Retrovirus-mediated Expression of Apolipoprotein A-I in the Macrophage Protects against Atherosclerosis in Vivo*

We have previously reported that the lack of apolipoprotein (apo) E expression by macrophages promotes foam cell formation in vivo. Because transgenic mice overexpressing human apoA-I from the liver (h-apoA-I TgN) are protected from the atherogenesis induced by apoE deficiency, we hypothesized that the presence of apoA-I in the vessel wall could reduce the negative effect of apoE deficiency on lesion growth. To address this issue, we used both retroviral transduction and transgenic approaches to produce in vivo systems where apoA-I is expressed from macrophages. In the retroviral transduction study, apoA-I-deficient (apoA-I−/−) mice reconstituted with apoE-deficient (apoE−/−) bone marrow cells that were infected with a retroviral vector expressing human apoA-I (MFG-HAI) had 95% lower atherosclerotic lesion area than that of recipients of apoE−/− bone marrow cells infected with the parental virus (MFG). To determine whether the protective effect of locally produced apoA-I was due to the lack of systemic apoA-I, we conducted a different experiment using h-apoA-I TgN mice as recipients of apoE−/− bone marrow with or without human apoA-I (driven by a macrophage-specific transgene defined as mϕ-AI). Aortic lesion area in apoE−/−/mϕ-AI → h-apoA-I TgN mice was decreased by 85% compared with apoE−/− → h-apoA-I TgN mice. These data demonstrate that expression of apoA-I from macrophages protects against atherogenesis without affecting plasma apoA-I and high density lipoprotein cholesterol levels.

Epidemiological studies have shown a strong inverse correlation between plasma high density lipoprotein (HDL)1 cholesterol levels and coronary heart disease (1–2). The protective effects of HDL are attributed to its ability to promote cholesterol efflux from cells and mediate the transport of cholesterol to the liver for catabolism (3). However, the molecular mechanisms of the HDL effect on atherogenesis remain unclear, and it is possible that a large portion of this effect may be due to events localized in the vessel wall.

Secretion of apolipoprotein (apo)E by macrophages has been proposed to prevent the conversion of arterial macrophages into foam cells by stimulating the efflux of free cholesterol. In support of this hypothesis, Brown and Goldstein (4) have shown that macrophage-derived foam cells release an excess of free cholesterol associated with apoE within HDL particles (4). In addition, we and others (5, 6) previously provided in vivo evidence that the lack of apoE expression by macrophages promotes foam cell formation. These data support a protective role for apoE expression by the macrophage in the early stages of atherogenesis. Furthermore, our studies indicated that macrophages and macrophage-derived foam cells are important players in the initiation and progression of atherosclerosis, and can modulate lesion development by enhancing cholesterol efflux and activating reverse cholesterol transport (7, 8).

Although apoE has a strong effect on cholesterol efflux in the macrophages (9), apoA-I is likely the physiological acceptor of free cholesterol from peripheral tissues and is present in plasma at a concentration about 20 times higher than that of apoE. ApoA-I is the primary structural protein component of HDL (10). A central role for this apolipoprotein in the synthesis and assembly of HDL is suggested by the tight correlation between plasma apoA-I and plasma HDL cholesterol levels (11, 12). Yancey et al. (13) have demonstrated that lipid-free apoA-I is more efficient than plasma HDL at promoting the efflux of cholesterol from macrophage-derived foam cells, indicating that recruitment of cholesterol by apoA-I represents a major pathway for cellular cholesterol efflux. The importance of cholesterol efflux in foam cell development or regression has been established by the recent discovery of the molecular cause of Tangier disease, a genetic condition characterized by low or absent HDL and early atherosclerosis. Lack of the ATP-binding cassette transporter 1 (ABCA1) gene (14–16) causes Tangier disease due to impaired cholesterol efflux. ABCA1 knockout mice have greatly reduced HDL cholesterol and apoA-I levels, and accumulation of foam cells in tissues. Substantial support for the concept that overexpression of human apoA-I reduces atherogenesis has been provided in studies utilizing rabbits fed a high fat diet (17), as well as transgenic C57BL/6 and hyperlipidemic mice (18–20). These studies show that increased levels of human apoA-I in plasma effectively delay the progression of atherosclerosis. However, it is still unclear whether local effects of apoA-I within the atherosclerotic lesion are responsible for some of these results.

