# Lack of Interleukin-1β Decreases the Severity of Atherosclerosis in ApoE-Deficient Mice

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***Objective***—Atherosclerosis is considered to be a chronic inflammatory disease and many cytokines participate in the development of atherosclerosis. We focused on the role of interleukin-1β (IL-1β), one of the proinflammatory cytokines secreted by monocytes/macrophages, in the progression of atherosclerosis.

***Methods and Results***—We generated mice lacking both apoE and IL-1β. The sizes of atherosclerotic lesions at the aortic sinus in apoE−/−/IL-1β−/−mice at 12 and 24 weeks of age showed a significant decrease of approximately 30% compared with apoE−/−/IL-1$\beta^{+/+}$ mice, and the percentage of the atherosclerotic area to total area of apoE−/−/IL-1β−/− at 24 weeks of age also showed a significant decrease of about 30% compared with apoE−/−/IL-1$\beta^{+/+}$. The mRNA levels of vascular cell adhesion molecule (VCAM)-1 and monocyte chemotactic protein-1 in the apoE−/−/IL-1β−/− aorta were significantly reduced compared with the apoE−/−/IL-1$\beta^{+/+}$. Furthermore, VCAM-1 was also reduced at the protein level in apoE−/−/IL-1β−/− aorta compared with apoE−/−/IL-1$\beta^{+/+}$.

***Conclusions***—The lack of IL-1β decreases the severity of atherosclerosis in apoE deficient mice, possibly through increased expressions of VCAM-1 and monocyte chemotactic protein-1 in the aorta. **(*Arterioscler Thromb Vasc Biol.* 2003;23:656-660.)**

**Key Words:** interleukin-1β ■ atherosclerosis ■ vascular cell adhesion molecule-1 ■ monocyte chemotactic protein-1

Atherosclerosis is a chronic arterial disease with two life-threatening complications, myocardial and cerebral infarctions. In response to atherogenic stimuli, mononuclear cells in the blood attach, adhere, and spread on the luminal surface of the arterial tree. These cells migrate across the endothelium and accumulate within the intima, particularly at the branches and bifurcations.[1] Substantial evidence has demonstrated that fully oxidized LDL (OxLDL) can contribute to atherogenesis, and many studies have indicated that macrophages in the artery wall take up OxLDL via scavenger receptors, leading to cholesterol ester accumulation and resulting in the formation of foam cells, the hallmark of the arterial fatty streak, which is recognized as the earliest atherosclerotic lesion.[2–5] Previous studies have found many kinds of scavenger receptors, including scavenger receptor class A, SR-BI, CD36, macrosialin, and LOX-1. However, adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1) are usually expressed on the endothelium. A major function of these adhesion molecules is to promote leukocyte recruitment from the vasculature into tissue through a series of events, including leukocyte rolling along the endothelial cell surface, firm adhesion and activation, and extravasation into the tissue.[6] Monocyte chemotactic protein-1 (MCP-1) seems to be the major chemotactic molecule generated within the vessel wall[7,8] and is found in macrophage-rich areas of atherosclerotic lesions.[9] Production of MCP-1 can be induced in several cell types, including endothelial cells, smooth muscle cells, and monocytes/macrophages, usually in response to inflammatory cytokines, such as interleukin-1 (IL-1), IL-4, tumor necrosis factor-α (TNF-α), and interferon-γ.[10,11] Actually, a large number of proinflammatory cytokines have been shown to be expressed in human atherosclerotic lesions, particularly in association with infiltrating monocytes and macrophages.[12] IL-1β, a proinflammatory cytokine with a variety of activities, is strongly induced in monocytes by direct contact with stimulated T lymphocytes. Both these cells are involved in immunoinflammatory diseases (such as rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus) and atherosclerosis.[13] IL-1β has been shown to have important effects on the cell types that constitute atherosclerotic lesions.[14–16]

In this study, we used apoE−/− mice as an animal model of atherosclerosis with hypercholesterolemia and established apoE−/−/IL-1β−/− mice by crossing with each mouse. To clarify the role of IL-1β in atherosclerosis, these two mice

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genotypes were compared regarding the development of atherosclerosis in the aorta. The results showed that the lack of IL-1β decreased the severity of atherosclerosis, and to define the mechanism we examined the effect of IL-1β on macrophages and adhesion molecules in the aorta.

