Neointimal formation in two apolipoprotein E-deficient mouse strains with different atherosclerosis susceptibility

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Abstract **C57BL/6 (B6) and C3H/HeJ (C3H) are two commonly used mouse strains that differ markedly in atherosclerosis susceptibility. In this study, we determined plaque formation after removal of the endothelium in the two strains carrying the mutant apolipoprotein E gene (apoE$^{-/-}$). At 10 weeks of age, male B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice underwent endothelial denudation of the left common carotid artery. Two weeks after injury, B6.apoE$^{-/-}$ mice developed significantly larger neointimal lesions in the vessel than their C3H.apoE$^{-/-}$ counterparts, although they had comparable plasma cholesterol levels on a chow diet. Feeding of a Western diet aggravated lesion formation in both strains, but the increase was more dramatic in B6.apoE$^{-/-}$ mice than in C3H.apoE$^{-/-}$ mice. Immunohistochemical and histological analyses demonstrated the presence of macrophage foam cells in neointimal lesions. We then compared neointimal growth in F1 mice reconstituted with bone marrow from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. No significant lesions were observed 2 weeks after endothelial denudation in the mice reconstituted with bone marrow from either donor. Thus, these data indicate that foam cell formation contributes to neointimal growth in the hyperlipidemic apoE$^{-/-}$ model and that neither endothelial cells nor blood cells alone explain the dramatic difference between B6 and C3H mice in plaque formation.**—Shi, W., H. Pei, J. J. Fischer, J. C. James, J. F. Angle, A. H. Matsumoto, G. A. Helm, and I. J. Sarembock. **Neointimal formation in two apolipoprotein E-deficient mouse strains with different atherosclerosis susceptibility.** ***J. Lipid Res.*** **2004.** 45: **2008–2014.**

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Atherogenesis is a complex process involving interactions among many types of cells, including endothelial cells, vascular smooth muscle cells, monocytes/macrophages, lymphocytes, and platelets (1). Endothelial cells have been considered to play a crucial role in the initiation and progression of primary atherosclerosis (2, 3). They oxidatively modify LDL, and in response to oxidized LDL or its components, endothelial cells express several inflammatory molecules, including vascular cell adhesion molecule-1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1), and macrophage colony-stimulating factor (M-CSF), that promote monocyte transmigration into the subendothelium and differentiation into macrophages (4–6). Subsequently, macrophages ingest modified LDL to give rise to foam cells, the hallmark of early atherosclerosis.

The availability of inbred mouse strains that differ in atherosclerosis susceptibility provides an experimental approach to identifying the role of various cell components involved in the development of atherosclerosis. C57BL/6 (B6) and C3H/HeJ (C3H) mice are two commonly used inbred strains that differ dramatically in their susceptibility to atherosclerosis. In both diet-fed and apolipoprotein E-deficient (apoE$^{-/-}$) models, B6 mice develop much larger atherosclerotic lesions than their C3H counterparts (7–9). Through reciprocal bone marrow transplantation, we have demonstrated that this difference in atherosclerosis susceptibility is not attributable to monocytes or other bone marrow-derived cells (9). In vitro, we have observed indirect evidence that endothelial cells contribute to the difference in atherosclerosis susceptibility. Indeed, endothelial cells from B6 mice exhibit a dramatic induction of VCAM-1, MCP-1, and M-CSF by oxidized LDL, whereas C3H endothelial cells exhibit minimal induction. Moreover, endothelial responses to oxidized LDL cosegregate with the size of atherosclerotic lesions, as observed in a set of recombinant inbred strains derived from B6 and C3H strains.

Endothelial denudation represents a direct method of identifying the role of endothelial cells in atherosclerosis and other biological processes (10). Complete removal of endothelial cells in the carotid arteries can be achieved in mice with a flexible wire or Epon-resin probe (10, 11).

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The early responses consist of adhesion of a layer of platelets, followed by adhesion of mononuclear inflammatory cells and by migration and proliferation of smooth muscle cells. However, recent studies have shown that intimal growth after arterial injury is very limited in most wild-type mouse strains, including B6 and C3H mice (12). De Geest et al. (13) found that abrasion of the endothelium of the common carotid artery did not produce neointimal lesions within 18 days in B6 mice. In contrast, apoE$^{-/-}$ mice, which were in the B6 genetic background, developed robust intimal lesions after injury (11, 13, 14). Accumulated evidence has suggested that foam cell formation is a mechanism for enhanced neointimal formation in apoE$^{-/-}$ mice (15, 16). We recently constructed a congenic strain of C3H.apoE$^{-/-}$ mice and found that this strain is extremely resistant to primary atherosclerosis despite its comparable hyperlipidemia to B6.apoE$^{-/-}$ mice on a chow diet (9). Therefore, in this study, we sought to determine whether C3H.apoE$^{-/-}$ mice are protected from injury-induced plaque formation after endothelial denudation.