ApoA-I is synthesized by the liver and small intestine but,
induce long-term expression and stem cell transmission of human apoA-I would enhance cholesterol efflux from the macrophages in vivo, resulting in delayed foam cell formation and reduced atherosclerosis. We have previously demonstrated that retroviral transduction of bone marrow can be used to protect against atherosclerosis development in mice. Our results support the concept that apoA-I expression from macrophages can reduce lesion growth induced by the lack of apoE in the vessel wall irrespective of the presence or absence of apoA-I in plasma.

**EXPERIMENTAL PROCEDURES**

**Animal Procedures**—C57BL/6, C57BL/6-TgApoA1R (transgenic 1460apoA-I expressing human apoA-I from the liver; h-apoA-I TgN), B6.129P2-P TatcI-H9262/3 apoA-I-deficient mice, and B6.129P2-PapoA-Imttl/H9262 (apoE deficient; apoE-/-) mice were all originally obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in our mouse colony. All mice used in these studies were maintained in microisolator cages on an autoclaved rodent chow diet (PMI Feeds Inc., St. Louis, MO) containing 4.5% fat. Mice were given autoclaved water acidified to pH 2.6. Butterfat diet (ICN, Aurora, OH) containing 19.5% fat, 1.25% cholesterol, and 0.5% cholic acid was initiated 4 weeks after bone marrow transplantation. Mice were healthy throughout these studies and showed no differences in feeding pattern and body weight. Animal care and experimental procedures were performed according to the regulations of Vanderbilt University’s Institutional Animal Care and Usage Committee.

**Generation of ApoE-/- Mice Expressing Human ApoA-I from the Macrophages**—The apoE-/- macrophage-apoA-I (apoE-/-/m6-AI) mice, which have the ability to express human apoA-I from the apoE-deficient macrophages, were generated as follows: an 868-bp cDNA of the human apoA-I gene was amplified by reverse transcription-polymerase chain reaction and was cloned into the EcoRI and BamHI sites of the SR-A promoter and 5'- of the human growth hormone splicing and polyadenylation sites (gift of Dr. Chris Glass (25)). The 7-kb fragment was isolated by digestion with ClaI and NolI, separated from the vector by agarose gel electrophoresis, and purified using Gelase (Epicentre Technologies, Madison, WI) and phenol/chloroform extraction. The fragment was injected into fertilized C57BL/6 oocytes and re-implemented into C57BL/6 foster mothers. Incorporation of the human apoA-I cDNA was confirmed by Southern blot analysis. The apoE-/-/m6-AI mice were generated by breeding apoE-/- mice with transgenic C57BL/6 mice expressing human apoA-I.

**Cloning of Human ApoA-I cDNA into the MFG Retroviral Vector**—The human apoA-I fragment was used for ligation into the MFG retroviral vector. The insert/vector junctions and the human apoA-I cDNA insert were sequenced and found to be correct and complete. Calcium phosphate transfection of COS-7 cells and subsequent Western blotting assay confirmed that expression of human apoA-I was driven from the construct.

**Preparation of Producer Cell Lines**—PT 67 cells, a viral packaging cell line, were transduced with either MFG or MFG-containing human apoA-I cDNA (MFG-HAI). After screening for human apoA-I expression levels, the clonal cell line, named PT 67/MFG-HAI, with the highest level of expression was selected for use with retroviral infection of bone marrow cells.

**Bone Marrow Transplantation (BMT)**—A retroviral transduction approach was performed as follows: bone marrow cells were collected from both C57BL/6 and apoE-/- mice by flushing femurs and tibias with RPMI 1640 medium (Life Technologies, Inc., Gaithersburg, MD) with 2% heat-inactivated fetal bovine serum and 10 units/ml of penicillin and streptomycin (Co. St. Louis, MO). The rest of the bone marrow manipulations were done as previously described (24). Lethally irradiated 7-week-old female apoA-I-/- recipient mice received 1.5 x 10^6 retroviral-transduced bone marrow cells per mouse in 0.2 ml of RPMI 1640 medium intravenously.

A transgenic approach was carried out as follows: Bone marrow cells were harvested from apoE-/- and apoE-/-/m6-AI mice. After total irradiation, bone marrow cells were used immediately for transplantation into lethally irradiated 6- to 8-week-old female recipient mice. C57BL/6 and h-apoA-I TgN mice used as recipients were given 3.0 x 10^6 cells in 0.2 ml of RPMI 1640 medium intravenously.

