Lipid retention in the arterial wall of two mouse strains with different atherosclerosis susceptibility

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Abstract LDL deposition in the subendothelium of arterial walls is the initial event in the development of atherosclerosis. The deposited LDL undergoes oxidative modification by arterial wall cells to become oxidized LDL and consequently contributes to atherosclerotic formation. Using mouse strains C57BL/6J (B6) and C3H/HeJ (C3H), which differ markedly in susceptibility to atherosclerosis, we determined whether variation in subendothelial retention of apolipoprotein B (apoB)-containing lipoproteins constitutes a genetic component in atherosclerosis. Lipoprotein retention was quantitated by Western blot analysis to detect the presence of apoB in aortic walls before foam cells developed. In both dietary and apoE-deficient models, B6 mice exhibited up to a 2-fold increase of apoB in the aortic wall compared with C3H mice. This increase could not be attributed to differences in plasma lipid levels of the two strains. In vitro, endothelial cells from C3H mice took up more acetylated and oxidized LDL but not native LDL and converted more native LDL to oxidized LDL than did endothelial cells from B6 mice. C3H mice expressed more scavenger receptor A in their aortic wall than B6 mice.▪ Thus, variation in the subendothelial retention of apoB-containing lipoproteins cannot explain the dramatic difference in atherosclerosis susceptibility between B6 and C3H mice, and endothelial cells may play a role in alleviating lipid accumulation in arterial walls.—Brown, M. D., L. Jin, M-L. Jien, A. H. Matsumoto, G. A. Helm, A. J. Lusis, J. S. Frank, and W. Shi. **Lipid retention in the arterial wall of two mouse strains with different atherosclerosis susceptibility.** *J. Lipid Res.* 2004. **45:** 1155–1161.

Supplementary key words low density lipoprotein retention • endothelial cells • scavenger receptor

Atherosclerotic disease begins with the accumulation of atherogenic plasma lipoproteins, predominantly LDL, in susceptible arteries (1). An increase in plasma LDL levels leads to an increased rate of entry of LDL into the intima and a higher level of LDL in the intima (2, 3). The accumulated LDL undergoes oxidative modification by arterial wall cells, including endothelial cells and smooth muscle cells, to become oxidized LDL (OxLDL). Even minimally oxidized LDL (MM-LDL) can increase the adherence and penetration of monocytes, partially by stimulating the release of monocyte chemotactic protein-1 (MCP-1) from endothelial cells (4). MM-LDL can also stimulate the release of macrophage colony-stimulating factor (M-CSF), vascular cell adhesion molecule-1 (VCAM-1), and other adhesion molecules and growth factors that promote monocyte transmigration into the subendothelium and differentiation into macrophages (5–7). More fully oxidized LDL is itself directly chemotactic for monocytes and is also one of the major ligands for scavenger receptors on macrophages, leading to foam cell formation (1).

OxLDL and acetylated LDL (AcLDL) can be rapidly taken up not only by macrophages but also by endothelial cells. In fact, the uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-labeled AcLDL is widely used for defining the identity of isolated endothelial cells (8). The receptors involved in the uptake of OxLDL and AcLDL are called scavenger receptors, which include SR-A (9), CD36 (10), CD68 (11), and LOX-1 (12). Studies have shown that endothelial cells express SR-A, CD36, and LOX-1 (12–16). Thus, we speculate that endothelial cells play a protective role in atherosclerosis by reducing the retention of OxLDL in the subendothelium.

The availability of various mouse strains that differ in atherosclerosis susceptibility provides an experimental approach to identifying a specific event in atherogenesis. C57BL/6J (B6) and C3H/HeJ (C3H) mice are two commonly used inbred strains that differ strikingly in atherosclerotic lesion formation when fed a high-fat/cholesterol

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diet with cholate or in the apolipoprotein E-deficient (apoE$^{-/-}$) background (17, 18). We recently found that endothelial cells of the two strains differ markedly with regard to the induction of inflammatory genes by OxLDL (19). In the present study, we determined whether variation in the retention of apoB-containing lipoproteins in the arterial wall is associated with the marked difference between B6 and C3H mice in atherosclerosis susceptibility, and we have now provided experimental evidence that genetic factors influence the accumulation of apoB-containing lipoproteins in the arterial wall and that endothelial cells may protect against atherosclerosis by alleviating the subendothelial retention of LDL.

