Transforming Growth Factor-β Mediates Balance Between Inflammation and Fibrosis During Plaque Progression

Esther Lutgens, Marion Gijbels, Marjan Smook, Peter Heeringa, Philip Gotwals, Victor E. Koteliansky, Mat J.A.P. Daemen

Abstract—The transition from stable to rupture-prone and ruptured atherosclerotic plaques involves many processes, including an altered balance between inflammation and fibrosis. An important mediator of both is transforming growth factor (TGF)-β, and a pivotal role for TGF-β in atherogenesis has been postulated. Here, we determine the in vivo effects of TGF-β inhibition on plaque progression and phenotype in atherosclerosis. Recombinant soluble TGF-β receptor II (TGFβRII:Fc), which inhibits TGF-β signaling, was injected in apolipoprotein E–deficient mice for 12 weeks (50 μg, twice a week intraperitoneally) as early treatment (treatment age 5 to 17 weeks) and delayed treatment (age 17 to 29 weeks). In the early treatment group, inhibition of TGF-β signaling treatment resulted in a prominent increase in CD3- and CD45-positive cells in atherosclerotic lesions. Most profound effects were found in the delayed treatment group. Plaque area decreased 37.5% after TGFβRII:Fc treatment. Moreover, plaque morphology changed into an inflammatory phenotype that was low in fibrosis: lipid cores were 64.6% larger, and inflammatory cell content had increased 2.7-fold. The amount of fibrosis decreased 49.6%, and intraplaque hemorrhages and iron and fibrin deposition were observed frequently. TGFβRII:Fc treatment did not result in systemic effects. These results reveal a pivotal role for TGF-β in the maintenance of the balance between inflammation and fibrosis in atherosclerotic plaques. (Arterioscler Thromb Vasc Biol. 2002;22:975-982.)

Key Words: atherosclerosis ■ transforming growth factor-β ■ inflammation ■ fibrosis

Transforming growth factor (TGF)-β is a growth factor that exerts many regulatory actions. It is known for its role in development, proliferation, migration, differentiation, and extracellular matrix biology, but it is also an important immunomodulator.1,2 These functions are best reflected in TGF-β1–deficient mice, which die in utero or perinatally because of widespread inflammation.3 Moreover, mice expressing a dominant-negative TGF-β receptor II (TGFβRII) under a T-lymphocyte–specific promoter in which TGF-β signaling is specifically blocked in T lymphocytes showed disruption of T-lymphocyte homeostasis and inflammation in many organs.4 On the other hand, mice with cardiovascular overexpression of TGF-β1 are embryonically lethal because of abnormal yolk sac vasculogenesis,5 and adenoviral gene therapy of TGF-β1 in the balloon-injured rat causes an increased extracellular matrix deposition in intimal lesions.6 Furthermore, mice overexpressing TGF-β1 under a liver- or kidney-specific promoter show excessive fibrosis in the targeted organ.7,8

Nowadays, atherosclerosis is considered to be a chronic inflammatory disease, and the balance between inflammation and extracellular matrix deposition is thought to be important for the maintenance of plaque stability in humans.9,10 Because TGF-β is an immunomodulator and a fibrosis modulator, we postulate that TGF-β might play a key role in the maintenance of the phenotype of an atherosclerotic plaque.

The first evidence of an important role for TGF-β in vascular disease has been obtained from studies in balloon-injured rats. TGF-β levels were increased 6 to 24 hours after balloon injury,11 and TGF-β overexpression12 or inhibition12 influences the disease process by an alteration of neointima formation, extracellular matrix deposition, or smooth muscle cell proliferation. Consistent with these results is the observation that patients with severe restenosis also exhibit increased serum levels of TGF-β.13