## Materials and Methods

### Animals

ApoE−/− mice in a C57BL/6J background were obtained from the Jackson Laboratory (Bar Harbor, Me) and IL-1β−/− mice, in a C57BL/6J background, were produced by gene targeting as described previously.[17] Each mouse was crossed to establish apoE−/−/IL-1β−/− mice. The mice were screened by polymerase chain reaction (PCR) analysis using blood DNA. PCR for apoE and IL-1β was performed using their selective primers. All mice were kept under specific pathogen-free conditions in an environmentally controlled clean room. During all the experiments, the animals were fed with normal diet containing 4.6% crude fat with less than 0.02% cholesterol (CLEA Japan, Inc., Tokyo). In this study, we used only male mice to exclude the sex difference. The experiments were performed according to the institutional guidelines for animal care of Gifu University.

### Lipid and Lipoprotein Analysis

Male mice at 12 and 24 weeks of age, after fasting for 16 hours, were sacrificed and blood was taken from the inferior vena cava after weighing. Serum concentrations of total cholesterol (TC) and triglyceride (TG) were measured enzymatically using an automatic analyzer (Hitachi 7600, Tokyo). HDL-C was measured by high performance liquid chromatography by determination of cholesterol using on line post-column enzymatic reaction as described previously.[18] Lipoproteins were separated on a superose 6 HR10/30 (Pharmacia, Fine Chemicals, Uppsala, Sweden).

### Atherosclerotic Lesion Analysis

Mice at 12 or 24 weeks of age were sacrificed and the heart and aorta were flushed with saline followed by 10% buffered formalin. The hearts were cut in half and the top half was embedded in either OCT compound or paraffin, sectioned at 10-μm thickness, mounted on slides, and stained with Oil red O. For quantitative analysis of atherosclerotic fatty streak lesions, a section was taken in the middle of the aortic valve. Quantitative analysis of the atherosclerotic lesions was performed on the section where the valves and their attachment sites were visible and two more sections 100 μm above and below the valves. The lesion size for each mouse was measured by NIH image 1.61 (public domain software), and we evaluated the sum of the area in 3 sections. En face analysis of the lesions in the entire aorta was also performed. After perfusion–fixation, the aorta was dissected out, opened longitudinally from the heart to the iliac bifurcations, pinned on a black wax pan, and stained with Oil red O. The total and the atherosclerotic areas of each aorta were measured by NIH image 1.61, and the percentage of the atherosclerotic lesion to total area was evaluated.

### Reverse Transcription (RT)-PCR Amplification

The aortas of 24-week-old mice were dissected and kept in liquid nitrogen. Total RNA was then extracted from the aortas using Isogen (Nippon Gene Co., Ltd., Tokyo) and determined by the absorbance at 260 nm. RT was performed with AMV Reverse Transcriptase XL (Takara Biochemicals, Otsu, Shiga) and real-time PCR on a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) was performed. The following oligonucleotide primer pairs were examined: VCAM-1 sense, 5′-TTT GCC GAG CTA AAT TAC AC-3′; antisense, 5′-ATT CTC CCA TAT TGA ACA ACT A-3′; ICAM-1 sense, 5′-TGC GTT TTG GAG CTA GCG GAC CA-3′; antisense, 5′-CGA GGA CCA TAC AGC ACG TGC AG-3′; MCP-1 sense, 5′-GCC CAG CAC CAG CAC CAG-3′; antisense, 5′-GGC ATC ACA GTC CGA GTC ACA C-3′. The optimum number of cycles was set for each gene product with uniform amplification. Each mRNA level was expressed as the ratio to β actin mRNA.