MATERIALS AND METHODS

Mice

Female B6, C3H, and B6.apoE$^{-/-}$ mice were purchased from Jackson Laboratories (Bar Harbor, ME). Female C3H.apoE$^{-/-}$ mice were generated in our laboratory by sequentially backcrossing B6.apoE$^{-/-}$ mice 10 times to C3H mice. ApoE$^{-/-}$ mice were fed a standard rodent chow containing 4% fat (Ralston-Purina Co.) throughout the entire period of experiments. Wild-type mice were fed either chow or a high-fat/cholesterol diet containing 15% fat, 1.25% cholesterol, and 0.5% cholic acid (TD 90221; Food-Tek, Inc.). All procedures were in accordance with current National Institutes of Health guidelines and were approved by the University Animal Care and Use Committee.

Lipoprotein isolation and modification

Human LDL ($d = 1.019$–1.069 g/ml) was isolated from the plasma of healthy donors by density-gradient ultracentrifugation as described (20), dialyzed with PBS containing 1 mM EDTA, filtered through 0.22 μm filters, and stored at 4°C. OxLDL was prepared by incubating LDL with 4 μM CuSO$_4$, as described by Watson et al. (21). LDL and OxLDL were labeled with the fluorescent probe DiI by the method described by Pitas et al. (22). A stock solution of the fluorescent probe DiI (Molecular Probes) was prepared by dissolving 3 mg of DiI per milliliter of DMSO; 250 μg of the DiI solution was added to 5 mg of LDL or OxLDL in lipoprotein-deficient serum and filtered. After incubating this mixture at 37°C for 8–15 h, the density was adjusted to 1.063 g/ml with potassium bromide. The labeled LDL or OxLDL was reisolated by ultracentrifugation at 40,000 rpm for 24 h at 15°C and dialyzed against sodium chloride. Pitas et al. (22) reported that the density of LDL was not altered by DiI labeling as determined by SDS-PAGE. Protein concentration was determined by the Lowry assay. DiI-labeled AcLDL was purchased from Biomedical Technologies.

Isolation and culture of endothelial cells

Endothelial cells were isolated from the thoracic aorta of mice by an established explantation technique (19). Initially, the aortic segments were placed on Matrigel and incubated in DMEM supplemented with 15% FBS, 1% penicillin/streptomycin, 90 μg/ml heparin, 60 μg/ml endothelial cell growth supplements, and 100 U/ml Fungizone. The vessel segments were removed once cell outgrowth was observed. The cells were passaged with Dispase and then plated into gelatin-coated culture dishes. The subsequent passages were performed with 0.25% trypsin-EDTA. Immunostaining for the von Willebrand factor and the DiI-labeled AcLDL uptake experiment confirmed that this method generated pure endothelial cells.

Protein preparation and Western blot analysis

The presence of apoB, SR-A, and α-actin in the descending thoracic aorta and of CD36, LOX-1, and GAPDH in cultured endothelial cells was determined by Western blot analysis. The aortic protein was prepared as we previously described (23). Briefly, the aorta was washed thoroughly with PBS containing 5 U/ml heparin and 1 mM EDTA through the left ventricle of the heart, cleaned of periadventitial fat and connective tissues, and snap-frozen in liquid nitrogen. The frozen aorta was mechanically broken up and then dispersed in lysis buffer containing 10 mM Tris, pH 8, 1 mM EDTA, 2.5% SDS, and 5% mercaptoethanol. The cultured endothelial cells were directly lysed with the lysis buffer. The resultant lysate was centrifuged at 500 g for 10 min at 4°C, and the supernatant was collected and used for Western blot analysis. Ten micrograms of protein was separated by electrophoresis on 4~12% Tris-polyacrylamide gels and electrophoretically transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies to mouse apoB-48 (generated in our laboratory; the specificity of this antibody was verified with an antibody for mouse apoB-48 from BioDesign International), SR-A (Serotec), α-smooth muscle actin (DAKO), CD36, LOX-1 (Santa Cruz), or GAPDH (Chemicon International) for 1 h and then incubated for 0.5 h with horseradish peroxidase-conjugated secondary antibodies. The signals were detected by the enhanced chemiluminescence detection method according to the manufacturer's instructions (ECL Western blotting; Amersham). The density of the bands was quantified with a densitometer (Molecular Dynamics).