In contrast to the large number of data that report a role for TGF-β on neointima formation, data regarding the effects of TGF-β on primary atherosclerosis are limited. The expression patterns of TGF-β1 to -β3 and of TGF-β receptor I (TGFβRI) and TGFβRII in atherosclerotic plaques are well known, but functional in vivo studies in primary atherosclerosis are sparse.14,15 However, the limited in vivo data available suggest a protective role for TGF-β in atherosclerosis. Some research,14 but not all,16 has reported that advanced plaque
cells contain mutations in the type II receptor that disable proper signaling and consequently decrease sensitivity of these cells for TGF-β. Furthermore, patients suffering from unstable angina exhibited decreased levels of TGF-β, indicating a correlation of low TGF-β levels with advanced atherosclerosis. ApoE-deficient (apoE−/−) mice that were treated with tamoxifen (an anti-estrogen) exhibited increased TGF-β levels that were associated with a decrease in initial lesion formation. Furthermore, in mice heterozygous for the deletion of the TGF-β1 gene (TGFβ1+/−/−) that were fed a high fat diet exhibited an increased endothelial activation, and lipid infiltration was observed in the vascular wall.  

Recently, Mallat et al20 treated apoE−/−/− mice with a neutralizing antibody against TGF-β1, -β2, and -β3 from weeks 6 to 15. They observed that atherosclerotic lesions contained a high inflammatory cell content and a decreased amount of collagen.

In the present study, we confirm and expand the data of Mallat et al20 with the use of a different approach. Inhibition of the TGF-β pathway with a soluble recombinant TGFβRII in apoE−/−/− mice, in an early treatment (weeks 5 to 17) and delayed treatment (weeks 17 to 29) setting, resulted in increased inflammation and decreased plaque fibrosis and was associated with intraplaque hemorrhages and iron and fibrin deposition. No systemic effects of recombinant soluble TGFβRII (TGFβRII:Fc) treatment were observed.

**Methods**

**TGFβRII:Fc Fusion Gene**

The recombinant mouse TGFβRII:Fc fusion gene, containing the extracellular domain of the mouse type II TGF-β receptor fused to the Fc portion of mouse IgG2a, was constructed as described previously. TGFβRII:Fc was purified, and its activity was tested in mink lung epithelial cells (Mv1Lu). In vivo studies confirmed the effectiveness the TGFβRII:Fc.

**Mice**

ApoE−/− (male, n=36) mice on a C57Bl6 background were obtained from Iffa Credo (Lyon, France). Mice were injected with TGFβRII:Fc fusion protein or the control mouse IgG twice a week for 12 weeks (100 µg/wk IP). The dosing regimen used in the present study was based on earlier in vivo studies using the same compound. Treatment started at the age of 5 weeks (early treatment, n=10 TGFβRII:Fc mice and n=10 control mice) or at the age of 17 weeks (delayed treatment, n=8 TGFβRII:Fc mice and n=8 control mice). After the experimental procedure, mice were euthanized after a 24-hour fast. Blood (0.5 to 1 mL) was obtained from the caval vein for lipoprotein analysis. The arterial tree was perfused, and the aortic arch, including its main branch points (brachiocephalic trunk, left common carotid artery, and left subclavian artery), was excised and fixed as described previously. The aortic arch including branch points was embedded longitudinally and cut into ~40 sections. A series of twenty 4-µm sections, which represented the central area of the arch with an intact morphology of the complete arch and branch points, were analyzed.

**Lipid Profile**

For the assessment of lipid profiles, standard enzymatic techniques, automated on the Cobas Fara centrifugal analyzer (Hoffmann-La Roche), were used. Total plasma cholesterol and HDL were measured by using kit No. 07 3663 5 and kit No. 543004 (Hoffmann-La Roche); total glycerol was measured by using kit No. 337-40A/337-10B (Sigma Chemical Co); and free glycerol was measured by using kit No. 0148270 (Hoffmann-La Roche). Standardized serum (Precipath) was used as an internal standard. LDL was calculated as follows: total cholesterol−[(total glycerol−free glycerol)/2.2]−HDL.