MATERIALS AND METHODS

Mice

Male B6.apoE$^{-/-}$ mice were obtained from The Jackson Laboratories (Bar Harbor, ME). C3H.apoE$^{-/-}$ mice were generated in our laboratory by initially crossing B6.apoE$^{-/-}$ mice with C3H mice. The resulting heterozygous apoE$^{+/-}$ mice were sequentially backcrossed to C3H mice for 10 generations, followed by brother-sister mating to generate homozygous apoE$^{-/-}$ mice. In one group, mice were fed a standard rodent chow diet containing 4% fat (Ralston-Purina Co., St. Louis, MO) throughout the entire experimental period; another group was fed a Western diet containing 42% fat, 0.15% cholesterol, and 19.5% casein without sodium cholate (TD 88137; Teklad, Madison, WI) for 1 week before and 2 weeks after endothelial removal. F1 mice were generated by crossing B6.apoE$^{-/-}$ with C3H.apoE$^{-/-}$ mice and were maintained on the chow diet. All procedures were carried out in accordance with current National Institutes of Health guidelines and approved by the University Animal Care and Use Committee.

Endothelium denudation

The procedure for removing the endothelium in the left common carotid artery was as previously described (10, 15). Briefly, mice were anesthetized by intramuscular injection with ketamine (80 mg/kg body weight; Ketaset, Aveco, Inc.) and xylazine (8 mg/kg; AnaSed, Lloyd Laboratories). Surgery was performed using a dissection microscope. The left external carotid artery was ligated distally and looped proximally with a 6-0 silk suture. Additional 6-0 sutures were looped around the common and internal carotid arteries for temporary control of the vessels during the procedure. A transverse arteriotomy was made in the left external carotid artery, and a flexible 0.014 inch angioplasty guide wire (for mice on the Western diet) or Epon-resin probe (for mice on the chow diet) was introduced and advanced ~1 cm toward the aortic arch. Endothelial denudation of the artery was achieved by repeated withdrawal for three passes. After removal of the wire or probe, the left external carotid artery was ligated and the skin incision was closed with a 5-0 sterile surgical gut (Ethicon, Inc.). Animals were allowed to recover in a warm box.

Bone marrow transplantation

Male F1 recipients derived from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice were lethally irradiated with a dose of 11 Gy at 6 weeks of age. Donor bone marrow was harvested from male B6.apoE$^{-/-}$ or C3H.apoE$^{-/-}$ mice by flushing femurs and tibias and prepared as previously described (17). Each recipient mouse was injected with 10^7 marrow cells in 0.3 ml of solution through the tail veins. Four weeks after transplantation, the mice underwent endothelial denudation as described above. They were maintained on the chow diet throughout the entire experimental period.

Tissue preparation and lesion quantification

Two weeks after endothelial denudation, mice were killed by cervical dislocation after isoflurane anesthesia. The carotid arteries were perfused in situ under 110 cm height water pressure initially with PBS to remove the blood and then with 4% paraformaldehyde via the left ventricle. The neck was then dissected en bloc and fixed in 4% paraformaldehyde for an additional 2 h. After fixation, the front soft tissues of the neck were dissected out, processed by using the standard histological technique, embedded in paraffin, and cross-sectioned in 10 μm thick sections. Parallel sections were subjected to van Gieson staining for elastic laminas and hematoxylin and eosin staining. As shown in **Fig. 1**, this processing method enables the evaluation of both injured and uninjured carotid arteries on the same section. Thus, the uninjured right carotid artery serves as a useful control. Morphometric measurements were made on the van Gieson-stained sections using Image Pro Plus 3.0 software (Media Cybernetics). Luminal, internal, and external elastic areas were measured. Intimal area was calculated by subtracting the luminal area from the area of the internal elastic lamina. Medial area was calculated by subtracting the area of the internal elastica from that of the external elastic lamina.

Immunohistochemical analysis

The presence of macrophages, smooth muscle cells, and endothelial cells in the arterial wall was determined by staining with antibodies to mouse macrophages (MOMA-2; Accurate Chemicals), to α-smooth muscle actin (1A4; Dako Corp.), and to the von Willebrand factor (Sigma) as previously described (18, 19). Briefly, paraffin-embedded sections were deparaffinized with xylene, followed by incubation with the primary antibodies. After a thorough wash, the slides were incubated with biotinylated sec-

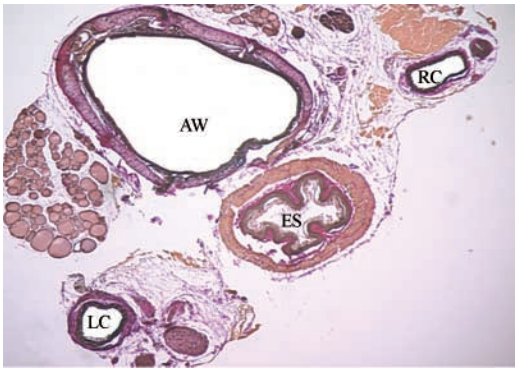

Fig. 1. A representative photomicrograph of a van Gieson-stained cross-section of the front soft tissues of the neck from a C57BL/6 apolipoprotein E-deficient (B6.apoE$^{-/-}$) mouse. The neck front soft tissues were dissected out after fixation and processed using standard histological technique. AW, airway; ES, esophagus; LC, left carotid artery; RC, right carotid artery. Original magnification, ×4.

ondary antibodies (Vector Laboratories). The reactions were visualized with peroxidase chromogen kits (Vector Laboratories).

