Elimination of macrophage-specific apolipoprotein E reduces diet-induced atherosclerosis in C57BL/6J male mice

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Abstract **Apolipoprotein (apo)E is synthesized in atherosclerotic lesions by macrophages, however, its role in lesions is not known. Whereas apoE could exacerbate atherosclerosis by promoting macrophage uptake of cholesterol-rich lipoproteins or modulating protective inflammatory responses, it could also restrict lesion formation by facilitating cholesterol efflux out of lesions. The role of apoE was examined in lethally irradiated male C57BL/6J wild-type (WT) mice that were repopulated with bone marrow cells (BMT) from either identical C57BL/6J mice (WT+WT BMT) or C57BL/6J apoE-deficient mice (WT+E−/− BMT). This enabled us to compare normal mice with mice possessing macrophages that did not express apoE. The participation of macrophage-derived apoE in atherosclerosis was assessed by placing the mice on an atherogenic diet. Male WT+E−/− BMT mice had significantly reduced lesion area in the aortic valves ($P < 0.01$) compared with male WT+WT BMT mice (~22,000 vs. ~49,000 μm^2/section, respectively). Further evaluation revealed that plasma cholesterol, lipoprotein cholesterol distribution, and plasma apoE were similar between the two groups, indicating that these known risk factors did not account for the differences in lesion area. However, the two groups were distinguished by the amount of apoE found in the lesions. ApoE antigen was expressed abundantly in WT+WT BMT lesions, whereas WT+E−/− BMT lesions contained little apoE. These findings indicate that the majority of apoE in lesions is synthesized locally by resident macrophages, and suggest that locally produced apoE can promote diet-induced atherosclerosis in male wild-type mice.**—Boisvert, W. A., and L. K. Curtiss. **Elimination of macrophage-specific apolipoprotein E reduces diet-induced atherosclerosis in C57BL/6J male mice.** *J. Lipid Res.* **1999.** 40: **806–813.**

Supplementary key words apolipoprotein E • bone marrow transplantation • atherosclerosis

Apolipoprotein (apo) E, a 34 kDa protein found on cholesterol-rich plasma lipoproteins, is a ligand for receptor-mediated hepatic clearance of these lipoproteins from the circulation (1, 2). The importance of apoE for cholesterol homeostasis is clearly demonstrated in apoE-deficient mice, which develop advanced atherosclerosis as a consequence of their severe hypercholesterolemia (3, 4). Although most apoE in circulation is synthesized by the liver (5), many extrahepatic tissues (6, 7) including macrophages produce apoE (8, 9). As demonstrated by replacing the bone marrow cells of these apoE-deficient mice with those of wild-type (WT) mice, apoE derived exclusively from macrophages reduces both the hypercholesterolemia and the atherosclerosis of apoE-deficient (E−/−) mice (10, 11).

Both apoE mRNA and antigen are absent in normal vessels, but they are abundant in fatty streak lesions of atherosclerosis (12, 13). However, the role of apoE in the microenvironment of the lesion is not clear. This apoE could be proatherogenic by promoting macrophage uptake and degradation of cholesterol-rich lipoproteins. Ingestion of apoE-enriched VLDL by macrophages leads to cholesteryl ester accumulation and foam cell formation (14–16). ApoE secreted by macrophages and acting in an autocrine fashion, enhances macrophage uptake of cholesterol-rich lipoproteins (17). Alternatively, apoE secreted by lesion foam cells could be atheroprotective by facilitating cellular cholesterol efflux. Cholesterol-loaded macrophages secrete apoE that can be transferred onto HDL and promote cholesterol efflux from the vessel (18). Expression of human apoE by transfected J774 macrophages, which normally do not synthesize apoE, induces a large increase in cholesteryl ester efflux (19). Additionally, cholesterol efflux from mouse peritoneal macrophages is lower when the cells are incubated with HDL devoid of apoE compared to incubation with normal HDL (20). Hence, apoE, either secreted by foam cells or present on HDL, partici-

Abbreviations: apo, apolipoprotein; WT, wild type; BMT, bone marrow transplant; E−/−, apo-E deficient mice; VLDL, very low density lipoproteins; HDL, high density lipoproteins; PBS, phosphate-buffered saline; FPLC, fast protein liquid chromatography; SDS, sodium dodecyl sulphate; IgG, immunoglobulin; PMA, phorbol myristate acetate; LDL, low density lipoproteins.

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pates in cholesterol efflux. Based on these in vitro findings, apoE could exacerbate lesion development by promoting macrophage uptake of atherogenic lipoproteins, or it could protect the vessel wall by promoting cholesterol efflux out of the lesion. In addition to its role in lipid metabolism, apoE alters lymphocyte function. ApoE is a potent inhibitor of lymphocyte activation and proliferation (21–23). Multimeric synthetic peptides of apoE also suppress lymphocyte activation (24, 25). Evidence of immune cell-mediated protection against atherosclerosis suggests that apoE also may influence immune responses that might be atheroprotective (26).