### Western Blot Analysis

The protein was obtained from 3 aortas of both genotypes at 24 weeks of age using lysing buffer (PBS with 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.1 mg/mL phenylmethyl sulfonyl fluoride, 0.3 TIU/mL aprotinin; Santa Cruz's recommended methods), and 50 μg of protein was separated on 10% SDS polyacrylamide gels and blotted onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA). For detection of VCAM-1 and MCP-1, they were immunoblotted with a primary goat polyclonal anti-VCAM-1 (R&D Systems, Minneapolis, MN) or anti–MCP-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The blots were visualized using ECL Western Blotting Detection Reagents (Amersham Biosciences, Piscataway, NJ).

### Statistical Analysis

Data are reported as means±SEM. Comparisons were performed by the Student *t* test using StatView 5.0 software. Results were considered significant at $P<0.05$

## Results

### Effect of IL-1β on Body Weight and Plasma Lipid Levels

To evaluate the effect of IL-1β on the body growth of mice and plasma lipid levels, we weighed the body and measured the concentrations of TC, HDL-C, and TG in plasma (see online Table I, which can be accessed at http://atvb.ahajournals.org). No significant differences in body weights and plasma lipid levels were found between the two groups of mice at 12 and 24 weeks of age.

### Evaluation of Atherosclerotic Lesions

Proximal aortic sections were stained with Oil red O to compare the atherosclerotic lesion size between apoE−/−/IL-1$\beta^{+/+}$ and apoE−/−/IL-1β−/− mice. We measured the atherosclerotic lesion area in 3 different site sections around the aortic valve per mouse. The atherosclerotic lesion sizes of apoE−/−/IL-1β−/− mice at 12 and 24 weeks of age (Figure 1D–F and J–L, respectively) were smaller than those of apoE−/−/IL-1$\beta^{+/+}$ mice (Figure 1A–C and G–I, respectively) at both weeks of age. The sum of the atherosclerotic lesions in apoE−/−/IL-1β−/− mice at 12 weeks of age (4039±242 mm$^2$; n=10) was significantly reduced by 33%, compared with that in apoE−/−/IL-1$\beta^{+/+}$ mice (6088±477 mm$^2$; n=9; $P<0.01$; see online Figure I, which can be accessed at http://atvb.ahajournals.org). Lesions in apoE−/−/IL-1β−/− mice at 24 weeks of age (8899±678 mm$^2$; n=13) were also significantly reduced by 32% compared with those in apoE−/−/IL-1$\beta^{+/+}$ mice (12998±442 mm$^2$; n=14; $P<0.0001$; Figure I).

Atherosclerotic lesions were also examined throughout the aorta. Although there was no difference in the atherosclerotic lesion area except in the area of the aortic sinus at 12 weeks of age, the percentage of the atherosclerotic lesion to entire aorta (20.3±1.5%; n=13) was significantly reduced in apoE−/−/IL-1β−/− mice compared with that of apoE−/−/IL-1$\beta^{+/+}$ mice at 24 weeks of age (30.7±1.7%; n=14; $P<0.05$; Figure 2).

### mRNA Levels of Adhesion Molecules and Chemokine in the Aorta

To investigate the effect of IL-1β on the development of atherosclerosis, we next paid attention to adhesion molecules

