Atherosclerosis in Mice Is Not Affected by a Reduction in Tissue Factor Expression


Objective.—To determine whether tissue factor (TF) contributes to the progression of atherosclerotic lesions in mice.

Methods and Results.—We determined the effect of a 50% reduction of TF levels in all cells on atherosclerosis in apolipoprotein E-deficient (apoE−/−) mice. No differences were observed in the extent of atherosclerosis in apoE−/−/TF+/− and apoE−/−/TF−/− mice fed regular chow for 34 weeks. Atherosclerosis could not be analyzed in apoE−/− mice expressing low levels of TF because of premature death of these mice. Macrophages are a major source of TF in atherosclerotic plaques. Therefore, in a second series of experiments, we investigated the effect on atherosclerosis of selectively reducing hematopoietic cell-derived TF by transplanting bone marrow from mice expressing low levels of TF into low-density lipoprotein receptor deficient (LDL−/−) mice. Atherosclerosis within the arterial tree and aortic root were similar in LDL−/− mice with low-TF bone marrow compared with control bone marrow (TF+/+ or TF−/−) after 4 and 16 weeks on an atherogenic diet. Furthermore, the cellular composition of the aortic root lesions was similar between the 2 groups.

Conclusions.—Our data indicate that either a 50% reduction of TF in all cells or a selective reduction in hematopoietic cell-derived TF does not affect the development of atherosclerotic lesions in mice. (Arterioscler Thromb Vasc Biol. 2006;26:555-562.)

Key Words: atherosclerosis ■ macrophages ■ mice ■ tissue factor
The role of TFPI in atherosclerosis was investigated by examining the effect of reducing TFPI levels by 50% in apoE\(^{-/-}\) mice.\(^{24}\) TFPI\(^{+/+}\) mice have increased TF activity. The apoE\(^{-/-}\)/TFPI\(^{+/+}\) mice exhibited a selective increase in atherosclerosis in carotid and iliac arteries compared with apoE\(^{-/-}\)/TFPI\(^{+/+}\) mice expressing wild-type levels of TFPI,\(^{24}\) suggesting a role for TFPI in atherosclerosis.

In the present study, we examined if TF plays a role in the progression of the atherosclerosis by examining the effect of a 50% global reduction of TF expression or by selectively reducing TF expression in hematopoietic cells. We used mice deficient in either apolipoprotein E (apoE\(^{-/-}\)) or the low-density lipoprotein receptor (LDLR\(^{-/-}\)) and mice expressing 100%, 50%, or \(\approx 1\)% of TF levels (low-TF mice).\(^{25,26}\) Reducing TF levels of apoE\(^{-/-}\) mice by 50% in all cells did not alter the amount of atherosclerotic lesions. Similarly, selectively reducing TF levels in hematopoietic cells in LDLR\(^{-/-}\) mice did not affect the percentage surface area of aortic lesions or aortic root lesion size and cellular composition.

**Methods**

**Mice**

All studies were approved by The Scripps Research Institute and University of Michigan Animal Care and Use Committees and comply with National Institute of Health Guidelines.

The apoE-deficient (apoE\(^{-/-}\)) mice on a C57Bl/6J background were purchased from Jackson Laboratories (Bar Harbor, Me). Low-TF mice express very low levels of human (h) TF (\(\approx 1\)% relative to mouse (m) TF) and completely lack mouse TF (mTF\(^{-/-}\)).\(^{25}\) The apoE\(^{-/-}\) mice were bred in-house with low-TF mice on a C57Bl/6J background to eventually generate apoE\(^{-/-}\)/TF\(^{-/-}\), apoE\(^{-/-}\)/TF\(^{+/-}\), and apoE\(^{-/-}\)/low-TF mice. For some experiments, apoE\(^{-/-}\) mice were bred with TF\(^{-/-}\) mice (a kind gift from Dr G. Broze) to generate apoE\(^{-/-}\)/TF\(^{+/-}\) and apoE\(^{-/-}\)/TF\(^{+/-}\). Mice were fed ad libitum either a standard mouse chow diet or, when stated, an atherogenic diet (15.8% fat, 1.25% cholesterol, and no cholic acid; No. 94059, Harlan, Teklad).