Two recent studies support the hypothesis that locally produced apoE inhibits lesion formation (27, 28). Expression of human apoE in the vascular endothelial and smooth muscle cells of normal mice reduces atherosclerosis (27). Also, macrophage expression of human apoE in apoE-deficient mice reduces lesion development (28). In both instances, human apoE is expressed in mice and presumed to function in a manner identical to murine apoE. However, these studies could be interpreted differently if human apoE does not behave like endogenous mouse apoE.

To address the role of macrophage apoE in atherosclerotic lesions without expressing a human transgene in mice, we lethally irradiated male C57BL/6 (WT) mice to eliminate their endogenous bone marrow cells, and reconstituted them with bone marrow from WT or apoE-deficient (E−/−) mice. The two groups of mice were placed on an atherogenic diet for 16 weeks and subsequently examined for atherosclerosis. Interestingly, the lesions were larger in the animals that received the WT bone marrow. After this experiment was completed, Fazio et al. (29) published a report using a similar experimental design. However, opposite results were obtained. Therefore, we repeated our initial work and confirmed our initial findings. Possible reasons for this discrepancy are discussed.

METHODS

Animals

C57BL/6J WT mice were obtained from the Rodent Breeding Facility of The Scripps Research Institute (La Jolla, CA). The C57BL/6J backcrossed apoE-deficient mice were purchased from Jackson Laboratories (Bar Harbor, ME). The animals were weaned at 4 weeks and fed a standard chow diet (Diet No. 5015, Harlan Teklad, Madison, WI). Blood was drawn into a heparin-coated capillary tube after an overnight fast (12 h) by retro-orbital puncture under methoxyflurane-induced anesthesia. Plasma was isolated by centrifuging the blood samples at 3000 *g* for 30 min at 4°C.

Irradiation and bone marrow transplantation (BMT)

Six-week-old male WT mice were given 1000 rads of total body irradiation to eliminate their bone marrow-derived stem cells. Details of irradiation, bone marrow cell extraction, and repopulation are described elsewhere (10). In our first study, 8 of 16 irradiated mice received 2×10^6 bone marrow cells from apoE-deficient mice (henceforth referred to as WT+E−/− BMT mice), whereas the other 8 received 2×10^6 bone marrow cells from genotypically identical WT mice (henceforth referred to as WT+WT BMT mice). In our second study of 20 mice, 7 mice were reconstituted with WT bone marrow and 13 were reconstituted with apoE-deficient bone marrow. After transplantation, all the mice were fed a chow diet for 4 more weeks before they were switched to an atherogenic diet containing 15.8% fat, 1.25% cholesterol, and 0.5% sodium cholate for an additional 16 weeks.

Atherosclerotic lesion analysis

Twenty weeks after BMT, animals were killed and their atherosclerosis was examined as described previously (30, 31). Briefly, after perfusing the animals with phosphate-buffered saline (PBS) and subsequently with formal–sucrose (4% paraformaldehyde and 5% sucrose in PBS, pH 7.4), the top half of the heart was removed and immersed in cold PBS for 2 h and then formal–sucrose overnight at 4°C. The hearts were embedded in OCT, snap frozen in liquid nitrogen, and stored at −70°C until sectioning. Serial sections of 10 μm thickness were cut through a 250 μm segment of the aortic valve, and five sections, each separated by 40 μm encompassing 200 μm of the valve, were examined from each mouse. The sections were stained with Oil Red O to reveal the bright red staining of the lesions and counterstained with hematoxylin. The Oil Red O-stained areas of each section were quantitated using a computer-assisted video imaging system as described in detail (31).

Plasma cholesterol and lipoprotein analyses

Total plasma cholesterol was measured by a colorimetric method using a kit from Sigma (St. Louis, MO). Lipoproteins were separated by fast protein liquid chromatography (FPLC) (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). A pool comprised of equal volumes of plasma (0.1 ml) from all mice per group was filtered and applied to a 1 × 30 cm Superose 6 column (Pharmacia) and eluted with 10 mm Tris-HCl buffer, pH 7.4, containing 0.15 m NaCl and 0.01% EDTA. Fractions (0.5 ml) were assayed for cholesterol with a fluorometric method using an enzymatic kit from Boehringer Mannheim Corp. (Indianapolis, IN) and for apoE by Western blotting as described below (32).