**Evaluation of Possible Systemic Effects**

To evaluate possible systemic effects of TGFβRII:Fc treatment, fluorescence-activated cell sorter (FACS) analysis (FACS calibur, Beckton Dickinson) with T-cell–specific antibodies was performed on spleen and lymph nodes of TGFβRII:Fc-treated (n=3) and control (n=3) mice of the delayed treatment group. Antibodies used for staining were anti-CD3 (clone 2C11, biotinylated), CD4, CD8 (clone 53-6.7, CD45RB [all Pharmingen]). In addition, >20 organs from each mouse (n=34 mice) were excised and analyzed on 4-µm sections stained with hematoxylin and eosin and with CD45.

**Histology and Morphometry**

For histological analysis of atherosclerosis, 4 sections (20 µm apart) were stained with hematoxylin and eosin. Atherosclerotic lesions were analyzed and classified according to American Heart Association (AHA) criteria. To obtain precise insights of the effects of TGFβRII:Fc treatment on plaque progression, all analyses were performed on separate lesion types, representing all stages of atherogenesis, as defined by the AHA. Because the data on plaque burden and inflammatory and fibrotic parameters between the separate early plaque stages (AHA type I, II, and III lesions) as well as between the separate advanced stages (type IV and V lesions) were similar, data were presented in 2 groups: initial lesions (AHA type II/III) and advanced lesions (AHA type IV/V). Morphometric parameters were determined as described previously.

**Immunohistochemistry**

Sections were immunolabeled with the following: α-smooth muscle actin (ASMA) monoclonal antibody, 1:500 (Sigma) as a marker for vascular smooth muscle cells and fibroblasts; Mac3 (1:30, Pharmingen) to detect macrophages; CD3 polyclonal antibody (A0452, 1:200; Dako) to detect T lymphocytes; CD45 (1:30, Pharmingen) to detect inflammatory cells; CD40 (1:30, Santa Cruz), CD68 (1:30, Santa Cruz), TGF-β (1:30, R&D Systems), factor VIII (1:500, Dako), and fibrinogen (1:800, Nordic Immunologies); and matrix metalloproteinase (MMP)-2 and MMP-9 (both 1:20), as described. Perls’ stain was used to detect iron. The relative amounts of cells were determined as described previously.

All measurements were performed by 2 independent investigators who were blinded regarding treatment group. Intraobserver as well as interobserver variation was <10%.

**Signaling**

To obtain more insight into the effects of TGFβRII:Fc treatment on downstream signaling effects, immunohistochemistry with antibodies against Smad2 (goat polyclonal antibody, 1:100; Santa Cruz), Smad3 (goat polyclonal antibody, 1:10; Santa Cruz), phospho-Smad2/3 (rabbit polyclonal antibody, 1:500; Santa Cruz), Smad4 (goat polyclonal antibody, 1:20; Santa Cruz), and Smad7 (goat polyclonal antibody, 1:100; Santa Cruz) was used.

**Statistical Analysis**

Data are expressed as mean±SEM. TGFβRII:Fc-treated apoE−/− mice were compared with control apoE−/− mice by a nonparametric Mann-Whitney U test. Data were considered statistically significant at P<0.05.

**Results**

**Mouse Study: General**

Survival rates were 100% in the early treatment study and 87.5% in both groups of the delayed treatment study. Body
weight and heart weight did not differ between the treatment groups, nor did the levels of plasma cholesterol (early treatment, 13.1 ± 1.0 mmol/L for TGFβRII:Fc versus 12.2 ± 0.4 mmol/L for control; delayed treatment, 11.8 ± 0.9 mmol/L for TGFβRII:Fc versus 13.8 ± 1.5 mmol/L for control), triglyceride (early treatment, 1.6 ± 0.2 mmol/L for TGFβRII:Fc versus 1.5 ± 0.1 mmol/L for control; delayed treatment, 1.0 ± 0.1 mmol/L for TGFβRII:Fc versus 1.1 ± 0.2 mmol/L for control), LDL (12.6 ± 0.9 mmol/L for TGFβRII:Fc versus 11.7 ± 0.4 mmol/L for control; delayed treatment, 11.4 ± 0.9 mmol/L for TGFβRII:Fc versus 13.3 ± 1.5 mmol/L for control), or HDL (early treatment, 0.2 ± 0.1 mmol/L for TGFβRII:Fc versus 0.3 ± 0.1 mmol/L for control; delayed treatment, 0.3 ± 0.0 mmol/L for TGFβRII:Fc versus 0.3 ± 0.1 mmol/L for control). Autopsy (>20 organs) revealed no abnormalities on sections stained with hematoxylin and eosin or with CD45 (data not shown); especially, there was no generalized inflammation in organs (please see online Figure I, which can be accessed at www.ahajournals.org), nor was there adventitial inflammation in normal arteries (please see online Figure IIa and IIb, which can be accessed at www.ahajournals.org) or atherosclerotic arteries (online Figure Iic and Iid), indicating that the effects described below were plaque specific and not due to a systemic effect. Furthermore, no signs of ischemic end-organ damage were observed either macroscopically or microscopically.