Plasma lipid measurements

Mice were fasted overnight before blood was collected through retro-orbital puncture under isoflurane anesthesia. Plasma total cholesterol, HDL cholesterol, and triglyceride were measured with enzymatic assays as previously described by Hedrick et al. (20).

Scanning electron microscopy

Mice were killed 1 h after the denuding procedure, and the injured carotid artery was fixed by perfusion with a solution containing 4% glutaraldehyde and 2% paraformaldehyde for 10 min through the left ventricle. The fixed carotid artery was cut open longitudinally, pinned flat onto Teflon sheets, and dehydrated with degrading ethanol. The tissues were mounted on scanning electron microscopy stubs with colloidal silver paste. After sputter-coating with gold/palladium, the specimens covering the entire length of the carotid artery were examined with a JEOL-100 CX electron microscope at the university.

Statistical analysis

All values are expressed as means ± SEM, with n indicating the number of mice. Student's *t*-test was used to determine the statistical differences between the two strains in morphometric measurements and lipid levels. Differences were considered statistically significant at $P < 0.05$.

RESULTS

At 10 weeks of age, male B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice underwent endothelial denudation of the left common carotid artery. Two weeks after the procedure, plaque formation in the vessel was examined by light microscopy. As shown in **Fig. 2**, endothelial denudation resulted in prominent intimal thickening of the artery in B6.apoE$^{-/-}$ mice when fed either chow or Western diet, whereas in C3H.apoE$^{-/-}$ mice, intimal thickening was barely detectable. On the chow diet, the average intimal area per section was 2,746 ± 343 μm^2 in B6.apoE$^{-/-}$ mice and 552 ± 415 μm^2 in C3H.apoE$^{-/-}$ mice ($P = 0.0161$, n = 3–5) (**Fig. 3**). On the Western diet, intimal area was 25-fold larger in B6.apoE$^{-/-}$ mice (25,814 ± 5,848 μm^2/section, n = 5) than in C3H.apoE$^{-/-}$ mice (922 ± 1,173 μm^2/section, n = 8; $P = 0.013$). Examination of the uninjured right carotid artery revealed no detectable intimal lesions in either strain on either diet. The area of the media was also increased in B6.apoE$^{-/-}$ mice on the Western diet compared with the chow diet, although the increase was not statistically significant (31,166 ± 2,733 vs. 22,497 ± 3,754 μm^2/section; $P = 0.068$). On the chow diet, C3H.apoE$^{-/-}$ mice had a slightly larger medial area than B6.apoE$^{-/-}$ mice (24,833 ± 2,643 vs. 22,497 ± 3,754 μm^2/section), whereas on the Western diet, B6.apoE$^{-/-}$ mice had a slight larger medial area than C3H.apoE$^{-/-}$ mice (31,166 ± 2,733 vs. 27,544 ± 3,102 μm^2/section).

Standard hematoxylin and eosin staining revealed the presence of numerous foam cells in the neointimal lesions (**Fig. 4A**). Immunohistochemical analysis with specific antibodies for macrophages and smooth muscle cells confirmed the abundance of macrophages and smooth muscle cells in the lesions (Fig. 4B, C). Double immunostaining with the antibodies further indicated that macrophages were more prominent at the core of neointimal lesions, whereas smooth muscle cells were more obvious at the cap of lesions (Fig. 4C). The presence of endothelial cells in the mid-portion of the common carotid arteries was evaluated by immunostaining with an antibody for the von Willebrand factor. In the uninjured right carotid artery, a thin layer of endothelial cells was observed lining the inner surface of the vessel (**Fig. 5A**). In contrast, endothelial cells were barely detectable in the injured left carotid artery of either strain (Fig. 5B, C).