Immunodetection of apoE

Plasma apoE was identified by Western blotting with a rabbit anti-rat apoE antibody that crossreacts with mouse apoE (a generous gift from Dr. K. Weisgraber, Gladstone Institute, San Francisco, CA). Samples (0.0020 ml) were diluted with 0.0455 ml deionized water and 0.0475 ml sample buffer (0.5 m Tris-HCl, pH 6.8–glycerol–10% (w/v) SDS–0.1% bromophenol blue–β-mercaptoethanol–deionized water 2.5:2:4:0.5:0.5:0.5 (v/v/v/v/v/v). This mixture (0.015 ml) was electrophoresed for 2 h at 125 V on a 6–20% gradient polyacrylamide gel containing 1% SDS. The gels were transferred at 25 V for 60 min to nylon membranes (Schleicher and Schuell, Keene, NH) with a semi-dry transfer cell (Bio-Rad, Hercules, CA). The membranes were incubated overnight at 4°C in blocking buffer (0.1% nonfat milk in PBS, pH 7.4), and for 2 h at room temperature with the antibody diluted 1:1000 in blocking buffer. After washing, the membranes were incubated for 1 h in a 1:2500 dilution of horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham, Buckinghamshire, England). Chemiluminescent detection of the apoE was performed with ECL Western detection reagent (Amersham). ApoE was quantitated by scanning the bands with a densitometer (Molecular Dynamics, Sunnyvale, CA).

Immunohistochemical detection of apoE

To detect apoE in peripheral blood leukocytes, mice were bled 16 weeks after transplantation. Approximately 0.2 ml of blood was washed twice in PBS and the red blood cells were lysed by

incubating in NH_4Cl lysis buffer. The leukocytes were washed and resuspended in RPMI medium supplemented with 5% fetal bovine serum, 10 mm HEPES, 2 mm l-glutamine, and 1% Pen-Strep. The cells were transferred to culture chamber slides (Nunc Inc., Naperville, IL) and exposed to 10 ng/ml of phorbol myristate (PMA). After 4 h at 37°C, non-adherent cells were removed and adherent cells were fixed overnight at 4°C with 1% paraformaldehyde. The cells were subjected to immunohistochemical analysis as described below.

Aortic valve sections of WT+WT BMT and WT+E−/− BMT mice of similar size and distribution of lesions were selected for this immunohistochemical detection, so that similarly staged lesions could be compared in the two groups. After fixing the sections for 2 min in acetone at −20°C, the protocol used for apoE detection was identical for both the adherent leukocytes and aortic valve sections. The tissues were incubated and washed successively at room temperature as follows: 1:50 normal goat serum for 1 h; 1:100 anti-apoE antibody in PBS containing 0.1% bovine serum albumin and 0.15% Triton X-100 for 2 h; 1:200 biotinylated anti-rabbit IgG (Vector Research, Minneapolis, MN) for 2 h; and 1:200 fluorescein isothiocyanate-conjugated streptavidin (PharMingen, San Diego, CA) for 1 h. The slides were mounted in fluorescence mounting media (Dako Corp., Carpinteria, CA) and viewed with a fluorescence microscope.

Statistical analysis

Lesion area was quantitated by calculating the mean lesion area of five aortic valve sections from each mouse, and comparing the mean lesion area of all mice in each group by Mann-Whitney U test. Sigma Plot version 2.0 (Jandel Corp., San Rafael, CA) was used for data analysis. Values in the text are given as mean ± SEM.

RESULTS

Four weeks after BMT the mice in our first study were bled to monitor repopulation with the donor bone marrow. The peripheral blood leukocytes were isolated, adhered to plastic, and stimulated with PMA. After fixation, the cells underwent immunofluorescent staining with an apoE-specific antibody. As shown in **Fig. 1**, the stimulated leukocytes of WT+WT BMT mice stained positive for apoE, whereas leukocytes from the WT+E−/− BMT mice showed only background staining. This staining confirmed successful repopulation of the WT+E−/− BMT mice.

Because macrophage-specific production of apoE was absent in the WT+E−/− BMT mice, plasma apoE was assessed by Western blot analysis to determine whether the absence of macrophage-derived apoE influenced the plasma levels of apoE. Plasmas of individual mice before and 20 weeks after transplantation were analyzed, and no differences in apoE levels were apparent (**Fig. 2**). Moreover, when the apoE was quantitated by densitometric scanning of the bands, no significant differences were detected. Interestingly, the total plasma apoE levels were reduced in all animals after they were placed on the high fat diet.