Freeze-fracture and deep-etch electron microscopy

The accumulation of lipids in the aortic walls of 6 week old apoE$^{-/-}$ mice on a chow diet was examined by high-resolution, freeze-etch electron microscopy as previously described by Tamminen et al. (24). Pieces of aorta from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice were removed from regions of predilection for atherosclerosis, placed endothelial side up on rectangles of gelatin, and ultrarapidly frozen in liquid nitrogen. Frozen pieces of aorta were fractured at -150°C to -110°C, and sections were examined with an electron microscope (JEOL 100CX).

Plasma lipid measurements

Mice were fasted overnight before blood was collected through retro-orbital veins under isoflurane anesthesia. Plasma total cholesterol, HDL cholesterol, and triglyceride were measured with enzymatic assays as previously described by Hedrick et al. (25).

Uptake of native LDL, AcLDL, and OxLDL by endothelial cells

Confluent endothelial cells in six-well plates were incubated with 2 ml of DMEM per well containing 1% FBS before they were treated in duplicate with 1 ml of DMEM per well containing 50 μg/ml DiI-labeled native LDL, OxLDL, or AcLDL. After 2 h of incubation at 37°C, the medium was removed and the cell monolayer was washed three times with PBS. The cells were then incubated with 1.5 ml of ethanol per well for 1 h at room temperature with gentle rotation to extract internalized LDL. The extracts were then centrifuged at 2,000 rpm for 10 min to remove cellular debris. The absorbance of the supernatant was determined at 547 nm (excitation) and 572 nm (emission). Cell protein of each well was determined by the Bio-Rad protein assay with BSA as the standard. Endothelial uptake of LDL was expressed as micrograms of LDL internalized per milligram of cell protein in 2 h.

Oxidation of native LDL by endothelial cells

Once endothelial cells grew to confluence in six-well plates, culture medium was removed and the cells were washed twice with Ham's F-10 medium. The cells were then incubated at 37°C with 2 ml of Ham's F-10 medium per well containing 250 μg/ml LDL for up to 48 h. After various periods of incubation, the LDL-containing medium was removed and butylated hydroxytoluene was added to prevent additional oxidation. The amount of oxidized lipids in the LDL-containing medium was assessed by measuring thiobarbituric acid-reactive substances (TBARS) as described by Morel and Chisolm (26). Briefly, 200 μl of TBARS agents (1.442 g of thiobarbituric acid and 3.15 g of Trizma base in 100 ml of water, pH 7.4) and 375 μl of 40% trichloroacetic acid in 1 N HCl were sequentially added to each sample (100 μl of 250 μg/ml LDL), and the resulting mixture was heated at 90°C for 45 min. After adding 900 μl of 0.9% NaCl solution, the samples were centrifuged at 2,500 rpm for 15 min. The absorbance of the supernatants was read at 532 nm, and the values were expressed as nanomolar malondialdehyde (MDA) equivalents per milligram of LDL protein.

The amount of OxLDL in the LDL-containing medium was also determined by enzyme-linked immunosorbent assay with a commercially available ELISA kit (Mercodia) according to the manufacturer's instructions.