Plasma lipid levels of mice were analyzed when they

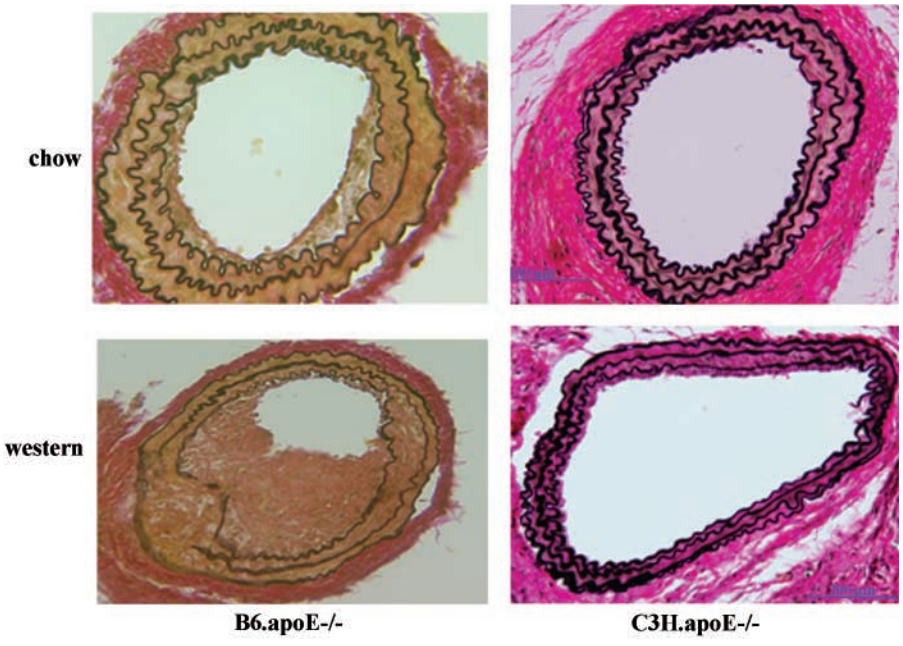

Fig. 2. Representative photomicrographs of cross-sections of injured carotid arteries from B6.apoE$^{-/-}$ and C3H/HeJ apoE-deficient (C3H.apoE$^{-/-}$) mice 2 weeks after endothelial denudation. The paraffin-embedded tissues were stained with van Gieson stain. Note the robust neointima formation in the B6.apoE$^{-/-}$ mice, particularly those fed a Western diet. Minimal neointima formation was seen in the C3H.apoE$^{-/-}$ mice after arterial injury. Original magnification, ×20.

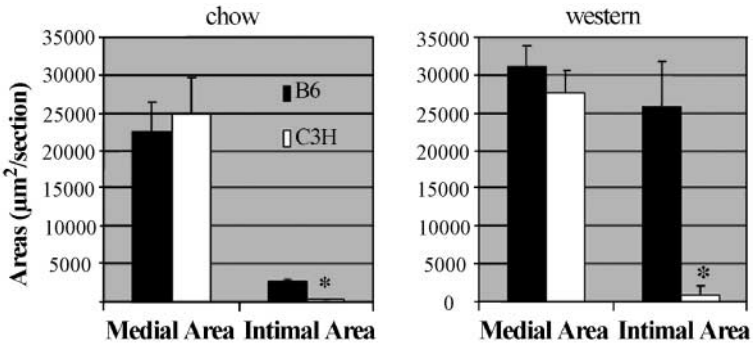

Fig. 3. Quantitative measurements of intimal and medial areas (μm^2/section) of the injured left carotid artery in B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice when fed a chow or Western diet. Note the significant increase in neointimal area in B6.apoE$^{-/-}$ mice on the Western diet and the moderate increase on the chow diet. Minimal neointima formation was seen in C3H.apoE$^{-/-}$ mice fed either the chow or Western diet after vascular injury. Values are means ± SEM for three to eight mice. * $P < 0.05$ vs. B6.apoE$^{-/-}$ mice.

were fed chow and Western diets. On the chow diet, both B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice had hyperlipidemia, which was accentuated by feeding the Western diet (**Fig. 6**). Compared with B6.apoE$^{-/-}$ mice, C3H.apoE$^{-/-}$ mice had slightly increased levels of total cholesterol and triglyceride on both chow and Western diets (n = 5–11; $P < 0.05$). There were no differences in HDL cholesterol levels between C3H.apoE$^{-/-}$ mice (20.2 ± 7.3 mg/l) and B6.apoE$^{-/-}$ mice (21 ± 2.8 mg/l) on the chow diet. On the Western diet, the HDL cholesterol level was significantly increased in C3H.apoE$^{-/-}$ mice (79.3 ± 8.3 mg/l; $P < 0.01$) but not in B6.apoE$^{-/-}$ mice (22.6 ± 5.9 mg/l).

Adherence of platelets and leukocytes to the surface of injured carotid artery was examined 1 h after endothelial denudation using scanning electron microscopy. As shown in **Fig. 7**, the exposed subendothelium was covered with a monolayer of platelets in both strains. Interestingly, leukocytes adherent to platelets were observed in B6.apoE$^{-/-}$ mice but not in C3H.apoE$^{-/-}$ mice. To further determine whether blood cells were responsible for the difference in the neointimal growth of the two strains, we performed bone marrow transplantation experiments in which F1 mice were reconstituted with bone marrow from their parents. Four weeks after transplantation, the F1 mice were subjected to endothelial denudation of the left common carotid artery and then maintained on the chow diet for 2 weeks. We found that F1 mice developed essentially no neointimal lesions in the injured artery regardless of whether they were reconstituted with the bone marrow from B6.apoE$^{-/-}$ or C3H.apoE$^{-/-}$ mice (331 ± 330 vs. 0 μm^2/section, n = 3–4) (**Fig. 8**).