Total plasma cholesterol levels in these mice also were virtually identical throughout the study. Before BMT and 4 weeks after BMT, the cholesterol levels were similar at ~3 mmol/L in both groups, indicating that BMT had no effect on plasma total cholesterol. After consuming the atherogenic high fat diet for 4 weeks (8 weeks after BMT),

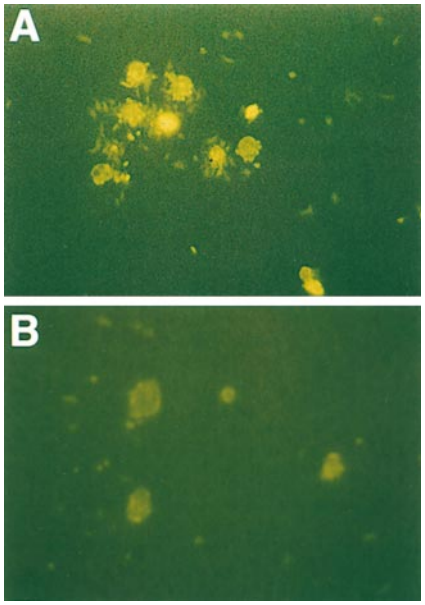

Fig. 1. ApoE expression by adherent peripheral blood leukocytes obtained 16 weeks after bone marrow transplantation. Cells were stimulated with PMA and allowed to adhere to the plastic slide chamber. ApoE expression was detected by immunofluorescence with an apoE-specific antibody as described in Methods. Panel A displays multiple cells from a WT+WT BMT mouse that stained brightly for mouse apoE. In contrast, the large macrophages from a WT+E−/− BMT mouse showed only background staining (panel B).

the cholesterol levels increased to 4.01 ± 0.28 mmol/L in the WT+WT BMT mice (n = 6) and to 4.24 ± 0.21 mmol/L in the WT+E−/− BMT mice (n = 6). After 16 weeks on the atherogenic diet, plasma cholesterol rose further to 4.40 ± 0.18 mmol/L in the WT+WT BMT mice (n = 6) and 4.71 ± 0.21 mmol/L in the WT+E−/− BMT (n = 6) mice. None of the mean time matched cholesterol values was significantly different between the two groups.

The distribution of cholesterol among the lipoprotein

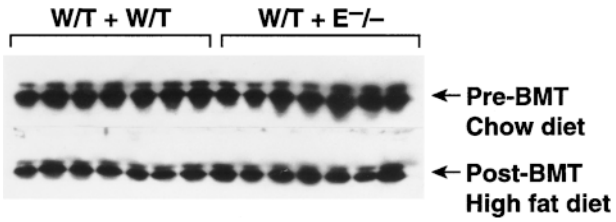

Fig. 2. Immunodetection of apoE in individual plasmas by Western blotting. To determine whether the absence of macrophage-derived apoE altered the circulating levels of apoE, 15 μl of a 1:47.5 dilution of plasma from irradiated mice was electrophoresed on a 6–20% gradient polyacrylamide SDS gel, transferred to a nylon membrane, and incubated with the apoE-specific antibody. ApoE was detected by chemiluminescence and quantitated by densitometric scanning of the bands. No significant differences in the apoE bands between the WT+WT BMT and WT+E−/−BMT mice were detected either before bone marrow transplantation on chow diet (upper bands) or 16 weeks after transplantation on the atherogenic diet (lower bands).

fractions was analyzed by separating the lipoproteins by column chromatography and measuring the total cholesterol content of each fraction (10). Before BMT, pooled plasma from both groups displayed the typical cholesterol distribution of chow-fed WT mice, with most of the cholesterol in HDL (**Fig. 3**). As expected, the profiles changed substantially at 16 weeks. After consuming the high fat diet, the majority of the cholesterol was in VLDL and LDL. Moreover, HDL cholesterol was approximately 50% of the levels observed with the chow diet. Nevertheless, this cholesterol distribution was similar in the plasmas of both WT+WT BMT and WT+E−/− BMT mice, indicating that bone marrow macrophage-derived apoE had no effect on the diet-induced lipoprotein distribution of cholesterol.

The distribution of apoE among the lipoprotein fractions also did not differ. As described previously, Western blotting of SDS-PAGE separated chromatography fractions corresponding to peak fractions of VLDL, IDL, LDL and HDL fractions can be used to identify the apoE lipoprotein distributions (32). In both the WT+WT BMT and the WT+E−/− BMT animals apoE was found predominately in the HDL fractions when the animals were fed the chow diet, whereas it was found predominately in the VLDL fraction when the animals consumed the high fat diet.

After 16 weeks on the atherogenic diet (which was 20 weeks after BMT), the mice were killed to assess lesion areas in the aortic valves. Oil Red O-stained fatty streak lesions were observed in all animals (**Fig. 4**). Mural lesions as well as lesions in the valve stem areas were common in the WT+WT BMT mice, whereas WT+E−/− BMT mice exhibited predominately valve stem lesions. The mean lesion areas of the WT+WT BMT and WT+E−/− BMT mice were 49,068 ± 7,825 μm^2 (n = 6) and 22,134 ± 2,976 μm^2 (n = 6), respectively (**Fig. 5A**). These differences were significant when analyzed by Mann-Whitney U test ($P < 0.01$). After this study was completed, a report by Fazio et al. (29) appeared. Using a similar design involving transplantation of wild-type C57BL/6J mice with bone marrow from apoE−/− mice, these researchers reported opposite results. To resolve this contradiction, our bone marrow transplantation studies were repeated. In a second study, 7 WT mice received WT bone marrow and 13 WT mice received apo E−/− bone marrow. All other experimental parameters were unchanged. As expected, the plasma apoE levels within each group were comparable both before and after consumption of the high fat diet. At killing, the mean lesion areas of the WT+WT BMT mice and WT+E−/− BMT mice were 48,783 ± 18,556 μm^2 and 21,342 ± 9,298 μm^2, respectively (Fig. 5B). These lesion areas were markedly similar to the areas quantitated in our first study and confirmed the data obtained with the first set of animals. Finally, as we initially observed, none of the animals had visible lesions in their aortas as assessed by staining the excised and longitudinally opened aortas with Sudan IV to stain the neutral lipids (data not shown).