Statistical analysis

Data are presented as means ± SEM, with n indicating the number of mice. ANOVA was used to compare differences between B6 and C3H mice in the production of TBARS. Student's t-test was used to compare differences between the two strains in expression levels of proteins on Western blotting and in plasma levels of lipids. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Lipid deposition in the arterial wall

Lipid deposition in the arterial wall of B6 and C3H mice was examined by Western blot analysis using both dietary and apoE$^{-/-}$ models. As shown in **Fig. 1**, there were no detectable apoB-containing lipoproteins in the aortic wall of wild-type B6 and C3H mice when fed a standard rodent chow diet. After 2 weeks on the atherogenic diet, both strains exhibited significant retention of apoB in the aortic wall. However, B6 mice showed a 1.5-fold increase in the optical density of the apoB band compared with C3H mice (29,628 ± 1,430 vs. 20,448 ± 2,362; $P = 0.039$). ApoB levels in the aortic wall of 6 week old apoE$^{-/-}$ mice were also examined when they were on the chow diet. At this stage, both apoE$^{-/-}$ strains are known to have no atherosclerotic lesions in the aorta (18, 27). Nevertheless, B6.apoE$^{-/-}$ mice exhibited a 1.7-fold increase over C3H.apoE$^{-/-}$ mice in the optical density of the apoB band (114,286 ± 11,044 vs. 65,787 ± 11,625; $P = 0.023$).

Freeze-fracture and deep-etch electron microscopy was used to determine the distribution of accumulated lipids in the aortic wall of B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. As shown in **Fig. 2**, in both apoE$^{-/-}$ mouse strains lipid particles were observed in the subendothelial space of the vessel. These lipid particles were sitting on extracellular matrix, such as collagen fibrils. Also, B6.apoE$^{-/-}$ mice
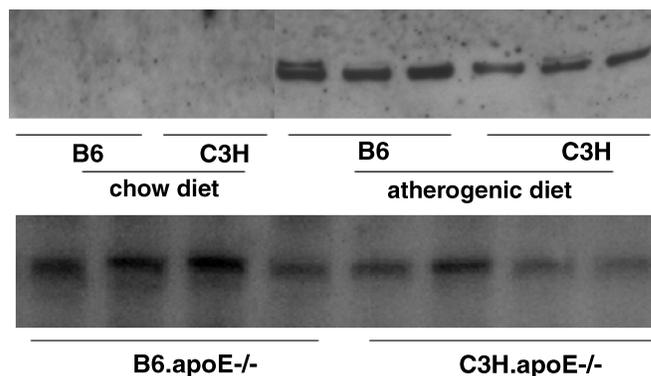
Fig. 1. Western blot analysis of apolipoprotein B (apoB) level in the aortic wall of C57BL/6J (B6) and C3H/HeJ (C3H) mice. Ten micrograms of protein prepared from the thoracic aorta was electrophoresed on 4~12% Tris polyacrylamide gels, transferred onto nitrocellulose membranes, and probed with an antibody to mouse apoB-48. Each lane represents one individual mouse. ApoB was not detectable in the arterial wall of wild-type B6 and C3H mice when fed a chow diet, whereas after 2 weeks on a high-fat/cholesterol diet containing cholate, B6 mice exhibited a significant increase in apoB deposition in their arterial wall compared with C3H mice (upper panel). The lower panel shows that B6 apoE-deficient (B6.apoE$^{-/-}$) mice had significantly more apoB in their aortic wall than C3H.apoE$^{-/-}$ mice at 6 weeks of age on the chow diet.
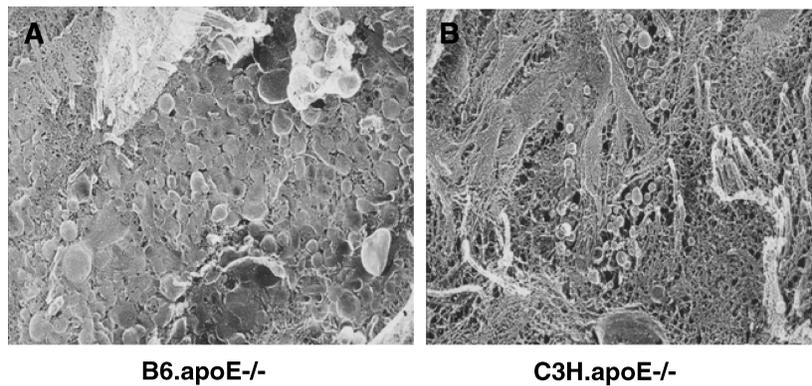
had more lipid particles present in the intima than did C3H.apoE$^{-/-}$ mice.