All recipient mice were maintained on acidified water (pH 2.6) containing 100 mg/liter neomycin and 5 x 10^-5 M polymer b (Monarch Pharmaceuticals, Inc., Bristol, TN) for 1 week prior to and 2 weeks following transplantation.

**Primary Cultures of Peritoneal Macrophage**—Cells were collected 3 days after intraperitoneal injection of 3% thiglycollate (Sigma) as described previously (26). Peritoneal cells were washed with Dulbecco’s PBS containing 2% FBS and plated on either plastic or nitrocellulose membranes for Western blot or chamber slides for immunocytochemical analyses. The medium was collected from each well at 72-h intervals and frozen with 1 ml phenylmethylsulfonyl fluoride for Western blot assay.

**Lipid Assay**—Fasting blood samples were collected by retroorbital venous plexus puncture using heparinized tubes under isoflurane anesthesia (Medeva Pharmaceuticals, Inc., Rochester, NY). Total cholesterol and triglyceride levels were measured as previously described (26). HDL cholesterol levels were determined on pooled serum samples using ACE HDL-C reagent on an automated analyzer (Schapirelli Biosystem Inc., Fairfield, NJ).

**Lipoprotein and Apolipoprotein Assays**—Agarose gel electrophoresis of apoE-deficient serum was performed using 20-ca/30 gel (BioRad Laboratories, Hercules, CA) according to the manufacturer’s instructions.

**Enzyme-linked Immunosorbent Assay for the Detection of Human ApoA-I**—Serum levels of human apoA-I-transduced bone marrow were determined by a specific ELISA system according to the following protocol. ELISA plates were coated with 50 nl/well of anti-human apoA-I monoclonal antibody (BioDesign International, Saco, ME) at a concentration of 1 µg/ml (in 0.1 x bicarbonate buffer) and incubated at 4 °C overnight. The wells were washed with phosphate-buffered saline/0.5% Tween 20 (PBST) twice and blocked with 10% fetal bovine serum in phosphate-buffered saline (PBS) for 2 h at room temperature. Diluted standards (BioDesign International) and serum samples were applied to the wells at 4 °C overnight. Plates were washed with PBST four times, and peroxidase-conjugated sheep anti-human apoA-I antibody (BioDesign International) was applied for 1 h at room temperature. After washing, 2-aminobis-3-ethylbenzthiazoline-6-sulfonic acid (Sigma) was added to each well. Color development was allowed to occur for 10–20 min. Absorbance was measured at 405 nm on a microplate reader ( Molecular Devices, Sunnyvale, CA). Serum human apoA-I levels were calculated by comparison to the standard curve.

**Western Blotting Analysis of ApoA-I**—Briefly, pooled serum was mixed with d = 1.006 NaCl solution and potassium bromide to increase the density to d = 1.210. Ultracentrifugation of lipoproteins was performed at 120,000 rpm for 5 h at 10 °C using an Optima TLX ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA). Three hundred microliters of supernatant was collected via a slicer system (Beckman Instruments). Amicon-Micro 100 columns (Millipore, Billerica, MA) were used to desalt and concentrate the fractionated sample and conditioned medium. Concentrated serum and medium were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4–20% gradient pre-cast polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA).

In the transduction study, the nitrocellulose membranes were incubated with a polyclonal anti-human apoA-I antibody (BioDesign International) as a primary antibody and a horseradish peroxidase-conjugated anti-goat IgG (Sigma) as a secondary antibody. Antibody bindings were visualized by chemiluminescence with the ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ).

**Immunochromical Analysis for Macrophage and ApoA-I**—The presence of human apoA-I in peritoneal macrophages was examined by immunocytochemistry. Peritoneal macrophages on a chamber slide were fixed in cold acetone and incubated with a polyclonal goat anti-human apoA-I antibody (Sigma) overnight. The samples were treated with a biotin polyclonal anti-goat IgG (BD Pharmingen, San Diego, CA). To detect macrophages in the arterial lesions, 5-µm serial cryosections of proximal aorta were fixed in cold acetone and incubated with a monoclonal rat antibody to mouse macrophages, MOMA-2 (Accurate Chemical & Scientific Corp., Westbury, NY) overnight at room temperature. The sections were treated with goat biotinylated antibody.
The baseline values were obtained on a diet of 4.5% fat rodent chow (Roden Diet 5010, PMI). Mice were fed an atherogenic high fat diet consisting of 19.5% fat, 1.25% cholesterol, and 0.5% cholic acid (Butter Fat Diet, ICPN) after 4 weeks post-BMT. At the indicated time points, blood samples were collected with heparinized tubes after a 4-h fast. Serum cholesterol and triglyceride levels were determined using Raichem reagent 80005 and a Sigma kit 339, adapted for microplate assay. HDL cholesterol levels were determined using ACE HDL-C reagent on an ACE clinical chemistry system. Values are in mg/dl (mean ± S.E.). The number of animals in each group at each time point is indicated by n.

