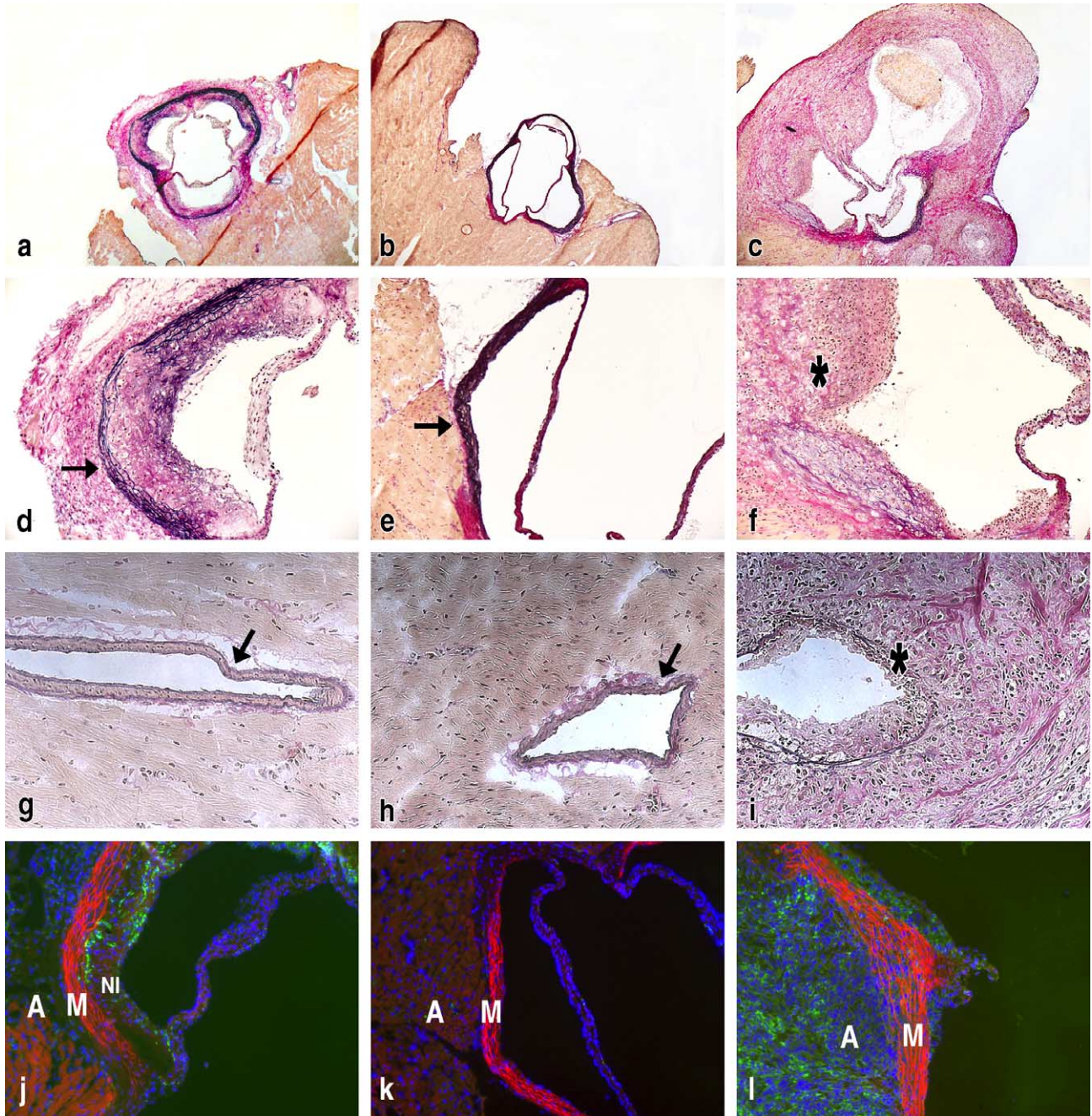was preserved in ApoE −/− IL-1Ra +/+ (Fig. 6a, d, and g)