Plasma lipid levels

Plasma lipid levels of B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice were examined when they were maintained on the chow diet (**Fig. 3**). Although both strains developed mild hyperlipidemia, C3H.apoE$^{-/-}$ mice had higher plasma levels of total cholesterol (480 ± 24 vs. 354 ± 10 mg/dl; $P = 0.00014$) and triglyceride (128 ± 23 vs. 37 ± 2.5 mg/dl; $P = 0.0017$) than did B6.apoE$^{-/-}$ mice. Because the HDL cholesterol levels were comparable between the two strains (20 ± 7 mg/dl in C3H.apoE$^{-/-}$ vs. 21 ± 3 mg/dl in B6.apoE$^{-/-}$), C3H.apoE$^{-/-}$ mice had a higher plasma LDL/VLDL level than did B6.apoE$^{-/-}$ mice. Thus, the variation in plasma LDL/VLDL levels was unlikely to explain the difference in arterial apoB levels of the two strains.

Internalization of LDL by endothelial cells

Uptake of native LDL, AcLDL, and OxLDL by endothelial cells from the aorta of B6 and C3H mice was examined after DiI-labeled LDL was incubated with the cells for 2 h at 37°C. As shown in **Fig. 4**, endothelial cells from the two strains internalized comparable amounts of native LDL (4.35 ± 0.87 μg/mg cell protein in B6 vs. 4.96 ± 1.29 μg/mg cell protein in C3H; $P = 0.72$). In contrast, endothelial cells from C3H mice showed more than 2-fold increases in the uptake of AcLDL (3.35 ± 0.50 vs. 1.59 ± 0.38 μg/mg cell protein; $P = 0.048$) and OxLDL (6.34 ± 0.28 vs. 3.04 ± 0.30 μg/mg cell protein; $P = 0.0013$).

Fig. 2. Freeze-etch electron photomicrographs of the intima of the aorta from 6 week old apoE$^{-/-}$ mice. Lipid particles are present in the subendothelium in both B6.apoE$^{-/-}$ mice (left) and C3H.apoE$^{-/-}$ mice (right). Lipid particles range in size from 30 to 66 nm. In B6.apoE$^{-/-}$ mice, lipid particles appear to be fusing to form larger particles ($\times 75,000 \sim 85,000$).

LDL oxidation by endothelial cells

Endothelial cells from the aorta of B6 and C3H mice were incubated with human native LDL in Ham's F-10 medium at a concentration of 250 μg/ml for up to 48 h. LDL oxidation was measured by assessing TBARS production. Without the presence of endothelial cells, TBARS formation increased slightly over time (6.9 nmol MDA/mg LDL at 48 h vs. 1.2 nmol MDA/mg LDL at 0 h; **Fig. 5A**). When endothelial cells from either strain were present, the production of TBARS increased significantly ($P < 0.05$). During the entire period of experiments, C3H endothelial cells displayed a significant increase in TBARS levels compared with endothelial cells from B6 mice (maximal amount, 33.6 ± 1.9 versus 18.3 ± 1.1 nmol MDA/mg LDL; $P = 0.0023$).

Immunoassays were used to further assess the amounts of OxLDL in the LDL medium that was incubated with endothelial cells from the two strains (Fig. 5B). There was more OxLDL in the LDL medium that was incubated with C3H endothelial cells than in the medium that was incu-

bated with B6 endothelial cells. The difference in the concentration of OxLDL in the medium was statistically significant after 12 h of incubation ($92,567 \pm 8,657$ U/l in C3H vs. $70,458 \pm 5,399$ U/l in B6; $P = 0.0483$).