DISCUSSION

In the present study, we investigated plaque formation after removal of the endothelium in two apoE$^{-/-}$ mouse strains that differ markedly in susceptibility to primary atherosclerosis. The key finding in our study was that atherosclerosis-susceptible B6.apoE$^{-/-}$ mice developed much larger intimal lesions after endothelial denudation than atherosclerosis-resistant C3H.apoE$^{-/-}$ mice. Previously, we observed that oxidized LDL induces marked expression of MCP-1, M-CSF, and VCAM-1 mRNAs in endothelial cells from B6 mice, whereas endothelial cells from C3H mice show small or no induction of these mRNAs (19). In recombinant inbred strains derived from B6 and C3H strains, we found that endothelial responses to oxidized LDL with regard to the induction of inflammatory genes cosegregate with the size of atherosclerotic lesions (9). Because MCP-1, M-CSF, and VCAM-1 are associated with monocyte recruitment to the arterial wall and differentiation into macrophages, it is plausible that the endothelium is a source of the difference between B6 and C3H mice in atherosclerosis susceptibility. Thus, in this study, we determined the role of endothelial cells in the control of atherosclerosis susceptibility by removing the endothelium. Unexpectedly, we found that endothelial denudation did

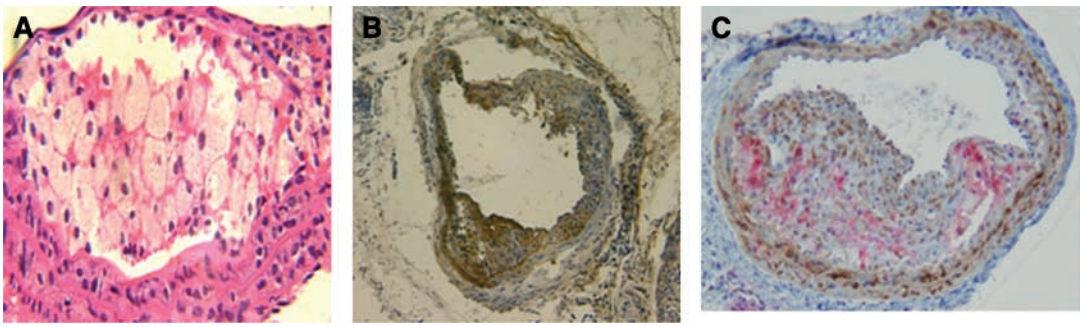

Fig. 4. The presence of macrophages and smooth muscle cells in neointimal lesions of B6.apoE$^{-/-}$ mice. A: Section stained with hematoxylin and eosin showing the presence of numerous foam cells in the lesion. B: Section stained with anti-mouse macrophage antibody MOMA-2 showing the presence of macrophages in both neointima and the media. C: Double immunohistochemical staining showing the differential distribution of macrophages and smooth muscle cells in a neointima lesion; macrophages stained red and smooth muscle cells stained brown. Original magnifications, ×20–40.

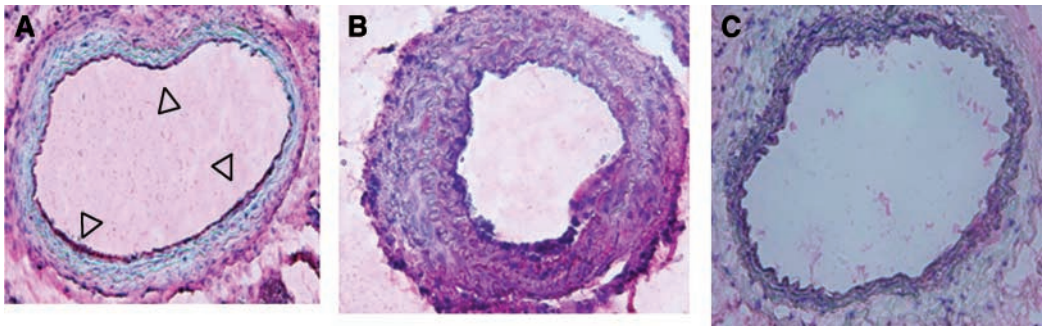

Fig. 5. Representative micrographs of cross-sections of mouse carotid arteries stained with an antibody for the von Willebrand factor. A: A normal carotid artery with an intact endothelial layer. Triangle symbols point to the endothelial layer stained with the von Willebrand factor antibody. B and C: Injured B6.apoE$^{-/-}$ (B) and C3H.apoE$^{-/-}$ (C) carotid arteries with barely detectable endothelial cells. Original magnification, ×20.

little to change the susceptibility. One explanation for this result is that other cell types in the arterial wall, such as vascular smooth muscle, also contribute to the difference in atherosclerosis susceptibility. Another explanation for the present finding is that endothelial denudation not only eliminates the role of endothelial cells but also gives rise to additional pathological changes that do not exist in the development of primary atherosclerosis. Indeed, after endothelial denudation, the subendothelial components are exposed to blood flow, which results in the deposition of platelets and fibrin, the adhesion of leukocytes, and the release of cytokines and mitogens from platelets, leukocytes, damaged endothelial cells, and smooth muscle cells (21). These cytokines and mitogens in turn stimulate smooth muscle cells in the media to transform from a quiescent contractile phenotype into a synthetic phenotype that proliferates and migrates from the media to the intima, leading to intimal growth. Thus, smooth muscle cells are central to the development of injury-induced intimal lesions. In contrast, macrophages are the major cellular components of atherosclerotic lesions at all stages and smooth muscle cells only become prominent in the advanced stages (1, 22).