**TABLE I**

| Donor     | Recipient | Serum lipid | Baseline | 4 weeks post-BMT | 8 weeks on butterfat diet | 12 weeks on butterfat diet |
|-----------|-----------|-------------|----------|-----------------|--------------------------|---------------------------|
| ApoE−/−/MFG | ApoAI−/− | Cholesterol | 20.8 ± 1 | 20.1 ± 1.0     | 30.5 ± 1.8               | 36.8 ± 3.0                |
| n = 8      |           | Triglyceride | 40.6 ± 3.0 | 38.9 ± 3.4      | 45.2 ± 3.4               | 40.9 ± 2.2                |
|           |           | HDL cholesterol | 7        | 10             | 12                        | 20                        |
| ApoE−/−  | ApoAI−/− | Cholesterol | 21.3 ± 1.9 | 15.6 ± 1.7     | 28.0 ± 3.7               | 35.5 ± 2.1               |
| n = 9      |           | Triglyceride | 40.0 ± 1.6 | 44.0 ± 2.5      | 45.6 ± 3.7               | 35.6 ± 3.1               |
|           |           | HDL cholesterol | 8        | 6              | 12                        | 20                        |
| ApoE−/− MFG-HAI | ApoAI−/− | Cholesterol | 20.6 ± 1.0 | 17.7 ± 1.8     | 24.6 ± 1.5               | 29.9 ± 2.8               |
| n = 8      |           | Triglyceride | 38.6 ± 4.2 | 43.8 ± 1.9      | 44.5 ± 3.5               | 35.7 ± 3.0               |
|           |           | HDL cholesterol | 7        | 10             | 10                        | 18                        |
| ApoE−/− MFG-HAI | ApoAI−/− | Cholesterol | 20.3 ± 1.3 | 15.1 ± 2.0     | 35.3 ± 2.9               | 38.5 ± 2.6               |
| n = 8      |           | Triglyceride | 48.5 ± 2.7 | 56.0 ± 4.5      | 49.1 ± 4.2               | 38.9 ± 3.5               |
|           |           | HDL cholesterol | 7        | 7              | 15                        | 26                        |

RESULTS

Retroviral Transduction Study

**Effects of Macrophage Expression of Human ApoA-I on the Lipid Profile**—Female apoA-I−/− mice were transplanted with bone marrow cells transduced with apoE−/−/MFG (n = 8), apoE−/− MFG (n = 10), apoE−/−/MFG-HAI (n = 8), or apoE−/− MFG-HAI (n = 8) and were placed on butterfat diet after 4 weeks post-BMT. In all groups, cholesterol levels increased throughout the study in a time-dependent manner. No relevant differences in serum among the groups were detectable at 16 weeks post-BMT (Table I), although in early time points some differences of between groups reached statistical significance. Agarose gel electrophoresis of serum lipoproteins showed no differences among groups before and after BMT; although the pre-β and β migrating bands (very low density lipoprotein and low density lipoprotein, respectively) increased in all groups on a butterfat diet (Fig. 1). HDL cholesterol levels increased in a time-dependent manner, but no differences were detected among the four groups (Table I).

**Expression of Human ApoA-I by Macrophages**—The expression of human apoA-I in either the apoE−/−/MFG-HAI or the apoE−/− MFG-HAI mice was confirmed as early as 4 weeks post-BMT and continued for the duration of the study. No human apoA-I was detected, before or after BMT, in recipients of marrow transduced with parental retrovirus (Fig. 2a). Determination of human apoA-I levels in serum by ELISA and Western blotting assay showed that, by 4 weeks after BMT, human apoA-I levels in apoE−/− MFG-HAI were around 20 ng/dl. The presence of human apoA-I in the media of cultured peritoneal macrophages of either the apoE−/−/MFG-HAI or the apoE−/− MFG-HAI was confirmed by Western blotting assay (Fig. 2b). The intracellular apoA-I was primarily in the cytoplasm as demonstrated by immunocytochemistry (Fig. 3). No signal was detected in macrophages of recipients of marrow transduced with parental retrovirus.