Expression of scavenger receptors

Expression of SR-A and α-actin in the thoracic aorta and of CD36, LOX-1, and GAPDH by cultured endothelial cells from the vessel was compared between B6 and C3H mice by Western blot analysis (**Fig. 6**). There was nearly a 2-fold increase in the optical density of the SR-A band in C3H mice than in B6 mice ($133,571 \pm 18,415$ vs. $68,486 \pm 9,277$; $P = 0.030$). The optical density of the α-actin band was not significantly different between C3H mice ($157,682 \pm 16,434$) and B6 mice ($160,663 \pm 14,567$; $P = 0.90$). The expression of CD36 by endothelial cells was slightly higher in B6 mice ($79,431 \pm 1,663$) than in C3H mice ($74,364 \pm 1,128$; $P = 0.0397$), whereas the expression of LOX-1 and GAPDH was comparable between the two strains ($P > 0.05$).
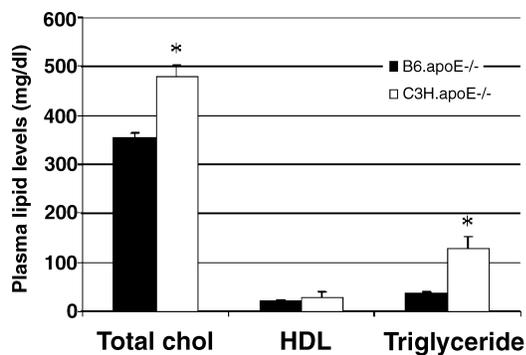
Fig. 3. Plasma levels of total and HDL cholesterol and triglyceride in B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice on the chow diet. Blood was collected after mice were fasted overnight. Plasma cholesterol and triglyceride were measured with enzymatic assays. Values shown are means $\pm$ SEM for 9–20 mice. * $P < 0.05$ versus B6.apoE$^{-/-}$ mice.
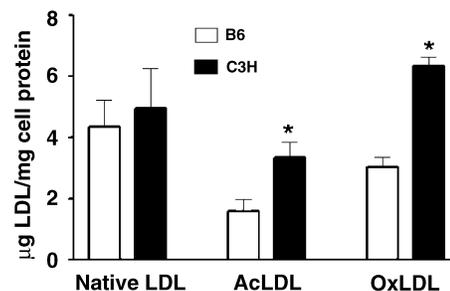
Fig. 4. Uptake of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled native LDL, acetylated LDL (AcLDL), and oxidized LDL (OxLDL) by endothelial cells from B6 and C3H mice. DiI-labeled LDLs were incubated in duplicate with confluent endothelial cells for 2 h at 37°C, and then the internalized LDLs were extracted for measurements of fluorescence intensity. Values are expressed as micrograms of internalized LDL per milligram of cell protein. Results shown are means $\pm$ SEM for endothelial cells from three mice of each strain. * $P < 0.05$ versus B6 mice.

A

B
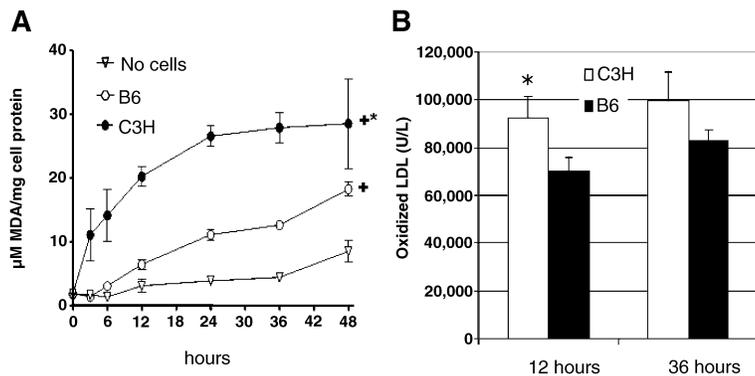
Fig. 5. Oxidation of LDL by endothelial cells isolated from the aorta of B6 and C3H mice. Confluent endothelial cells were incubated for up to 48 h with Ham's F10 medium containing 250 μg/ml human native LDL. After various periods of incubation, the levels of oxidized lipids in the medium were evaluated by measuring thiobarbituric acid-reactive substance formation and expressed as nanomolar malondialdehyde (MDA) per milligram of LDL protein (A) or by measuring OxLDL with an ELISA kit and expressed as units per liter of supernatant (B). Values shown are means ± SEM for five separate experiments using endothelial cells from five individual mice of each strain. * $P < 0.05$ versus B6 mice; + $P < 0.05$ versus no cells.