Recent studies have indicated that intimal growth after endothelial denudation is very limited in most wild-type mouse strains, including B6 and C3H mice (12). De Geest et al. (13) even found that abrasion of the endothelium of the common carotid artery did not produce neointima by 18 days in B6 mice. In contrast, apoE$^{-/-}$ mice, which were in the B6 genetic background, developed pronounced intimal lesions in the carotid arteries after removal of endothelial cells (11, 13, 14). Thus, absence of apoE, hyperlipidemia, or both appear to be responsible for the increased neointimal formation of B6.apoE$^{-/-}$ mice. However, the current finding that C3H.apoE$^{-/-}$ mice were resistant to neointima formation despite their comparable hyperlipidemia and absence of apoE suggests that the enhanced neointimal growth of B6.apoE$^{-/-}$ mice may result from the excessive response of the vasculature to these two factors. Indeed, B6.apoE$^{-/-}$ mice have been shown to have an increased susceptibility to foam cell formation compared with C3H.apoE$^{-/-}$ mice under comparable hyperlipidemia (9, 23). Accordingly, in this study, we demonstrated the presence of numerous foam cells in the neointimal lesions, which was accentuated by feeding of the Western diet (Figs. 3, 4). Also, previous studies have provided indirect evidence to support the conclusion that foam cell formation is an important mechanism for the increased intimal formation of B6.apoE$^{-/-}$ mice. Manka et al. (15) have observed large numbers of macrophages in the neointima and media of injured vessels. Quarck et al. (16) further demonstrated the presence of oxidized LDL in the neointimal lesions of the mice.

The mechanisms underlying foam cell development in

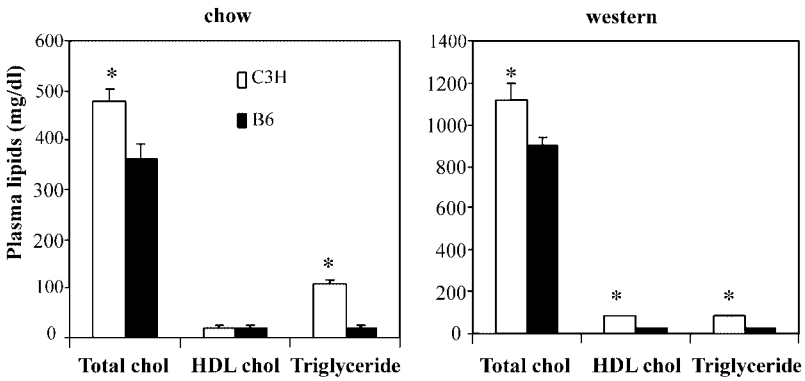

Fig. 6. Plasma total cholesterol (chol), HDL cholesterol, and triglyceride (mg/dl) levels of B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice on chow and Western diets. Blood was obtained after overnight fasting. Values are means ± SEM for 5–11 mice. * $P < 0.05$ vs. B6.apoE$^{-/-}$ mice. Note the significant increase in total cholesterol, more marked in the C3H.apoE$^{-/-}$ mice with a 2-fold increase after Western diet feeding.

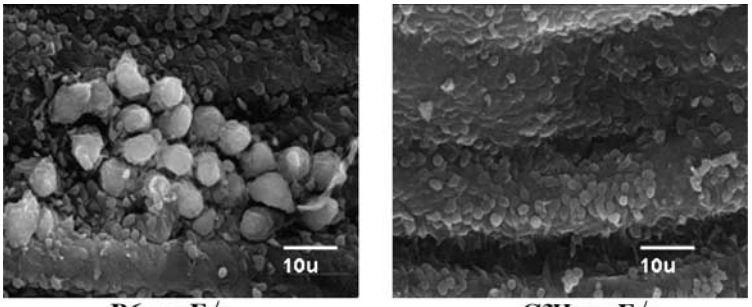

Fig. 7. Representative scanning electron micrographs of mouse carotid arteries 1 h after endothelial denudation. Complete endothelial denudation was achieved, and the exposed subendothelium was covered by a monolayer of platelets. Note leukocyte adhesion to platelets in B6.apoE$^{-/-}$ but not in C3H.apoE$^{-/-}$ mice.

the absence of endothelial cells in apoE$^{-/-}$ mice are unknown. However, it is known that denudation of the endothelium leads to exposure of the subendothelial components of the vessel wall, which leads to the deposition of platelets and fibrin, leukocyte adhesion and infiltration, and release of cytokines and mitogens from platelets, leukocytes, and smooth muscle cells (10, 24). These cytokines and mitogens then stimulate infiltrated monocytes to transform into macrophages in the intima of injured artery. Macrophages ingest lipids accumulated in the intima to become foam cells. The hyperlipidemia of apoE$^{-/-}$ mice has been shown to result in significant deposition of apoB-containing lipoproteins in the arterial wall (25). Moreover, Linton et al. (26) have demonstrated that macrophages ingest native LDL and consequently contribute to atherosclerotic lesion formation.

C3H.apoE$^{-/-}$ mice had slightly increased levels of plasma cholesterol and triglyceride compared with B6.apoE$^{-/-}$ mice on the chow diet. Thus, the resistance of C3H.apoE$^{-/-}$ mice to intimal lesion formation was unlikely to be associated with plasma lipid levels. Nevertheless, on the Western diet, C3H.apoE$^{-/-}$ mice had a higher HDL level than B6.apoE$^{-/-}$ mice. The higher HDL level probably contributed to the decreased lesion formation of C3H.apoE$^{-/-}$ mice on the Western diet.