**Effects of Human ApoA-I on Atherosclerosis**—Quantitative analysis of aortic atherosclerosis was performed on Oil Red O-stained sections of the proximal aorta. In agreement with our previously published data (5), removal of apoE from macrophages worsened atherosclerosis. The mean lesion area was 7-fold greater in the apoE−/− MFG mice (n = 10) than in the
Procedures.

Lanes 4/H9262 concentrated down to 15 columns. Lanes 1
lanes 4 were subjected to 4 a
bated for 72 h, and media were collected as described under
human apoA-I secreted into culture medium. Macrophages were incu-
apoE serum.

Lane 3 apoE was 18-fold less than in the apoE
MFG-HAI (n = 835; p < 0.05) (Fig. 4). The mean lesion area in the
apoE -/-. apoE -/-/mφ-AI mice had about 250 mg/dl human apoA-I in serum, and the production of apoA-I by macrophages did not affect total hu-
mouse was detected at the base of the
pattern that only partially overlaps with the
macrophage-positive area (Fig. 5d). In contrast, human apoA-I in
apoE -/-/apoA-I/-/- double knockout mice reconstituted with
apoE -/-/MFG-HAI, apoE -/-/MFG-HAI, apoE -/- MFG-HAI, respectively.

To determine whether the effect of local expression of apoA-I
was due to the lack of circulating apoA-I in the recipient mice and also to see if small amounts of local apoA-I can have an even more protective effect in the presence of large amounts of systemic apoA-I, we conducted a different experiment using
apoA-I TgN mice (data not shown).

Effects of Macrophage Expression of Human ApoA-I on the Lipid Profile—Total cholesterol levels in the apoE -/- h-apoA-I TgN mice and the apoE -/-/mφ-AI h-apoA-I TgN mice were increased in a time-dependent manner, with no differences between groups (Table II). Lipoprotein distribution by agarose gel electrophoresis showed no differences between the
apoE -/- h-apoA-I TgN mice and the apoE -/-/mφ-AI h-apoA-I TgN mice (data not shown).

Expression of Human ApoA-I by Macrophages—Human apoA-I was detected at all time points (including the pre-BMT time point) in the apoE -/- h-apoA-I TgN mice and the apoE -/-/mφ-AI h-apoA-I TgN mice, but not in the apoE -/-/mφ-AI C57BL/6 mice (Fig. 6a). At 16 weeks post-BMT, the apoE -/- h-apoA-I TgN mice and the apoE -/-/mφ-AI h-apoA-I TgN mice had about 250 mg/dl human apoA-I in serum, and the production of apoA-I by macrophages did not affect total hu-

Macrophage ApoA-I Is Protective against Atherosclerosis

Localizaton of Human ApoA-I in the Atherosclerotic Plaque—Because of the small vessel wall lesion area in apoA-
recipient mice due to hypolipidemia, the analysis of hu-
anapolA-I from artery wall macrophages was con-
conducted in apoE -/-/apoA-I/-/- double knockout recipient mice. The aortic lesions in both apoE -/- and apoE -/-/apoA-I/-/- double knockout mice consisted mainly of foam cells that stained with the anti-macrophage antibody MOMA-2 (Fig. 5, a and c). Mouse apoA-I in apoE -/- mice was detected at the base of the
lesion, in a pattern that only partially overlaps with the macrophage-positive area (Fig. 5b). In contrast, human apoA-I in
apoE -/-/apoA-I/-/- double knockout mice reconstituted with
apoE -/-/MFG-HAI stained mainly the macrophages in the foam cell lesion (Fig. 5d).