DISCUSSION

The deposition of apoB-containing lipoproteins, primarily LDL, in the subendothelial space of the arterial wall is the initial event in the development of atherosclerosis. In this study, we determined whether variation in the retention of apoB-containing lipoproteins in the arterial wall constitutes a genetic component in atherosclerosis through the use of B6 and C3H mouse models. The major finding was that atherosclerosis-susceptible B6 mice had significantly more apoB in their aortic wall than did atherosclerosis-resistant C3H mice. We also found that endothelial cells from the aorta of C3H mice had increased abilities to transform native LDL to OxLDL and to take up OxLDL in comparison with endothelial cells of B6 mice.

We used both dietary and apoE$^{-/-}$ models to determine the retention of apoB-containing lipoproteins in the aortic wall before foam cells developed in B6 and C3H mice. Wild-type mice were fed a high fat/cholesterol diet containing cholate for 2 weeks, and 6 week old apoE$^{-/-}$ mice were raised on the chow diet before they were used for the assessment of apoB in the aortic wall. At these stages, both strains are known to have no detectable atherosclerotic lesions in the aorta. Indeed, Paigen et al. (28) have reported that foam cells can only be observed at the aortic root after B6 mice are fed the atherogenic diet for 6 weeks and that in C3H mice there are essentially no foam cells even after they are fed the atherogenic diet for up to 12 months. For apoE$^{-/-}$ mice, Nakashima et al. (27) have demonstrated that foam cells develop in the aorta at 8 weeks of age. Thus, the variation in apoB levels in the aortic wall observed in the present study was unlikely to be associated with the differences between the two strains in the number of foam cells.

Lipoprotein retention in the arterial wall is well dependent on the level of lipoproteins in the plasma (2). However, we found that variation in plasma lipid levels cannot explain the difference in apoB retention in the aortic wall because in the apoE$^{-/-}$ background, C3H mice had a higher plasma LDL level but displayed a lower apoB level in the aortic wall than did B6 mice. Nevertheless, in the dietary model, the higher plasma HDL level could partially account for the lower arterial apoB level of C3H mice when fed the atherogenic diet (Fig. 1). On the atherogenic diet, the two strains had similarly increased plasma levels of LDL/VLDL, whereas the HDL level was decreased in B6 but not in C3H mice (29). HDL has been shown to promote cholesterol efflux from cells of the arterial wall through absorption of cell membrane cholesterol at the cell surface and through its apoA-I and apoA-II moieties (30). We also found that there was no detectable apoB accumulation in the aortic wall when wild-type B6 and C3H mice were fed the chow diet. On the low-fat/cholesterol chow diet, both strains have comparable plasma levels of lipoproteins, which consist predominantly of HDL (28, 29). The high plasma HDL level probably plays a key role in preventing the deposition of apoB-containing lipoproteins in the arterial wall.

SR-A

Actin

CD36

LOX-1

GAPDH

B6.apoE-/-   **C3H.apoE-/-**
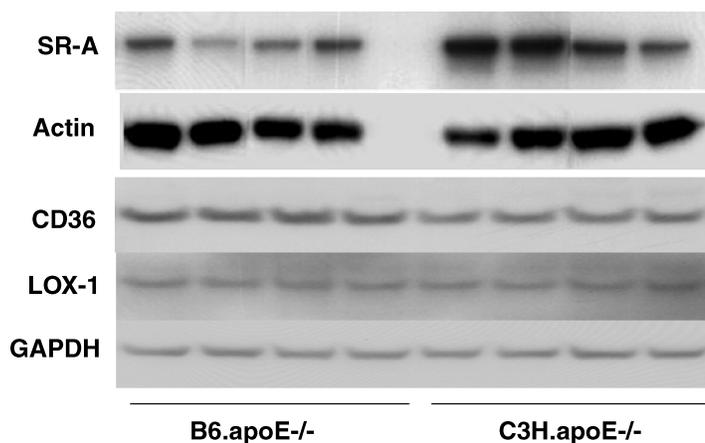
Fig. 6. Western blot analysis of SR-A and α-actin proteins in the aorta and of CD36, LOX-1, and GAPDH proteins in endothelial cells from B6 and C3H mice. Ten micrograms of protein was electrophoresed on 4~12% Tris polyacrylamide gradient gels, electroblotted onto nitrocellulose membranes, and probed with antibodies for the proteins. Each lane represents one individual mouse.