In the present study, we observed differences between the two strains in the adhesion of leukocytes at the site of arterial injury. Indeed, leukocyte adhesion to injured arterial walls was seen in B6.apoE$^{-/-}$ mice but not in C3H.apoE$^{-/-}$ mice at 1 h after endothelial removal. There are several potential explanations for the increased leukocyte adherence to the denuded endothelium/platelet layer of the B6 mice, which was not seen in the C3H mice: *1*) differences in the expression of adhesion molecules such as P-selectin and platelet/endothelial cell adhesion molecule-1 on the surface of the platelets or of the exposed smooth muscle cells; *2*) differences in acute endothelial denudation; and *3*) differences in the response to the mechanical force of injury or in the actual force imparted on the arterial wall, which may be attributable to differences in vessel diameter and in the compliance of the vessels. The latter two explanations seem unlikely because complete endothelial denudation had been achieved for both strains and because the two strains had comparable diameters of the uninjured right common carotid arteries.

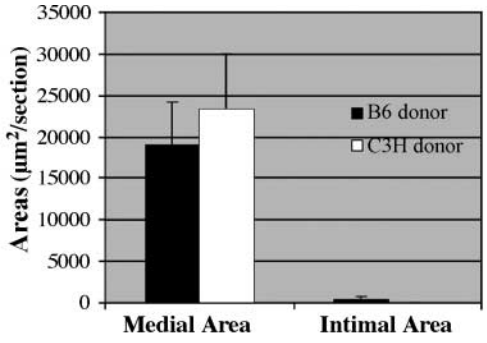

Fig. 8. Quantitative measurements of intimal and medial areas (μm^2/section) of the left carotid artery 2 weeks after endothelial denudation in F1 mice reconstituted with bone marrow from B6.apoE$^{-/-}$ and C3H.apoE$^{-/-}$ mice. Mice were subject to endothelial denudation injury 4 weeks after bone marrow transplantation and were maintained on the chow diet during the experiment. Values are means ± SEM for three to four mice.

To determine whether the difference in leukocyte adhesion could contribute to the different neointima growth of the two strains, we carried out the bone marrow transplantation study in which F1 mice were reconstituted with bone marrow from their parents. Unexpectedly, the F1 mice reconstituted with B6.apoE$^{-/-}$ bone marrow showed little increase in lesion formation compared with the mice reconstituted with C3H.apoE$^{-/-}$ bone marrow, suggesting that the bone marrow-derived cells contributed little to the differential neointima growth of the two strains. Nevertheless, we cannot exclude the possibility that the high resistance of F1 mice after lethal irradiation may have overwhelmed the effect of bone marrow-derived cells on neointima growth. High-dose irradiation has been shown to prevent neointima formation in both humans and animal models (27, 28).

In summary, we have observed that atherosclerosis-susceptible B6.apoE$^{-/-}$ mice developed much larger plaques than C3H.apoE$^{-/-}$ mice after denudation of the endothelium. Our study has demonstrated that foam cell formation contributes to injury-induced intimal growth in the hyperlipidemic apoE$^{-/-}$ model and that neither endothelial cells nor blood cells alone explain the dramatic difference between B6 and C3H mice in plaque formation.