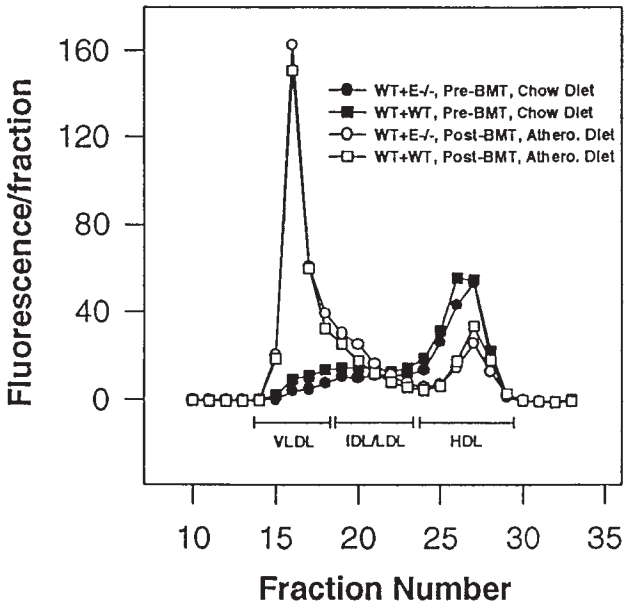

Fig. 3. FPLC profiles of plasma lipoproteins in WT+WT BMT and WT+E−/− BMT mice before and after transplantation. These FPLC cholesterol profiles represent pooled plasmas obtained before BMT on a chow diet (pre-BMT) and 16 weeks after BMT (12 weeks after initiation of the atherogenic diet) (post-BMT). The chromatographic separation of the lipoproteins was performed with 0.1 ml of pooled plasma from each group with a Superose 6 column. Cholesterol in each 0.5 ml fraction was measured by a fluorometric assay described in Methods.

Our final assessment was an examination of apoE expression within the lesions. To do this, lesions of similar size and morphology from both groups were studied. ApoE, detected by immunofluorescence, was abundant throughout the lesions of WT+WT BMT mice, whereas WT+E−/− BMT mice displayed little or no lesion apoE staining (**Fig. 6**) even though the plasma of these mice contained ample apoE (Fig. 2). Although it is inappropriate to quantitate immunofluorescent staining intensities, apoE antigen was unmistakably and consistently more prominent in the lesions of the WT+WT BMT compared to the WT+E−/− BMT mice. This was true irrespective of the size and location of the lesion and confirmed that the presence or absence of abundant lesion apoE was determined by the bone marrow donor phenotype. More importantly, because replacement of WT bone marrow with apoE-deficient bone marrow resulted in significant reduction of lesion area in our mice, these observations imply that removal of mouse macrophage apoE can be atheroprotective in WT male mice.

DISCUSSION

ApoE is expressed within atherosclerotic lesions. Although it can enter the artery wall from the periphery, most is believed to be synthesized locally by resident macrophages, as evidenced by abundant apoE mRNA detected in lesions of both humans and rabbits (12, 13). Yet, despite its presence, the role of local apoE in atherosclerosis is still controversial. To define the role of endoge-