In the present study, we found that endothelial cells from C3H mice internalized significantly more OxLDL and AcLDL than endothelial cells from B6 mice, although the uptake of native LDL was comparable between the two strains. Moreover, we found that C3H endothelial cells had an increased transformation of native LDL to OxLDL compared with B6 endothelial cells. These differences in endothelial function could contribute to the variation in the retention of apoB-containing lipoproteins in the aortic wall of the two strains. Thus, it was likely that hyperlipidemia resulted in the accumulation of LDL in the subendothelial space, where it underwent oxidative modification by endothelial cells and vascular smooth muscle cells to become OxLDL. The OxLDL was then taken up and degraded by overlying endothelial cells, leading to reduction in accumulated LDL in the subendothelium. It appeared that because the endothelial cells of C3H mice modified LDL more rapidly and subsequently took up more OxLDL than did the endothelial cells of B6 mice, the deposition of LDL would be less in C3H than in B6 mice. Endothelial cells may modulate the accumulation of apoB-containing lipoproteins in the arterial wall through other mechanisms, such as influencing the kind of subendothelial matrix that is responsible for the lipoprotein retention and the nature of the lipoproteins that are available for retention.

The uptake of OxLDL and AcLDL is mediated by scavenger receptors, including SR-A, CD36, and LOX-1, on cellular membranes. In this study, we found that C3H mice expressed significantly more SR-A protein than B6 mice in the aorta. Thus, SR-A might contribute to the increased uptake of OxLDL and AcLDL by endothelial cells in C3H mice. B6 mice expressed slightly more CD36 than C3H mice, and the two strains expressed comparable levels of LOX-1 in their endothelial cells. Therefore, CD36 and LOX-1 could not explain the difference between the two strains in the endothelial uptake of OxLDL and AcLDL.

There are greater than 100-fold differences between B6 and C3H mice in the size of aortic lesions in either the dietary or the apoE$^{-/-}$ model (18, 28). Obviously, the less than 2-fold difference in subendothelial LDL retention is unlikely to explain the marked difference in lesion formation of the two strains. Recently, we observed a marked difference in the response of endothelial cells of the two strains to OxLDL. OxLDL induced a pronounced expression of inflammatory and oxidative stress genes in the endothelial cells of B6 mice, but in the cells of C3H mice, the induction was minimal (18, 19). The endothelial cells of C3H mice produced more OxLDL, but this might have little impact on the pathogenesis of atherosclerosis in the mice as a result of the insensitivity of their cells to the LDL. Recently, we found that endothelial cells rather than bone marrow-derived cells account for the difference between B6 and C3H mice in atherosclerosis susceptibility (18). Endothelial cells of B6 mice exhibited a dramatic induction of MCP-1, M-CSF, VCAM-1, and heme oxygenase-1 in response to OxLDL, whereas endothelial cells from C3H mice showed little induction. MCP-1, M-CSF, and VCAM-1 are primarily associated with the recruitment and differentiation of monocytes. Therefore, if endothelial cells of C3H mice were unable to recruit monocytes to the artery wall and promote their differentiation into macrophages, it might be expected that monocytes would have no chance to exert their effect on atherosclerosis.

In summary, we have observed the difference between B6 and C3H mice in the subendothelial retention of LDL, which could not explain the difference in their susceptibility to atherosclerosis. We have also observed that endothelial cells play a role in alleviating subendothelial LDL retention. Although scavenger receptors on macrophages have been shown to promote atherosclerosis (31–34), these receptors on endothelial cells could play a protective role against the disease. However, it is worth noting that because the uptake of LDL and the expression levels of CD36 and LOX-1 were assayed in vitro using endothelial cells, our findings may not exactly reflect the situation in vivo.

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