LDLR-deficient (LDLR\(^{-/-}\)) mice, backcrossed onto a C57Bl/6J background, were purchased from Jackson Laboratories and bred in-house. The mice were weaned at 4 weeks and fed ad libitum either a standard mouse chow diet (regular chow; Purina 7012, Harlan, Teklad) or an atherogenic diet for 4 weeks (21.2% fat, 1.25% cholesterol; No. 96121, Harlan, Teklad) and 16 weeks (15.8% fat, 1.25% cholesterol, and no cholic acid; No. 94059, Harlan, Teklad).

apoE\(^{-/-}\) and LDLR\(^{-/-}\) mice are different models of atherosclerosis. apoE\(^{-/-}\) mice have high very-low-density lipoprotein levels, whereas LDLR\(^{-/-}\) mice have high LDL. In bone marrow transplantation experiments, macrophage apoE will reduce the hypercholesterolemia and protect the mice from atherosclerosis.\(^{27}\) Therefore, we used LDLR\(^{-/-}\) mice for the bone marrow transplantation experiments because macrophage LDL receptor does not alter lipid levels.\(^{27}\)

**Irradiation and Bone Marrow Transplantation**

LDLR\(^{-/-}\) mice (8 weeks old) were subjected to 13 Gy (1300 rad) \(\gamma\)-irradiation from a cesium 137 irradiator (Gamaccell 40; Atomic Energy of Canada, Mississauga, ON, Canada) to ablate endogenous bone marrow-derived cells and stem cells. All irradiated mice were injected with \(2 \times 10^6\) bone marrow cells from control (either mTF\(^{+/+}\) or mTF\(^{-/-}\)/ATF\(^{+/-}\)) or low-TF mice via the retro-orbital sinus. Mice were allowed to recover for 4 weeks before being fed an atherogenic diet.

**Genotyping of DNA From Bone Marrow Recipient Mice**

Blood was collected after euthanasia and DNA was prepared from peripheral blood mononuclear cells. Analysis of the levels of the wild-type (WT) and low-TF allele was used to demonstrate bone marrow reconstitution. The WT mouse (mTF) allele was detected by polymerase chain reaction (PCR) using a forward primer, 5' -ATGAGGAGCTGTGGTTAAGGTCGCAAGA- and a reverse primer, 5' -TGCAGTAAATCGAGTGTCTGCGCAT-; that are located upstream of exon 1 of the mTF allele (559bp). The mutant TF allele was detected using the mTF forward primer, 5' -CAAGAGGAGCAGGGTCGCTTCG- in conjunction with a reverse primer, 5' -CACGGAGAACCGGTTCGACCCATTCCG- that is located within the neo cassette (700 bp).

**TF Activity**

Functional TF activity in homogenized unjured carotid arteries was determined using a previously described chromogenic assay.\(^{28}\) Briefly, 30 \(\mu L\) of carotid artery homogenate was combined with 90 \(\mu L\) of a reaction mixture containing 3 nmol/mL human FVIIa (Haemtech), 100 nM human FX (Haemtech), 8.3 mmol/L CaCl\(_2\), and 0.33 mmol/L Spectrozyme FXa (American Diagnostica) in Tris-buffered saline, pH 7.4, with 1 mg/mL bovine serum albumin. After 45 minutes of incubation at 37°C, the optical density was measured at 405 nm and the TF activity was expressed in pmol/L as determined by reference to a standard curve of recombinant human repiled TF (rTF) (American Diagnostica). This analysis provides data on the relative levels of TF activity in the carotid arteries of WT and TF\(^{-/-}\) mice and does not accurately measure TF levels because of the inefficiency of human FVIIa binding to murine TF.\(^{29}\)

The procoagulant activity of peritoneal macrophages was determined using a single-stage clotting assay. Peritoneal macrophages, elicited by thioglycollate, were collected from TF\(^{+/+}\), TF\(^{+/+}\)/hTF\(^{-/-}\) and low-TF mice and \(2 \times 10^6\) cells were plated per well of a 6-well plate. Cells were treated with lipopolysaccharide (LPS) (1 \(\mu g/mL\); Escherichia coli serotype OB111; Sigma) or vehicle for 6 hours, then scraped and stored at \(-80°C\). The cells were subsequently lysed with N-octyl-\(\beta\)-D-glucopyranoside and diluted in 25 mmol/L Hepes-saline, pH 7.4, before combining with mouse pool plasma (Sigma) in a Start 4 clotting machine (Diagnostica Stago). The reaction was initiated by the addition of 20 mmol/L CaCl\(_2\) in 25 mmol/L Hepes-saline, pH 7.4, and the time to clot was determined. TF activity expressed in relative units/\(1 \times 10^6\) cells was calculated by reference to a standard curve of mouse brain extract.