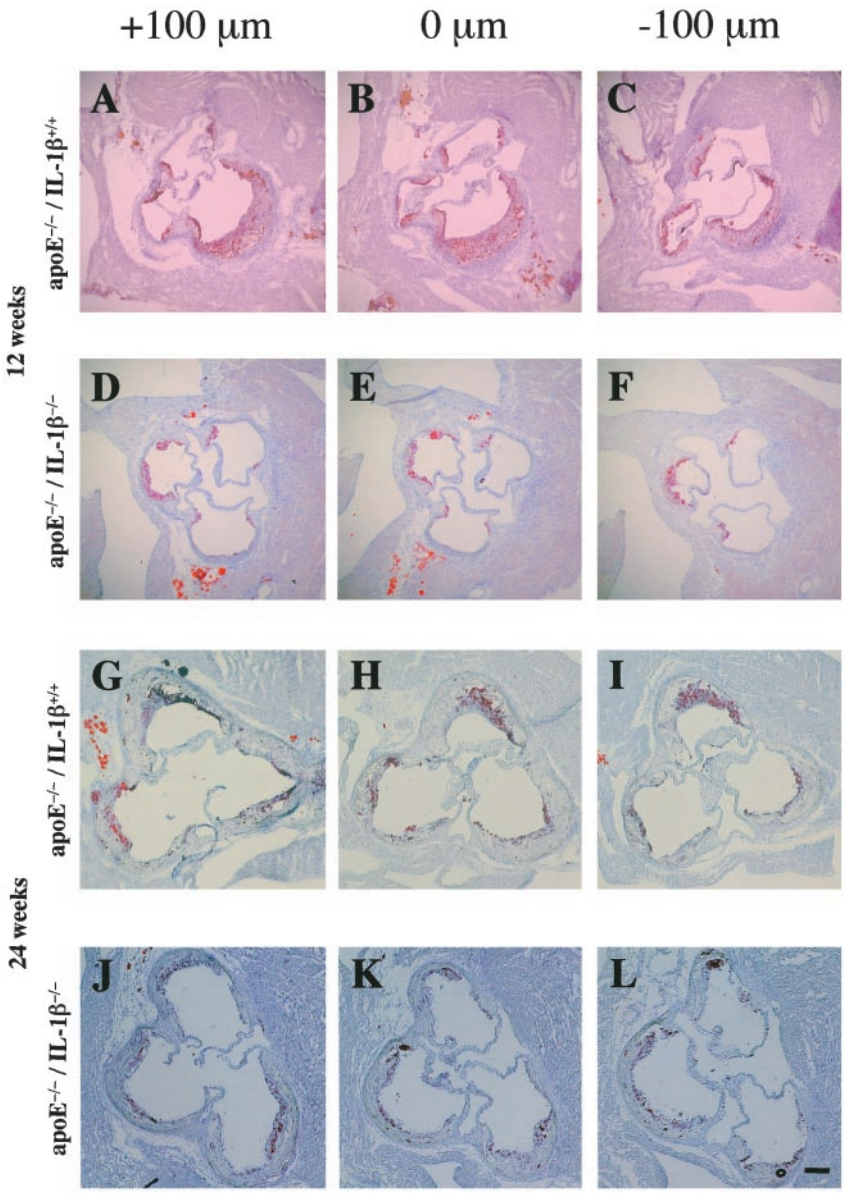


**Figure 1.** Microscopic appearances around the aortic valves of male mice at 12 (A–F) and 24 (G–L) weeks of age. ApoE−/−/IL-1$\beta^{+/+}$ mice (A–C and G–I) and apoE−/−/IL-1$\beta$−/− mice (D to F and J to L) were fed on a normal diet. We observed 3 sections around the aortic valve per mouse at 100-$\mu$m intervals. The section where all aortic valves are visible was regarded as 0 $\mu$m. The left (+100 $\mu$m) and right (−100 $\mu$m) photomicrographs are sections 100 $\mu$m above and below the 0 $\mu$m section, respectively. All sections were stained with Oil red O (magnification, ×40. Bar in L equals 200 $\mu$m).