Macrophages were incubated for 72 h, and media were collected as described under “Experimental Procedures.” Media were concentrated with Microcon-YM 10 columns. Lanes 1 and 2 contain 15 µl of the indicated dilution of human serum. Lanes 3–7 contain the equivalent of 1500 µl of culture media concentrated down to 15 µl. Lane 3 is non-conditioned DMEM, and lanes 4–7 contain macrophage media from apoE +/+ MFG, apoE -/- MFG, apoE +/+ MFG-HAI, or apoE -/- MFG-HAI, respectively.

apoE +/+ MFG mice (n = 8) (9776 ± 1835 (mean ± S.E.) versus
1431 ± 448 µm²; p < 0.05) (Fig. 4). The mean lesion area in the
apoE +/+ MFG-HAI mice (n = 8) (1158 ± 569; p < 0.05) was eight times smaller than in the apoE -/- MFG. In the apoE -/- MFG-
HAI mice (n = 8), the mean lesion area (546 ± 335; p < 0.05) was 18-fold less than in the apoE -/- MFG mice.
Macrophage ApoA-I Is Protective against Atherosclerosis

**FIG. 5.** Immunohistochemical detection of macrophages and apolipoprotein A-I in proximal aorta of apoE<sup>−/−</sup> (a and b) and apoE<sup>−/−</sup> MFG-HAI → apoE<sup>−/−</sup> apoA-I<sup>−/−</sup> double knockout (c and d). Sections were stained for macrophages and apoA-I as described under “Experimental Procedures.”

Man apoA-I or HDL cholesterol levels in recipient mice (data not shown). Western blot analysis showed that peritoneal macrophages in the apoE<sup>−/−</sup>/mφ-AI → h-apoA-I TgN mice secreted human apoA-I in culture medium, whereas macrophages from the apoE<sup>−/−</sup> → h-apoA-I TgN mice did not (Fig. 6b).

**Effects of Human ApoA-I on Atherosclerosis—**Quantitative analysis of aortic atherosclerosis was carried out as in the first study. The mean lesion area was 36-fold greater in the apoE<sup>−/−</sup> → C57BL/6 mice (n = 20) than in the apoE<sup>−/−</sup> → h-apoA-I TgN mice (n = 8) (15.37 ± 2865 versus 424 ± 208; p < 0.0001) (Fig. 7). The mean lesion area in the apoE<sup>−/−</sup>/mφ-AI → h-apoA-I TgN mice (n = 10) (58 ± 21; p = 0.05) was seven times smaller than in the apoE<sup>−/−</sup> → h-apoA-I TgN mice.

**DISCUSSION**

In the present study, transduction and transgenic approaches were used to determine whether apoA-I expression by macrophages can protect against atherosclerosis development in apoA-I-deficient or human apoA-I-overexpressing mice. ApoA-I<sup>−/−</sup> recipient mice reconstituted with the apoE<sup>−/−</sup> marrow developed significantly more aortic atherosclerosis than did recipients of the apoE<sup>−/−</sup> marrow, confirming our previously reported observation in C57BL/6 mice (5). In this setting, the expression of human apoA-I from macrophages decreased atherosclerosis in both apoA-I-deficient and apoA-I-overexpressing mice. This indicates that human apoA-I produced by apoE-negative macrophages affects atherosclerotic lesion formation. Our studies clearly establish that macrophage apoE is involved in foam cell formation and that, in the absence of apoE, apoA-I produced by macrophages re-establishes an appropriate cholesterol homeostasis.

Macrophages express apoE abundantly in atherosclerotic lesions, and apoE secretion by macrophages has been proposed to be a protective process, which prevents foam cell formation by stimulating cholesterol efflux and/or facilitating reverse cholesterol transport from the arterial wall (5, 9, 8). Bellosta et al. (7) have reported that macrophage-specific expression of the human apoE3 transgene in apoE-null mice induced a dramatic reduction in atherosclerosis susceptibility. We showed that low level expression of apoE secretion by arterial macrophages in apoE-deficient mice delays atherogenesis (24). In addition, we and others demonstrated that lack of apoE expression by the macrophage promotes foam cell formation in wild-type mice (5, 6), supporting a protective role for apoE expression by the macrophage in the early stage of atherogenesis. The present study confirms these observations by showing that the mean lesion area in aortic cross-sections was increased by 85% in mice reconstituted with apoE<sup>−/−</sup> MFG compared with apoE<sup>−/−</sup> MFG mice, in the absence of differences in plasma lipid lipoprotein levels or apoE expression in serum. These data also support the concept that apoE expression from macrophages is able to modulate atherogenesis even if normal levels of apoE are present in serum.