**Analysis of Atherosclerosis**

Assessment of the atherosclerotic lesions was performed in 2 different ways.

**Method 1**

After euthanasia, the vasculature of the mice was perfused with phosphate-buffered saline (PBS), pH 7.4, followed by formalin (4% paraformaldehyde and 5% sucrose in PBS, pH 7.4). The aorta from the proximal ascending aorta to the bifurcation of the iliac artery was dissected and stained with Sudan IV. The aortas were photographed and images digitized. The total arterial surface area and total lesion area were determined using Adobe Photoshop 5.0.2 and NIH Scion Image Software. The extent of lesion development was reported as percentage of the total area of a given artery that was occupied by atherosclerotic lesions.

Lesions of the aortic root were analyzed as previously described.\(^{30,31}\) The proximal aortic root and adjoining portion of the heart from LDLR\(^{-/-}\) mice was removed, immersed in formalin for 6 hours and subsequently embedded in OCT and stored at \(-70°C\) until sectioning. Serial sections (10 \(\mu m\) in thickness) were cut through a 250-\(\mu m\) segment of the aortic root, where all 3 valve leaflets are present. For each mouse, 4 sections separated by 40 \(\mu m\) were examined. Each section was stained with oil red O, counterstained with Gill’s Hematoxylin #1 (Fisher Scientific) and images were digitized. Total lesion cross-sectional area, including the...
intima, lipid cores, and fibrotic components, was calculated for each cross-section and mean cross-sectional areas were calculated for each animal.

Method
After euthanasia, the vasculature of the mice was perfused with PBS followed with zinc formalin. The aorta and its major branches (left and right distal carotid, brachiocephalic, left and right iliac, left and right subclavian arteries) were dissected and stained with Sudan IV. The images were analyzed and the extent of lesion development was reported as percentage of the total area of a given artery that was occupied by atherosclerotic lesions using Image-Pro Plus software (Media Cybernetics, Marietta, Ga).

Immunohistochemistry
Serial frozen tissue sections of aortic root lesions were stained immunohistochemically with MOMA-2 and α-actin to identify macrophages and smooth muscle cells, respectively, and with anti-TF antibody to detect the cellular location of TF expression. Sections were fixed and quenched in acetone/0.3% H2O2 for 30 minutes and rehydrated in either distilled water or PBS/0.1% Tween-20 for 10 minutes. All sections were blocked in 1% bovine serum albumin/PBS for 30 minutes. Sections were incubated with primary antibody and incubated overnight at 4°C at the following dilutions: rat anti-mouse MOMA-2 (Serotec) at 1:25 and rabbit anti-human TF polyclonal antibody, a generous gift from Dr T. Edgington, at 1:1000. RTU-HRP–labeled mouse anti-human α-actin (DAKO) was used as directed by manufacturers’ instructions and incubated for 2 hours at room temperature. Nonspecific IgG was blocked in 1% bovine serum albumin/PBS for 30 minutes. Sections were incubated with horse radish peroxidase (HRP) conjugated rabbit anti-rat IgG at 1:200 (Bio-Jackson) and the antigen/antibody complexes were visualized with a 30-minute exposure to Vectastain ABC Elite solution (Vector Laboratories). TF was visualized using DAKO envision plus HRP-labeled polymer (DAKO). All sections were counterstained with Mayer’s Hematoxylin. Stained sections were dehydrated and mounted.

Plasma Cholesterol and Triglyceride Levels
Mice were fasted for 6 hours before collection of venous blood from the retro-orbital sinuses into a heparinized capillary tube. Plasma was isolated by centrifugation at 3000 g for 5 minutes at 4°C and stored at −20°C. Enzymatic measurements of total cholesterol and triglyceride levels were performed using the Infinity Cholesterol kit (Thermo Electron Corporation) and Triglyceride GPO (Raichem), respectively.