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REFERENCES

1. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
2. Berliner, J. A., M. Navab, A. M. Fogelman, J. S. Frank, L. L. Demer, P. A. Edwards, A. D. Watson, and A. J. Lusis. 1995. Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics. *Circulation.* **91:** 2488–2496.
3. Steinberg, D. 2002. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat. Med.* **8:** 1211–1217.
4. Khan, B. V., S. S. Parthasarathy, R. W. Alexander, and R. M. Medford. 1995. Modified low density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. *J. Clin. Invest.* **95:** 1262–1270.
5. Cushing, S. D., J. A. Berliner, A. J. Valente, M. C. Territo, M. Navab, F. Parhami, R. Gerrity, C. J. Schwartz, and A. M. Fogelman. 1990. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proc. Natl. Acad. Sci. USA.* **87:** 5134–5138.
6. Rajavashisth, T. B., A. Andalibi, M. C. Territo, J. A. Berliner, M. Navab, A. M. Fogelman, and A. J. Lusis. 1990. Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature.* **344:** 254–257.
7. Paigen, B., A. Morrow, C. Brandon, D. Mitchell, and P. Holmes. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis.* **57:** 65–73.
8. Qiao, J. H., P. Z. Xie, M. C. Fishbein, J. Kreuzer, T. A. Drake, L. L. Demer, and A. J. Lusis. 1994. Pathology of atheromatous lesions in inbred and genetically engineered mice. Genetic determination of arterial calcification. *Arterioscler. Thromb.* **14:** 1480–1497.
9. Shi, W., N. J. Wang, D. M. Shih, V. Z. Sun, X. Wang, and A. J. Lusis. 2000. Determinants of atherosclerosis susceptibility in the C3H and C57BL/6 mouse model: evidence for involvement of endothelial cells but not blood cells or cholesterol metabolism. *Circ. Res.* **86:** 1078–1084.
10. Lindner, V., J. Fingerle, and M. A. Reidy. 1993. Mouse model of arterial injury. *Circ. Res.* **73:** 792–796.
11. Zhu, B., D. G. Kuhel, D. P. Witte, and D. Y. Hui. 2000. Apolipoprotein E inhibits neointimal hyperplasia after arterial injury in mice. *Am. J. Pathol.* **157:** 1839–1848.
12. Kuhel, D. G., B. Zhu, D. P. Witte, and D. Y. Hui. 2002. Distinction in genetic determinants for injury-induced neointimal hyperplasia and diet-induced atherosclerosis in inbred mice. *Arterioscler. Thromb. Vasc. Biol.* **22:** 955–960.
13. De Geest, B., Z. Zhao, D. Collen, and P. Holvoet. 1997. Effects of adenovirus-mediated human apo A-I gene transfer on neointima formation after endothelial denudation in apoE-deficient mice. *Circulation.* **96:** 4349–4356.
14. Zhu, Y., P. M. Farrehi, and W. P. Fay. 2001. Plasminogen activator inhibitor type 1 enhances neointima formation after oxidative vascular injury in atherosclerosis-prone mice. *Circulation.* **103:** 3105–3110.
15. Manka, D., R. G. Collins, K. Ley, A. L. Beaudet, and I. J. Sarembock. 2001. Absence of P-selectin, but not intercellular adhesion molecule-1, attenuates neointimal growth after arterial injury in apolipoprotein E–deficient mice. *Circulation.* **103:** 1000–1005.
16. Quarck, R., B. De Geest, D. Stengel, A. Mertens, M. Lox, G. Theilmeier, C. Michiels, M. Raes, H. Bult, D. Collen, P. Van Veldhoven, E. Ninio, and P. Holvoet. 2001. Adenovirus-mediated gene transfer of human platelet-activating factor-acetylhydrolase prevents injury-induced neointima formation and reduces spontaneous atherosclerosis in apolipoprotein E-deficient mice. *Circulation.* **103:** 2495–2500.
17. Shi, W., X. Wang, N. J. Wang, W. H. McBride, and A. J. Lusis. 2000. Effect of macrophage-derived apolipoprotein E on established atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2261–2266.
18. Manka, D., S. B. Forlow, J. M. Sanders, D. Hurwitz, D. K. Bennett, S. A. Green, K. Ley, and I. J. Sarembock. 2004. Critical role of platelet P-selectin in the response to arterial injury in apolipoprotein-E-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1124–1129.
19. Shi, W., M. E. Haberland, M. L. Jien, D. M. Shih, and A. J. Lusis. 2000. Endothelial responses to oxidized lipoproteins determine genetic susceptibility to atherosclerosis in mice. *Circulation.* **102:** 75–81.
20. Hedrick, C. C., L. W. Castellani, H. Wong, and A. J. Lusis. 2001. In vivo interactions of apoA-II, apoA-I, and hepatic lipase contributing to HDL structure and antiatherogenic functions. *J. Lipid Res.* **42:** 563–570.
21. Bennett, M. R., and M. O'Sullivan. 2001. Mechanisms of angioplasty and stent restenosis: implications for design of rational therapy. *Pharmacol. Ther.* **91:** 149–166.
22. Nakashima, Y., A. S. Plump, E. W. Raines, J. L. Breslow, and R. Ross. 1994. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Thromb.* **14:** 133–140.
23. Matsushima, Y., T. Sakurai, A. Ohoka, T. Ohnuki, N. Tada, Y. Asoh, and M. Tachibana. 2001. Four strains of spontaneously hyperlipidemic (SHL) mice: phenotypic distinctions determined by genetic backgrounds. *J. Atheroscler. Thromb.* **8:** 71–79.
24. Zhu, B., C. A. Reardon, G. S. Getz, and D. Y. Hui. 2002. Both apolipoprotein E and immune deficiency exacerbate neointimal hyperplasia after vascular injury in mice. *Arterioscler. Thromb. Vasc. Biol.* **22:** 450–455.
25. Brown, M. D., L. Jin, M. L. Jien, A. H. Matsumoto, G. A. Helm, A. J. Lusis, J. S. Frank, and W. Shi. 2004. Lipid retention in the arterial wall of two mouse strains with different atherosclerosis susceptibility. *J. Lipid Res.* **45:** 1155–1161.
26. Linton, M. F., V. R. Babaev, L. A. Gleaves, and S. Fazio. 1999. A direct role for the macrophage low density lipoprotein receptor in atherosclerotic lesion formation. *J. Biol. Chem.* **274:** 19204–19210.
27. Verheye, S., P. K. Coussement, M. Y. Salame, P. Fallahi, J. Cui, N. A. Chronos, S. B. King, I. R. Crocker, and K. A. Robinson. 2001. High-dose external beam irradiation inhibits neointima formation in stented pig coronary arteries. *Int. J. Radiat. Oncol. Biol. Phys.* **51:** 820–827.
28. Crocker, I., and K. A. Robinson. 2002. Rationale for coronary artery radiation therapy. *Semin. Radiat. Oncol.* **12:** 3–16.