FACS analysis of lymph nodes and spleen revealed no differences in the amount of CD3-positive cells (T cells) or in the activation status of the T cells between the groups (CD4/CD8 ratio, CD25+T cells), confirming the absence of systemic inflammatory effects of TGFβRII:Fc treatment (online Figure Ie through IIg).

Signaling

In atherosclerotic plaques of the early and delayed TGFβRII:Fc treatment groups, immunoreactivity of Smad2 and phospho-Smad2/3 (please see online Figure IIIa and IIIb, which can be accessed at www.ahajournals.org) decreased, whereas immunoreactivity of Smad3, Smad4, and Smad7 did not change. The decrease in phospho-Smad2/3 and the decrease in Smad2 indicate the in vivo inhibition of TGF-β signaling.

Plaque Burden

We analyzed 43 lesions in the aortic arches of TGFβRII:Fc-treated apoE−/− mice (n = 10) and 47 lesions of the control mice (n = 10) in the early treatment study and 40 lesions in the aortic arches of TGFβRII:Fc-treated apoE−/− mice (n = 7) and 41 lesions of the control mice (n = 7 mice) in the delayed treatment study. In the early treatment study, total plaque burden was not affected. However, in the delayed treatment study, total plaque area of the aortic arch decreased 37.5% after TGFβRII:Fc treatment (early treatment, 111 125 ± 24 924 μm² for TGFβRII:Fc versus 138 246 ± 23 574 μm² for control, P < 0.05; delayed treatment, 547 359 ± 68 266 μm² for TGFβRII:Fc versus 876 869 ± 68 847 μm² for control, P < 0.05). The number of plaques did not change after TGFβRII:Fc treatment (Figure 1a and 1b). Interestingly, TGFβRII:Fc treatment altered the distribution of AHA lesion types. In the early treatment group, the absolute and relative amounts of type Va lesions increased after TGFβRII:Fc treatment (72.7% for TGFβRII:Fc versus 30% for control, as percentage of all advanced lesions), whereas the absolute and relative amounts of type IV lesions (18.2% for TGFβRII:Fc versus 70% for control, as percentage of all advanced lesions) had decreased. Similar results were obtained in the delayed treatment group (Figure 1c and 1d). In that group, the number of Va lesions (fibrous cap and large lipid core) had increased (64.0% for TGFβRII:Fc versus 39.1% for control), whereas the number of Vc lesions (fibrous lesions) was decreased (8% for TGFβRII:Fc versus 30.4% for control). This indicates that TGFβRII:Fc treatment induces a plaque phenotype with large lipid cores and thin fibrous caps. Individual plaque area per lesion type had significantly decreased in type IV lesions of the early treatment study and in type V lesions of the delayed treatment study (Figure 1c and 1d).

Plaque Composition

Besides the decrease in plaque area, the composition of the plaques significantly differed after TGFβRII:Fc treatment compared with control treatment.