Fig. 6. Histological examination of inflammation in aortic roots and coronary arteries. ApoE −/− IL-1Ra +/+ (a, d, g, j), ApoE +/+ IL-1Ra −/− (b, e, h, k), and
ApoE −/− IL-1Ra −/− (c, f, i, l) mice were fed a cholesterol-rich diet for 7 weeks. Van-Gieson Miller staining (determination of collagen or fibrotic tissue in
red and elastin areas in purple) shows a complete destruction of elastic lamina and presence of fibrotic tissue in aortic valves (c, f) and coronary arteries (i) of
ApoE −/− IL-1Ra −/− mice as compared to aortic valves (a, d) and coronary arteries (g) of ApoE −/− IL-1Ra +/+ and ApoE +/+ IL-1Ra −/− mice (b, e and
h). Arrow indicates the elastic lamina and * indicates the destruction of the elastic lamina. CD68 staining (j, k, and l) shows massive infiltration of macrophages
(FITC green signal) in the adventitia of aortic valves of ApoE −/− IL-1Ra −/− mice (l) and in the neointima of aortic valves of ApoE −/− IL-1Ra +/+ (j) but
not in ApoE +/+ IL-1Ra −/− mice (k). Sections are counterstained with Evans blue (red signal) and DAPI (blue nuclear signal). Original magnifications ×25
(a through c); ×100 (d through f and j through l); and ×200 (g through i). A, adventitia; M, media; and NI, neointima.

and ApoE +/+ IL-Ra −/− mice (Fig. 6b, e, and h). Immunostaining with an anti-CD68 antibody was performed to demonstrate the presence of macrophages in vascular lesions. Histological analysis of aortic roots demonstrated the presence of CD68-positive cells in the neointima in ApoE −/− IL-1Ra +/+ mice (Fig. 6j), whereas negative staining was observed in ApoE +/+ IL-Ra −/− mice (Fig. 6k). In accordance with the findings in aortic arches, massive macrophage infiltrate was present in the adventitia and intima of ApoE −/− IL-1Ra −/− mice (Fig. 6l).

4. Discussion

In the present study, we showed that overexpression of either sIL-1Ra or icIL-1Ra1 can inhibit the development of atherosclerotic lesions in ApoE −/− mice. In addition, we showed that production of sIL-1Ra mRNA was present in the aortic roots of wild-type and ApoE −/− mice and was further increased with the development of atherosclerotic lesions after cholesterol-rich diet. In contrast, low levels of icIL-1Ra1 mRNA were mainly present in some ApoE −/− mice after diet. By crossing ApoE −/− mice with IL-1Ra −/− mice, we observed that double deficient mice exhibited a severe form of aortic inflammation with massive infiltration of macrophages in the adventitia, lipid accumulation in macrophages, and marked destruction of the elastic lamina. ApoE −/− IL-1Ra +/+ developed atherosclerosis, whereas ApoE +/+ IL-1Ra −/− had no signs of atherosclerosis or vascular inflammation. These results indicate that IL-1 plays a critical role in the pathogenesis of atherosclerosis and vascular inflammation only in the context of ApoE deficiency.

IL-1Ra is produced as four different peptides. sIL-1Ra and icIL-1Ra1 have distinct mRNAs, use different first exons, and are regulated by specific 5′upstream promoters [25]. The icIL-1Ra2 mRNA possesses an additional exon between icIL-1Ra1 and sIL-1Ra first exons [26]. IcIL-1Ra3 is produced by the use of an alternative translation start site primarily from sIL-1Ra mRNA [27,28]. Both sIL-1Ra mRNA and icIL-1Ra1 mRNA are detected in human atherosclerotic coronary arteries. However, human endothelial cells produced only icIL-1Ra1 upon stimulation with transforming growth factor-β and lipopolysaccharide/phorbol 12-myristate 13-acetate (PMA) [29]. Our results indicate that sIL-1Ra mRNA is the predominant isoform in normal and atherosclerotic aortic roots. This finding suggests that, at least in mice, sIL-1Ra may be more important than icIL-1Ra1 in the control of atherogenesis. However, icIL-1Ra3 peptide is also produced from sIL-1Ra mRNA, and may thus also participate in the regulation of IL-1 activities.