Statistical Analyses
All results were presented as means±standard error of the mean (SEM). Data were analyzed by Student t test or by Mann-Whitney rank sum test for nonparametric data (SigmaStat, v.3.1, SYSTAT 2004). A value of P<0.05 was considered significant.

Results
Effect of Reducing TF by 50% on Atherosclerotic Lesion Formation in the Vasculature of ApoE−/− Mice
A previous study showed that a 50% reduction of TFPI activity detected in the carotid arteries of apoE−/−/TF−/− mice (2.8±0.2 pM, n=8) was approximately half that of apoE−/−/TF+/− mice (5.1±0.5 pM, n=6, P<0.001).

Next, apoE−/−/TF−/− and apoE−/−/TF+/− mice were fed regular chow for 34 weeks and plasma cholesterol levels and atherosclerotic lesions in the arterial tree were quantitated. The plasma cholesterol level was not different between apoE−/−/TF−/− and apoE−/−/TF+/− mice (mean±SEM: 683±69 mg/dL versus 795±56 mg/dL; n=5 to 9). Whole-mount arterial trees were stained with Sudan IV and the percentage lesion coverage of the arterial tree was determined in the 2 groups (Figure 1). Atherosclerotic lesions had developed, albeit to different extents, in all the regions of the arterial tree that were examined. No significant differences were observed in lesion development between the apoE−/−/TF−/− and apoE−/−/TF+/− mice groups (Figure 1). apoE−/−/TF−/− mice were also bred with low-TF mice to generate apoE−/−/TF−/−/hTF+ (apoE−/−/TF+/−) and apoE−/−/TF−/−/hTF− (apoE−/−/low-TF) mice. However, we observed a very high mortality rate in apoE−/−/low-TF mice fed an atherogenic diet that precluded analysis of the atherosclerotic lesions at 16 weeks. All of these mice (7/7) died by 12 weeks.

Effect of Reducing Hematopoietic Cell TF on Atherosclerotic Lesion Development in LDLR−/− Mice
TF is primarily expressed by macrophages in atherosclerotic lesions. In addition, monocytes and macrophages are the major hematopoietic cell type that can express TF. Therefore, we used a genetic approach to determine the effect of reducing TF in hematopoietic cells on the development of atherosclerosis. Figure 2 shows the levels of TF activity in peritoneal macrophages isolated from TF−/−, TF+/−, and low-TF mice. Unstimulated low-TF macrophages expressed 21-fold and 43-fold lower levels of TF compared with TF+/− and TF−/−, respectively. Similar, but slightly less dramatic differences were observed using LPS stimulated peritoneal macrophages (Figure 2). These results indicated that there was a significant reduction in TF in peritoneal macrophages from low-TF mice compared with either TF+/− or WT mice.
Therefore, both WT and TF$^{+/+}$ bone marrow were used as controls in our experiments.

Bone marrow from either control or low-TF mice was transplanted into LDLR$^{-/-}$ recipients to determine the contribution of hematopoietic cell-derived TF on atherosclerotic lesion progression. Bone marrow reconstitution in recipient mice was determined by PCR analysis of peripheral blood cell DNA from the LDLR$^{-/-}$ mice transplanted with either TF$^{+/+}$ or low-TF bone marrow. The wild-type mouse TF allele was detected in bone marrow from LDLR$^{-/-}$ mice transplanted with TF$^{+/+}$ bone marrow, but not in mice transplanted with low-TF bone marrow (Figure 3A). This demonstrated efficient irradiation and bone marrow reconstitution in these mice.