and MCP-1. The mRNA was extracted from the aorta of each mouse at 24 weeks, because we did not extract enough RNA to perform RT and real-time PCR from the lesion around the aortic valve alone (Figure 3). The level of VCAM-1 mRNA in apoE−/−/IL-1$\beta$−/− mice (0.64±0.05, n=6) was significantly reduced by 39% compared with apoE−/−/IL-1$\beta^{+/+}$ mice (1.06±0.16, n=5; $P<0.05$). Although the level of ICAM-1 mRNA in apoE−/−/IL-1$\beta$−/− mice (0.21±0.05) was also reduced compared with apoE−/−/IL-1$\beta^{+/+}$ mice (0.33±0.05), no significant difference was observed between them ($P=0.11$). However, the level of MCP-1 mRNA in apoE−/−/IL-1$\beta$−/− mice (0.37±0.05, n=6) was significantly decreased by 58% compared with apoE−/−/IL-1$\beta^{+/+}$ mice (0.87±0.13, n=5; $P<0.01$; Figure 3). These observations show that IL-1$\beta$ induces the development of atherosclerosis, at least because of the induction of mRNA of VCAM-1 and MCP-1 in the aorta.

## Adhesion Molecule and Chemokine in the Aorta by Western Blot Analysis

To examine whether the decreased mRNA of VCAM-1 and MCP-1 reflects each protein level, we performed Western blot analysis. As shown in Figure 4, VCAM-1 was reduced in apoE−/−/IL-1$\beta$−/− compared with apoE−/−/IL-1$\beta^{+/+}$ mice. This finding was compatible with the result of mRNA determined by RT-PCR. In contrast, MCP-1 was detected in neither apoE−/−/IL-1$\beta$−/− nor apoE−/−/IL-1$\beta^{+/+}$ mice by Western blot analysis.

## Discussion

In this study, we compared apoE−/−/IL-1$\beta^{+/+}$ and apoE−/−/IL-1$\beta$−/− mice to investigate the effect of IL-1$\beta$ on the development of atherosclerosis. There was no significant difference in plasma lipid levels between two genotypes (Table I). However, Devlin et al[19] reported that IL-1 receptor antagonist increased plasma TC concentrations, especially non-HDL cho-

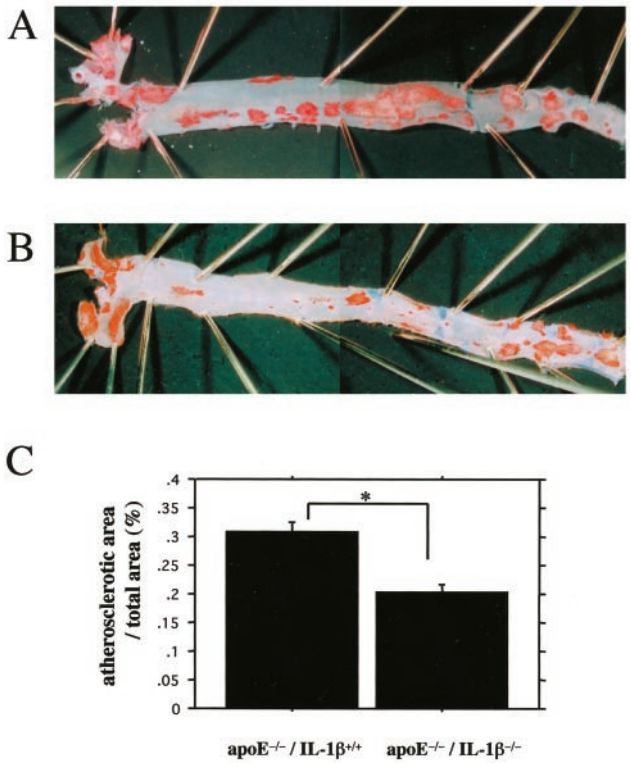


**Figure 2.** Macroscopic findings of total aortae of apoE−/−/IL-1$\beta^{+/+}$ (A, n=14) and apoE−/−/IL-1$\beta$−/− male mice (B, n=13) at 24 weeks of age. These aortae were stained with Oil red O. Comparison of the percentage of the atherosclerotic lesion to total aorta area between two genotypes (C, $^{*}P<0.05$). Values are the mean±SEM.

lesterol, using knockout and transgenic mice of IL-1 receptor antagonist. Although this discrepancy in plasma lipid levels between our study and theirs is unclear, it might be the result of the differences of mice model and the diet.