Although the inverse relationship between HDL cholesterol, apoA-I, and atherosclerosis risk has long been recognized, the mechanism by which HDL and apoA-I lower the risk of coronary heart disease is unclear. The process of cholesterol efflux remains the focus of the majority of studies aimed at understanding the protective role of HDL and apoA-I. Rubin et al. (18) created human apoA-I transgenic mice to evaluate the effect of apoA-I on the development of atherosclerosis. Total plasma apoA-I and HDL cholesterol concentrations were increased 2-fold in transgenic animals compared with control animals. The expression of the human apoA-I transgene from the liver resulted in complete protection from the development of atherosclerosis in transgenic animals. One mechanism by which apoA-I and HDL may act to prevent atherosclerosis progression in this model is the enhancement of cholesterol efflux. In vitro studies confirmed that cholesterol efflux is increased when FusAH hepatoma cells are incubated with sera from apoA-I transgenic versus control rabbits (17). Paszty et al. and others (20, 19) introduced the human apoA-I transgene into the hypercholesterolemic apoE knockout background to examine if increases in apoA-I and HDL cholesterol levels are also effective in minimizing the harmful effect of apoE deficiency. The mean lesion area of apoE knockout mice expressing human apoA-I from the liver was 6-fold lower than that of apoE knockout mice. These studies demonstrate that elevated HDL cholesterol levels due to high levels of human apoA-I can influence atherosclerosis susceptibility in hypercholesterolemic apoE null mice. Moreover, it was recently reported that systemic apoA-I modulates atherogenesis in apoE negative mice after bone marrow transplantation (28). In this study, wild-type marrow was transplanted into apoE-negative or apoE/ apoA-I-double-negative mice. Macrophage-derived apoE lowered plasma cholesterol in both mouse types, but the extent of aortic atherosclerosis was 45–60% larger in the double mutants, indicating that the absence of circulating apoA-I increases susceptibility to vascular damage in this model.

In the present studies, human apoA-I was present in the serum of apoA-I-deficient recipients reconstituted with either apoE<sup>−/−</sup> MFG-HAI or apoE<sup>−/−</sup> MFG-HAI for as long as 16 weeks post-BMT. The small amount of human apoA-I in plasma (about 20 ng/dl) did not affect HDL cholesterol levels, but the atherosclerotic lesion area in apoE<sup>−/−</sup> MFG-HAI mice was significantly decreased compared with apoE<sup>−/−</sup> MFG animals. These results indicate that large changes in plasma HDL cholesterol levels are not needed to modulate atherogenesis and that apoA-I secretion from macrophages and macrophage-derived foam cells in the vessel wall can affect lesion growth. We also confirmed in our transgenic study that small amounts of human apoA-I produced by macrophages decreased atherosclerotic lesion area in apoE<sup>−/−</sup>/mφ-AI → h-apoA-I TgN mice compared with in apoE<sup>−/−</sup> → h-apoA-I TgN mice even though high levels of serum HDL cholesterol and human apoA-I levels were present in plasma in the recipients. In normolipidemic human plasma, the concentration of lipid-free apoA-I has been estimated to be ~8% of the total plasma apoA-I pool (29). This small amount of lipid-free apoA-I is able to enter the subendo-
thelial space of the arterial wall wherein it promotes the removal of excess cholesterol from peripheral cells. If human apoA-I produced by macrophages and macrophage-derived foam cells in the subendothelial space promotes cholesterol efflux without affecting plasma HDL levels, then high levels of HDL cholesterol in plasma may not be the obligatory condition to obtain benefits at the level of the vessel wall. In the present study, human apoA-I secreted by the macrophage in subendothelial space added benefits to the protective effects of high levels of plasma apoA-I.

Gene therapy has been considered a promising alternative for treatment of cardiovascular disease (30). Several approaches (31, 32) have been used to evaluate the impact of the cellular and molecular mechanisms of the atherogenesis, but a useful clinical approach for cardiovascular disease is yet to be developed. Our data support the view that expression of apoA-I from the macrophage is a target for gene therapy of atherosclerosis.

In conclusion, we show that macrophage-specific expression of human apoA-I has a significant impact on foam cell formation without affecting serum lipids or lipoprotein levels. Further studies will be necessary to identify the molecular mechanism of the anti-atherogenic effect of apoA-I secretion by the macrophage.

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Retrovirus-mediated Expression of Apolipoprotein A-I in the Macrophage Protects against Atherosclerosis in Vivo

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