We performed 3 independent bone marrow experiments, one with TF$^{+/+}$ bone marrow as a control (experiment 1) and 2 with TF$^{+/+}$ bone marrow as a control (experiments 2 and 3). In experiment 2, we analyzed plasma triglyceride levels in the 2 groups of mice. Triglyceride levels were not altered by bone marrow transplantation or by the atherogenic diet and were similar in the LDLR$^{-/-}$/TF$^{+/+}$ and LDLR$^{-/-}$/low-TF groups (Figure 3B) (mean concentration [mg/dL±SEM]: 0 weeks, 55±3 versus 59±3; postdiet, 67±5 versus 60±4). In experiments 2 and 3, we analyzed cholesterol levels in the 2 groups. In experiment 2, plasma cholesterol levels were determined before initiating the atherogenic diet and after 16 weeks on an atherogenic diet (Figure 3C). As expected, plasma cholesterol dramatically increased after 16 weeks on an atherogenic diet. A small difference in cholesterol levels was observed at 16 weeks between the LDLR$^{-/-}$/TF$^{+/+}$ and LDLR$^{-/-}$/low-TF groups (mean concentration [mg/dL±SEM]: 0 weeks, 284±14 versus 253±6; 16 weeks, 917±36 versus 1064±41; $P=0.02$). However, no differences in cholesterol levels were observed in experiment 3 at any time point (Figure 3C) (mean concentration [mg/dL±SEM] at 16 weeks for the control and low-TF groups were 1171±108 and 1361±83, respectively).

We investigated whether reducing TF in hematopoietic cells affects early atherosclerotic lesion development (experiment 1). Analysis of lesion development in LDLR$^{-/-}$/TF$^{+/+}$ and LDLR$^{-/-}$/low-TF transplanted mice was performed after

![Figure 2. Procoagulant activity of peritoneal macrophages obtained from TF$^{+/+}$, TF$^{+/−}$, and low-TF mice under (A) basal conditions and (B) stimulated with LPS (1 μg/mL) for 6 hours. Data are presented as mean±SEM.](http://atvb.ahajournals.org/)

![Figure 3. A, PCR of peripheral blood cell DNA from LDLR$^{-/-}$ mice transplanted with bone marrow from WT or low-TF mice demonstrating efficient ablation and reconstitution with donor bone marrow. B, In experiment 2, plasma triglyceride (TG) levels were determined before initiating the atherogenic diet regime and after 16 weeks on an atherogenic diet. C, In experiments 2 and 3, cholesterol levels were determined at various times. Black squares represent LDLR$^{-/-}$/TF$^{+/+}$ and LDLR$^{-/-}$/low-TF transplanted mice. Horizontal bars represent the means of the plasma triglyceride or cholesterol levels.](http://atvb.ahajournals.org/)
4 weeks on an atherogenic diet. At 4 weeks, whole-mount arterial trees were stained with Sudan IV and the percentage lesion coverage was determined in the aorta and its major arteries (Figure 4). No significant differences in percentage lesion coverage between the LDLR<sup>−/−</sup>/TF<sup>−/−</sup> and LDLR<sup>−/−</sup>/low-TF groups were observed in any vessel except the carotid artery, whereas LDLR<sup>−/−</sup>/low-TF mice. B, Percentage aortic lesion coverage was not significantly different (P=0.4) between the LDLR<sup>−/−</sup>/TF<sup>−/−</sup> and LDLR<sup>−/−</sup>/low-TF groups (Figure 5B) (mean %±SEM: 8.5±0.8 versus 9.6±0.9). Similar results were observed in experiment 2 (mean %±SEM: LDLR<sup>−/−</sup>/TF<sup>−/−</sup> 6.1±0.3, n=10, versus LDLR<sup>−/−</sup>/low-TF 6.9±0.4; n=12).

Lesion area in the aortic root was also determined in the 2 groups. Serial transverse sections of the aortic root were stained with oil red O to allow quantification of the extent of the atherosclerotic lesions (Figure 5C). Lipid-laden lesions were observed within the vessel wall as well as on the heart valves. However, only vessel wall lesions were quantified because the heart valves are often damaged during the dissection/fixing process. In experiment 3, the area of the lesions was not affected by a reduction of TF in hematopoietic cells in LDLR<sup>−/−</sup> mice (Figure 5D) (mean area [μM<sup>2</sup>×10<sup>3</sup>]±SEM: LDLR<sup>−/−</sup>/TF<sup>−/−</sup> 5.7±0.5 versus LDLR<sup>−/−</sup>/low-TF 6.4±0.5). Similar results were observed in experiment 2 (mean area [μM<sup>2</sup>×10<sup>3</sup>]±SEM: LDLR<sup>−/−</sup>/TF<sup>−/−</sup> 5.5±0.9, n=10, versus LDLR<sup>−/−</sup>/low-TF 8.2±1.8, n=12).