The present study showed that atherosclerotic lesions at the aortic sinus of apoE−/−/IL-1$\beta$−/− mice were significantly reduced compared with apoE−/−/IL-1$\beta^{+/+}$ mice at both 12 weeks and 24 weeks of age (Figures 1 and I). It was also shown that the percentage of the atherosclerotic lesion to the entire aorta of apoE−/−/IL-1$\beta$−/− mice was significantly reduced compared with apoE−/−/IL-1$\beta^{+/+}$ mice at 24 weeks. These findings suggest that IL-1$\beta$ as one of the proinflammatory cytokines possibly promotes atherosclerosis. Although there was no difference in the atherosclerotic lesion area except in the area of the aortic sinus at 12 weeks of age, localized differences in hemodynamic conditions, including differences in velocity profiles, wall shear stress, and recirculation zones, may be implicated in the differential atheroma localization between the aortic sinus and aorta.[20,21] Atherosclerosis is regarded as a chronic inflammatory disease, and a complex multifaceted disease that begins with monocyte adherence to the activated endothelium and extracellular matrix leading to plaque formation.[9,22,23] Two major processes are involved in the development of atherosclerosis. One is the recruitment of monocytes to the aortic intima, and the other is the foam cell formation of macrophages and/or migrated smooth muscle cells.

In our experiments, we examined the mRNA level of adhesion molecules, VCAM-1 and ICAM-1, and the chemotactic molecule,

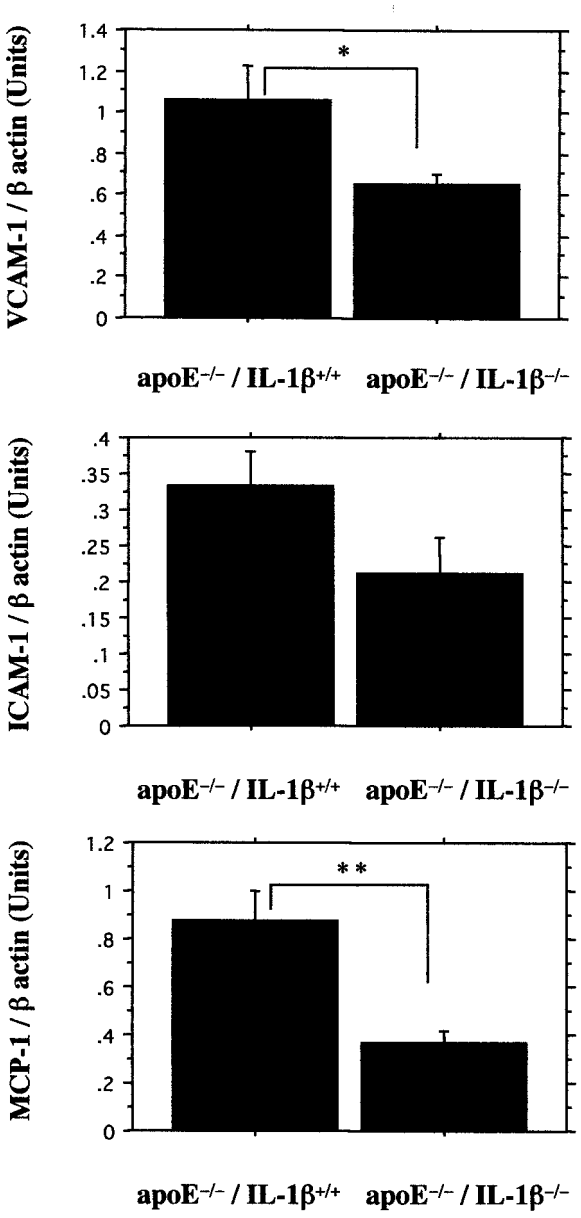


**Figure 3.** Comparisons of the level of adhesion molecules, VCAM-1, ICAM-1, and MCP-1. RNA was extracted from the total aorta at 24 weeks of age, and RT- and real-time PCR were performed ($^{*}P<0.05$, $^{**}P<0.01$). Values are the mean±SEM (apoE−/−/IL-1$\beta^{+/+}$; n=5, apoE−/−/IL-1$\beta$−/−; n=6).