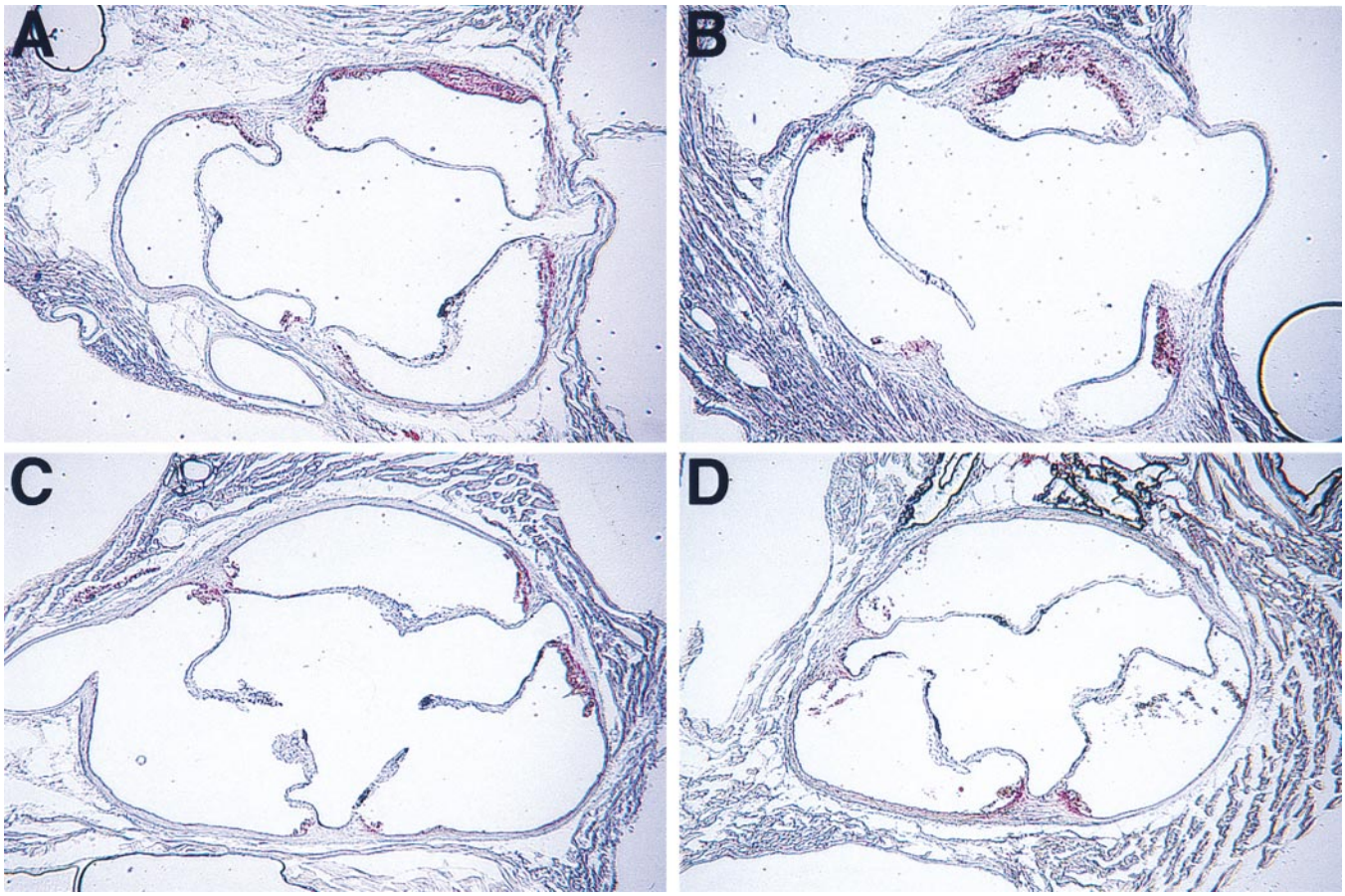

Fig. 4. Fatty streak lesions in representative aortic valve sections from WT+WT BMT (panels A and B) and WT+E−/− BMT (panels C and D) mice 20 weeks after transplantation. These frozen sections were stained with Oil Red O and counterstained with hematoxylin to reveal the bright red staining of the fatty streak lesions (×20).

nous macrophage apoE in atherosclerosis, we studied a mouse phenotype that lacked only macrophage apoE. This phenotype was generated by us and by Fazio et al. (29) by lethally irradiating WT mice and repopulating them with bone marrow from apoE-deficient mice. In both studies the traditional factors that promote lesion formation such as a high fat cholate-containing diet, high plasma cholesterol, high VLDL, IDL and LDL cholesterol, low plasma apoE, and low HDL cholesterol were not different in animals receiving either WT or apoE−/− bone marrow. However, replacing the bone marrow of WT mice with bone marrow of apoE-deficient mice essentially eliminated the local expression of apoE antigen in the fatty streak lesions that formed after 16 weeks on an atherogenic diet. In contrast, mice also lethally irradiated, but repopulated with WT bone marrow and placed on the same atherogenic diet, expressed apoE in their lesions. It can be concluded that most lesion apoE is synthesized locally and the macrophage is a predominant source of this apoE. Thus, in our studies as well as those of Fazio et al. (29), the only observed distinction was the expression of apoE by resident intimal macrophages.