Because there was no difference in the gross morphology and size of the lesions from the 2 groups, we analyzed the cellular composition in the aortic root lesions. Serial sections were stained with MOMA-2 and smooth muscle α-actin to detect macrophages and smooth muscle cells, respectively (Figure 6A). A nonspecific IgG served as a negative control (Figure 6A). The lesions from both groups showed a high degree of MOMA-2-positive macrophages in the center of the lesion, whereas α-actin–positive smooth muscle cells were predominantly located in the region of a fibrous cap or lesion coverage between the LDLR<sup>−/−</sup>/TF<sup>−/−</sup> (n=7; black bar) and LDLR<sup>−/−</sup>/low-TF (n=8; white bar) mice fed an atherogenic diet for 4 weeks. AA, ascending aorta; S, subclavian; C, carotid; BC, brachiocephalic artery; AB, abdominal aorta; IL, iliac artery. Data are expressed as means±SEM. A statistically significant difference was observed in the carotid artery (P<0.05). Male mice were used for this experiment.
beneath the lesion in the media region of the vessel wall. No differences in the cellular composition were observed between the LDLR⁻/⁻/TF⁺/+ and LDLR⁻/⁻/low-TF mice.

Aortic root lesions were also stained with an anti-human TF polyclonal antibody that cross-reacts with mouse TF. In the LDLR⁻/⁻/TF⁺/+ group, TF was predominantly expressed by macrophages in the center of the lesions, as well as in VSMCs in the fibrous cap (Figure 6B). In contrast, TF staining in the LDLR⁻/⁻/low-TF group was predominantly in VSMCs with low levels in the macrophages, suggesting that macrophages within the lesion were predominantly derived from the transplanted low-TF bone marrow (Figure 6B).

Discussion
This study assessed whether TF expression contributed to the progression of atherosclerosis in mice. First, we reduced TF expression by 50% in all cells but observed no effect on atherosclerosis in apoE⁻/⁻ mice after 34 weeks on a regular chow diet. Second, we used bone marrow transplantation to selectively reduce TF expression in hematopoietic cells. LDLR⁻/- mice receiving low-TF bone marrow had similar levels of atherosclerosis to LDLR⁻/- mice that received control bone marrow after 4 and 16 weeks on an atherogenic diet. These data suggest that the level of TF expression, particularly in macrophages, does not influence the development of atherosclerosis in mice. Our results are consistent with a previous study showing that genetically depleting fibrinogen did not affect atherosclerosis in apoE⁻/⁻ mice. In addition, treatment of mice with anti-coagulant warfarin did not reduce atherosclerosis. However, a recent study demonstrated a reduction of lesion size in FVIII-deficient apoE⁻/⁻ mice after 8 weeks on an atherogenic diet and, to a lesser extent, at 16 weeks. These studies suggest that the role of coagulation in the development of atherosclerotic lesions is complex and may play a more prominent role in early lesion development in mice.