MCP-1, in the aorta by real-time PCR. MCP-1 belongs to the group of CC chemokines that are involved in the recruitment of leukocytes to inflammatory sites and might be critically involved in monocyte/macrophage recruitment to early atherosclerotic lesions.[24] We showed that mRNA of VCAM-1, ICAM-1, and MCP-1 was decreased in apoE−/−/IL-1$\beta$−/− mice. Furthermore, we determined VCAM-1 and MCP-1 by Western blot analysis to further confirm the effect of IL-1$\beta$ on atherosclerosis. VCAM-1 in apoE−/−/IL-1$\beta$−/− aorta at 24 weeks of age was significantly reduced compared with that of the apoE−/−/IL-1$\beta^{+/+}$ aorta, although MCP-1 was detected in neither the apoE−/−/IL-1$\beta$−/− nor apoE−/−/IL-1$\beta^{+/+}$ aorta possibly because of the low sensitivity.

These findings indicate that the lack of IL-1$\beta$ causes the downregulation of VCAM-1 and MCP-1 at both the mRNA and

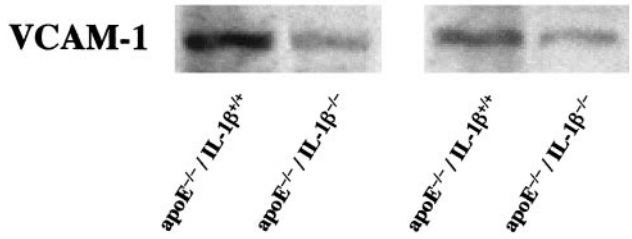


**Figure 4.** Comparison of the VCAM-1 by Western blotting. The protein was obtained from the aortae of 3 animals in both genotypes at 24 weeks of age and 50 $\mu$g of protein was applied per lane. Two independent experiments were performed.

protein levels in the aorta. Previous studies have shown that antibody blockade of VCAM-1 significantly reduced monocyte rolling and adhesion in perfused carotid arteries isolated from apoE−/− mice[25,26] and that local overexpression of MCP-1 at the vessel wall induces the infiltration of macrophages and formation of atherosclerotic lesions.[27] This likely reflects an important function of VCAM-1 and MCP-1 in the recruitment of monocytes to the arterial intima. There has also been a report showing that IL-1$\beta$ is closely associated with atherosclerosis. Platelet-endothelium interactions play a central role in hemostatic and inflammatory mechanisms within the vessel wall, and activated platelets induce the secretion of IL-1$\beta$ and MCP-1 from cultured endothelial cells.[14] In the presence of IL-1 antagonists, both platelet-induced secretion of MCP-1 and surface

of ICAM-1 of human umbilical vein endothelial cells were significantly reduced.[15] It has also been shown that IL-1$\beta$ promotes proliferation of smooth muscle cells in the presence of platelet-derived growth factor in vitro study.[28] Thus, there is a possibility that the lack of IL-1$\beta$ might affect smooth muscle cells to antiatherogenesis in our study.

In conclusion, IL-1$\beta$ deficiency induced an approximately 33% reduction in atherosclerotic lesions in apoE−/− mice. The present study suggests that IL-1$\beta$ exerts an atherogenetic action by enhancing the expression of VCAM-1 and MCP-1 in the aorta, which possibly increases the recruitment of monocytes/macrophages to the intima.

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