Macrophage apoE could enhance atherogenesis by increasing the affinity of atherogenic lipoproteins for macrophages and smooth muscle cells that would favor foam cell formation. Additionally, the retention of atherogenic lipoproteins in the intima (by virtue of their apoE content) could promote their modification into even more atherogenic forms (33). We propose an additional mechanism whereby locally produced apoE might promote lesion formation. ApoE inhibits activation and proliferation of lymphocytes (21–25). The role of T lymphocytes in atherosclerosis has been examined (34). Although it was concluded that antigen-specific immune responses played a minor role in atherogenesis related to severe hypercholesterolemia, these same studies demonstrated that T cells play an important modulatory role in atherosclerosis under conditions of moderate hypercholesterolemia (34). C57BL/6J wild-type mice on a high fat diet are not severely hypercholesterolemic. Because atherosclerosis is more severe in class I major histocompatibility (MHC)-deficient C57BL/6J mice, which lack cytolytic T lymphocytes and have impaired natural killer cell activity (35), immune effector cells may protect against the chronic inflammatory reaction that characterizes atherosclerosis. Therefore, in the absence of apoE, activation and atheroprotective lymphocyte responses may predominate. Evidence that lymphokines such as interferon-γ can retard lesion formation (36, 37) supports this hypothesis, and the effect of apoE on inflammatory responses in the lesion needs to be explored.

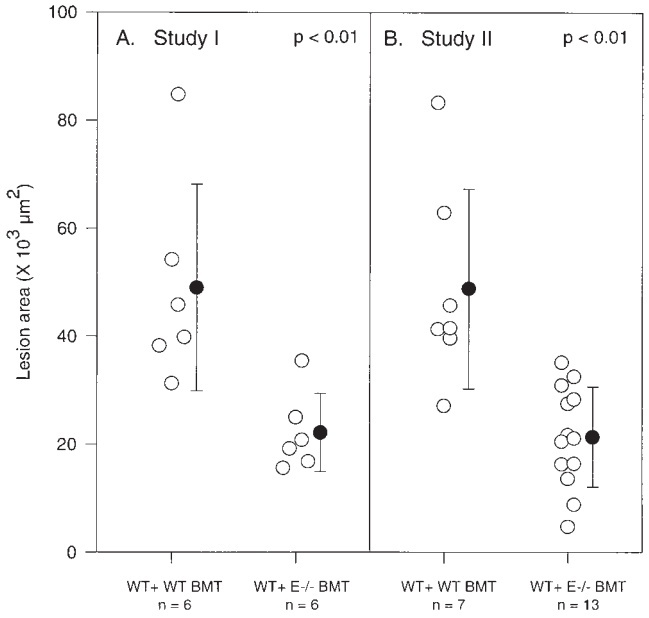

Fig. 5. Lesion area of the aortic valve sections. The Oil Red O-stained area of each section was quantitated with a computer-assisted video imaging system. For each mouse, five sections, separated by 40 μm and representing 200 μm of the aortic valve segment, were measured. In the first study (panel A) 6 mice from each group were used for this analysis, and each open circle on the graph represents the lesion area of one mouse. In the second study (panel B) 7 and 13 mice received bone marrow from WT and apoE−/− donors, respectively. The mean ± SD is represented by the closed circle with bars.

TABLE 1. Comparison of atherosclerosis in three studies

Study	Wild-Type Mice (n)	Transplanted Bone marrow	Mean Lesion Area
			μm^2
I	6	WT	49,068 ± 7,825[a]
	6	E−/−	22,134 ± 2,976
II	7	WT	48,743 ± 18,556[a]
	13	E−/−	21,342 ± 9,298
Fazio et al. (29)	11	WT	1,622 ± 458[a]
	14	E−/−	16,090 ± 3,419

Values given as mean ± SEM.
[a] $P < 0.01$, compared to E−/− transplanted group in each study.

Macrophage apoE also could be atheroprotective. Macrophage apoE could prevent foam cell formation by stimulating an efflux of free cholesterol from lesion macrophages (19). It also could facilitate reverse cholesterol transport of excess cholesterol (9) perhaps in concert with HDL (8, 38). Using a similar BMT experimental design, Fazio et al. (29) concluded that local apoE expression by macrophages is atheroprotective. As summarized in **Table 1**, the aortic lesion area in their studies was 10-fold greater in the WT + apoE−/− BMT mice (n = 14). In striking contrast, in our studies, the aortic lesion area was significantly reduced in WT animals who received apoE−/− BMT.