TF is primarily expressed by macrophages in atherosclerotic lesions. Furthermore macrophages are the first TF-expressing cell type to infiltrate, proliferate, and accumulate in early as well as advanced lesions. Therefore, in this study we investigated whether hematopoietic cell TF plays a role in atherosclerotic lesion progression in LDLR⁻/- mice by transplanting LDLR⁻/- mice with bone marrow from either control or low-TF mice. In one experiment, we observed a significant difference in cholesterol levels between the 2 groups. However, no differences in cholesterol levels were observed in a second experiment. This indicates that there are no consistent differences in cholesterol levels between the 2 groups. We did not observe any differences in the lesion surface area in the aorta or most of the major arterial branches in the 2 groups at 4 or 16 weeks on an atherogenic diet except for a significant increase in lesion area in the carotid artery in the low-TF group at 4 weeks. The reason for this difference is not clear. One limitation of the study is that only female mice were used in the experiments in which the mice were fed a high-fat diet for 16 weeks. Interestingly, the degree of atherosclerosis in the aorta and aortic root of the low-TF group was consistently higher, although was not statistically significant than the control. These results suggest that TF may be protective in atherosclerosis by stabilizing the plaque. Indeed, in support of this notion, dramatic reduction in TF expression in apoE⁻/⁻ mice leads to premature death (see below).
The cellular composition of a lesion may determine its stability and propensity to plaque disruption in humans.\textsuperscript{35} We hypothesized that reducing macrophage derived TF in LDLR\textsuperscript{−/−} mice may alter the cellular composition of the aortic root lesions. Infiltration and proliferation of inflammatory cells, such as monocytes, as well as VSMCs, may be influenced by TF expression via local generation of coagulation proteases and the activation of PARs. For instance, TF/FVIIa enhances the migration of macrophages and VSMCs in vitro and fibrin matrices provide a support for the migration of inflammatory cells and VSMC.\textsuperscript{15,17,36–38} However, we observed a similar distribution and content of MOMA-2–positive macrophages and α-actin–positive VSMCs within lesions of LDLR\textsuperscript{−/−} mice with either low-TF or control bone marrow. TF expression in macrophages in the LDLR\textsuperscript{−/−}/low-TF group was dramatically reduced compared with the control group. This is consistent with our study showing that bone marrow derived macrophages from low-TF mice express very low levels of TF.\textsuperscript{26} In contrast, TF expression by VSMCs in the fibrous cap and vessel wall was similar in both groups of LDLR\textsuperscript{−/−} mice. Our data do not support a role for macrophage TF on the development of atherosclerosis or the composition of the lesion. However, our studies do not exclude a role for VSMC-derived TF in this process. Recently, we generated mice that have a floxed TFPI. Breeding of these mice with mice that express Cre recombinase under the control of a VSMC-specific promoter will allow us to investigate the effect of deleting TF, specifically in VSMCs, on the development of atherosclerotic plaques.

A recent study showed that apoE\textsuperscript{−/−} mice with a 50% reduction of TFPI had increased levels of atherosclerotic lesions in the carotid and iliac arteries compared with controls.\textsuperscript{24} This increase in lesion size could be because of an increase in TF activity or TF-independent effects of TFPI. Our data showing that a 50% reduction in TF did not affect atherosclerosis in apoE\textsuperscript{−/−} mice suggest that these results may not be caused by an increase in TF activity. However, it is difficult to compare the studies because one increases TF activity and the other decreases TF activity. It is also possible that TFPI might affect atherosclerosis in a TF-independent manner. For instance, the presence of TFPI may inhibit the activation of the endothelium and reduce atherosclerosis.

Analysis of atherosclerotic lesions in apoE\textsuperscript{−/−}/low-TF mice fed an atherogenic diet was not possible because of the premature death of these mice. All 7 apoE\textsuperscript{−/−}/low-TF mice died by 12 weeks. At present, we do not know the cause of sudden death. Low-TF mice are prone to fatal lung hemorrhages, but this occurs at a much lower frequency than observed for the apoE\textsuperscript{−/−}/low-TF mice.\textsuperscript{39} A recent study reported a high rate of sudden death of apoE\textsuperscript{−/−} mice fed an atherogenic diet over a 24-week period.\textsuperscript{40} The frequency of plaque rupture in the brachiocephalic artery was similar in the survivors and the nonsurvivors. However, some of the mice had myocardial infarctions, suggesting that plaque stability in the coronary circulation may cause sudden death. We speculate that low levels of TF may further destabilize atherosclerotic plaques in apoE\textsuperscript{−/−} mice fed a high-fat diet, thus increasing the frequency of myocardial infarctions and sudden death. Further studies are needed to analyze the cause of death of the apoE\textsuperscript{−/−}/low-TF mice.

A major distinction between the lesions in atherosclerosis-prone mice and humans is the formation of mural thrombi secondary to plaque fissure in the latter.\textsuperscript{41,42} Although our study suggests that TF in mice is not involved in progression of atherosclerosis, indirect evidence from studies of atherosclerosis in humans suggest that TF may play an important role in human vascular disease.\textsuperscript{8,11,12} Therefore, our studies do not exclude a role for TF in the progression of atherosclerotic lesions in humans.

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References


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