In ApoE −/− mice, overexpression of either sIL-1Ra or icIL-1Ra1 exerts similar protective effects regarding the development of atherosclerotic lesions. Consistently, we recently showed that the occurrence of collagen-induced arthritis is completely prevented in both sIL-1Ra and icIL-1Ra1 Tg mice [19]. As significant amounts of icIL-1Ra1 are also present in the circulation, we cannot infer from our data to which extent the protective effect of icIL-1Ra1 is linked to its secretion, as compared to its intracellular action.

The results of recent studies as well as our findings demonstrate that the balance between IL-1 and IL-1Ra is involved in atherogenesis. ApoE −/− mice lacking IL-1β develop less atherosclerotic lesions [30]. In addition, the role of endogenous IL-1Ra has recently been investigated by using IL-1Ra +/− mice which have lower circulating levels of IL-1Ra. ApoE −/− IL-1Ra +/− mice exhibited more severe atherosclerotic lesions than ApoE −/− IL-1Ra +/+ mice, particularly during early stages of the disease. Partial IL-1Ra deficiency alters the composition of atherosclerotic plaques with increased numbers of macrophages and higher levels of mRNA for MCP-1, ICAM-1, and VCAM-1 [31]. These findings suggest that partial IL-1Ra deficiency may be sufficient to promote atherogenesis in the context of particular genetic disorders associated with hypercholesterolemia. The analysis of the IL-1Ra gene (IL-1RN) polymorphism in patients provides an additional support for the role of endogenous IL-1Ra. The association of the IL-1RN*2 allele, a polymorphic variant of IL-1RN consisting in two 86-bp tandem repeats in intron 2, and presence of coronary artery disease and carotid atherosclerosis in patients has been recently described [32,33]. Most interestingly, human umbilical vein endothelial cells isolated from IL-1RN*2 homozygous individuals produced 2- to 3-fold less icIL-1Ra1 than cells from subjects with another more common genotype IL-1RN*1; heterozygous individuals (IL-1RN*1/*2) producing intermediate levels of IL-1Ra [29]. This finding provides a potential link between the results of epidemiological studies and the role of the IL-1/IL-1Ra balance in atherogenesis in humans.

The most impressive finding of our study is the presence of massive vascular inflammation in ApoE −/− IL-1Ra −/− with marked macrophage infiltration in the adventitia and severe destruction of elastic lamina. These results suggest that IL-1Ra deficiency is associated with uncontrolled vascular inflammation in ApoE −/− mice. The mice used in these experiments were obtained by crossing DBA/1 and C57BL/6 mice suggesting a potential role of the background in our findings. However, during the preparation of our manuscript, Isoda et al. described the presence of severe aortic inflammation with identical histological characteristics in ApoE −/− IL-1Ra −/− mice bred into the C57BL/6 background, thus strongly supporting the role of IL-1Ra in the regulation of vascular inflammation in ApoE −/− mice [31]. As recently demonstrated in mice partially deficient in IL-1Ra, the recruitment of macro-

phages in vascular lesions is likely to be related to enhanced expression of several downstream molecules regulated by IL-1 such as adhesion molecules and chemokines [31]. Moreover, the occurrence of severe arteritis mimicking some of the pathologic manifestations of panarteritis nodosa in IL-1Ra −/− bred into a mixed 129×MF1 background supports a role for IL-1Ra in the pathophysiology of vasculitis [9]. Interestingly, our data show that ApoE +/+ IL-1Ra −/− have no signs of vascular pathology. This is consistent with previous results in other mouse backgrounds, including C57BL/6, BALB/cA and DBA/1, indicating that IL-1Ra deficiency alone is not sufficient to promote the development of arteritis.

Acknowledgments

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