The conflicting data of these two reports requires a careful comparison between them. First, the sexes of the mice were different. Although we both used inbred WT C57BL/6J mice, and apoE−/− mice from the same C57BL/6Jx129 founder after it had been backcrossed nine generations into a C57BL/6J background, we used only male mice, and Fazio et al. (29) used only female mice. Second, the contents of the diets and the feeding schedules were different. The Fazio diet was obtained from ICN and contained 19.5% fat, 1.25% cholesterol, and 0.5% cholic acid, whereas ours was from Harlan Teklad and contained 15.8% fat along with 1.25% cholesterol and 0.5% sodium cholate. Our mice were fed this high fat

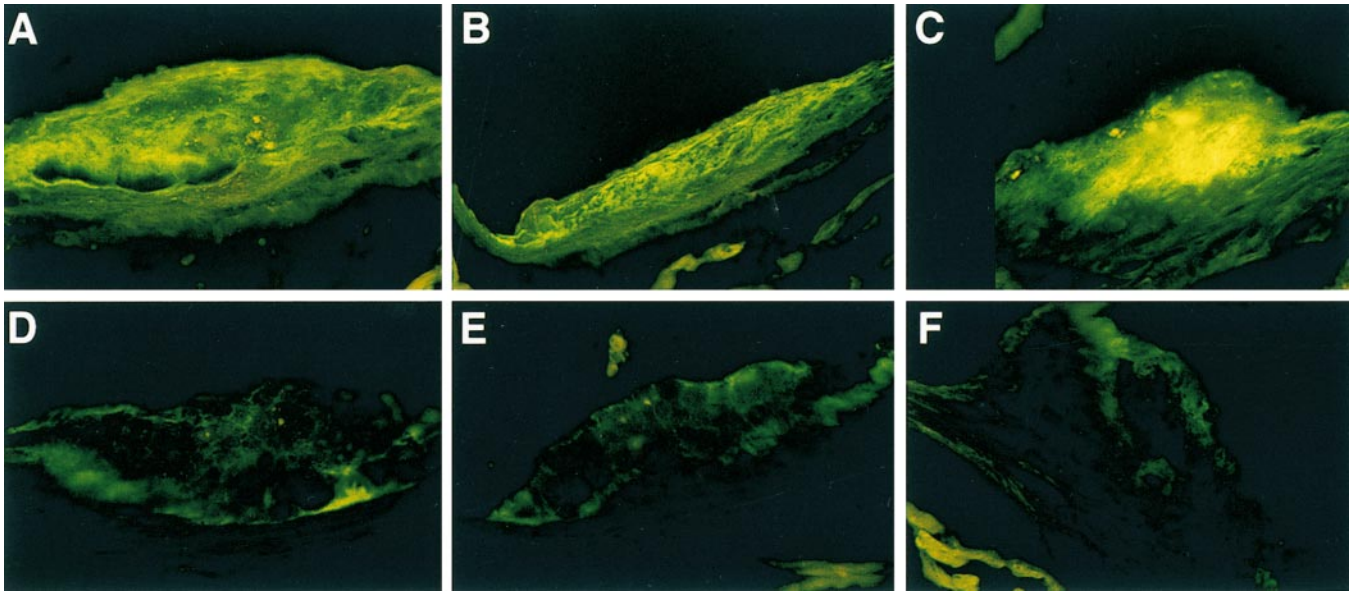

Fig. 6. Immunofluorescent detection of apoE antigen in fatty streak lesions. Frozen sections of the heart aortic valves were prepared 20 weeks after transplantation. Sections of similar distribution and size of lesions were selected for this analysis. Detection of apoE antigen was achieved by immunofluorescence as detailed in Methods. Panels A, B, and C are sections from WT+WT BMT mice that show abundant fluorescent staining, whereas similar size sections from WT+E−/− BMT mice (panels D, E, and F) show little or no staining.

diet for 16 weeks, 4 weeks after BMT, and their mice were fed the high fat diet for 13 weeks, 8 weeks after BMT. Finally, the amount of radiation used differed slightly (we used 1000 vs. 900 rads). Nevertheless, both studies resulted in quite comparable lesion areas in the WT mice reconstituted with apoE−/− bone marrow (Table 1). In fact, the major difference was not in the apoE−/− BMT mice, but in the WT BMT mice. Their WT + WT BMT female mice had lesion areas of 1,622 nm^2, whereas, our WT + WT BMT male mice had lesion areas of 49,000 nm^2 (Table 1).

Although we do not know the reason for this discrepancy in lesion areas, data on sex differences in lesion areas have been reported. Two studies have examined C57BL/6 mice of both sexes. Both Purcell-Huynh et al. (39), and Qiao et al. (40) reported that female mice fed comparable high fat diets for 15–18 weeks developed larger lesion areas than their male counterparts (~14,000 vs. 2,000–8,000 nm^2, respectively). Thus, the report of the low lesion areas in the female mice used by Fazio et al. (29) are not consistent with these previous observations, and a detailed analysis of the effects of hypercholesterolemia on males and females is warranted.

Other differences also could account for the conflicting results. For example, environmental factors such as number of animals per cage have been reported to influence the extent of disease. Finally, more information must be obtained on the direct effects of total body irradiation on atherosclerosis. Nevertheless, our studies have confirmed that the majority of apoE in the lesion is synthesized locally by lesion macrophages. Moreover, our studies establish that the elimination of this macrophage-derived apoE in the lesion can retard the development of atherosclerosis in male mice fed a high fat diet.

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