Initial Atherosclerotic Plaques (Fatty Streaks)

Further analysis of initial lesion development in the aortic arch revealed that the relative and absolute numbers of CD3-positive cells (T lymphocytes) increased after TGFβRII:Fc treatment: relative numbers were 5.0 ± 1.0% for TGFβRII:Fc versus 1.8 ± 1.2% for control (P < 0.05) for early treatment and 4.9 ± 0.6% for TGFβRII:Fc versus 2.0 ± 1.0% for control (P < 0.05) for late treatment; absolute numbers were 10 ± 5 for TGFβRII:Fc versus 3 ± 1 for control for early treatment and 2 ± 1 for TGFβRII:Fc versus 1 ± 0 for control for late treatment. The relative and absolute numbers of CD45-positive cells also increased after TGFβRII:Fc treatment: relative numbers are shown in Figure 1c; absolute numbers were 3 ± 1 for TGFβRII:Fc versus 1 ± 0 for control for early treatment and 9 ± 4 for TGFβRII:Fc versus 2 ± 1 for control for late treatment. Furthermore, CD40 and CD40L immunoreactivity in initial lesions of the early treatment and delayed treatment studies also increased after TGFβRII:Fc treatment. Macrophage content decreased in initial lesions of the treated mice of the early treatment study (82.1 ± 2.2% for TGFβRII:Fc versus 73.5 ± 2.0% for control, P < 0.05; delayed treatment, 74.4 ± 5.6% for TGFβRII:Fc versus 70.8 ± 3.4% for control, P < 0.05). Collagen content (Figure 1f), α-smooth muscle actin content (early treatment, 0.4 ± 0.2% for TGFβRII:Fc versus 0.9 ± 0.5% for control, P < 0.05; delayed treatment, 0.7 ± 0.5% for TGFβRII:Fc versus 0.8 ± 0.8% for control), and MMP-2 and MMP-9 immunoreactivity did not differ.

Advanced Atherosclerotic Plaques

In advanced lesions, the effects of inhibition of TGF-β signaling on plaque composition were even more pronounced. Lipid cores of the TGFβRII:Fc treatment group were 84.2% and 64.6% larger than in control-treated mice,
respectively (Figures 1g, 2a, and 2b), and lipid core expansion was accelerated (148.2% versus 50% increase in lipid core content from weeks 17 to 29, \( P<0.05 \); Figure 1g). Inflammatory cell content was significantly increased after TGFβRII:Fc treatment, as reflected by macrophage cell content (relative numbers for early treatment were 60.8±4.8% for TGFβRII:Fc versus 64.2±5.7% for control; relative numbers for delayed treatment were 60.9±2.3% for TGFβRII:Fc versus 43.6±2.7% for control, \( P<0.05 \)), CD3 cell content (relative numbers for early treatment were 3.7±0.4% for TGFβRII:Fc versus 2.0±0.7% for control; relative numbers for delayed treatment were 5.0±0.6% for TGFβRII:Fc versus 2.0±0.5% for control; absolute numbers for early treatment were 5±2 for TGFβRII:Fc versus 1±1 for control; and absolute numbers for delayed treatment were 8±2 for TGFβRII:Fc versus 4±1 for control), and CD45-positive cell content (Figures 1e, 2c, and 2d; absolute numbers for early treatment were 6±1 for TGFβRII:Fc versus 2±1 for control; absolute numbers for delayed treatment were 12±2 for TGFβRII:Fc versus 6±1 for control). CD40 and CD40L (Figures 2e and 2f) immunoreactivity was also significantly increased after TGFβRII:Fc treatment. In addition, the amount of fibrosis, reflected by the collagen content, had decreased 50.3% in the delayed treatment study (Figures 1f, 2g, and 2h). MMP-2 and MMP-9 immunoreactivity was increased. The amount of α-smooth muscle actin–positive
cells did not differ (early treatment, 1.2±0.3% for TGFβRII:Fc versus 1.0±0.2% for control, P>0.05; delayed treatment, 1.0±0.3% for TGFβRII:Fc versus 1.0±0.2% for control, P>0.05).

Interestingly, the increase in inflammation and decrease in fibrosis in plaques of mice treated with TGFβRII:Fc were associated with a significant increase in the frequencies of recent and older intraplaque bleedings and fibrin and iron deposition in the delayed treatment group (Figures 1h and 3). This was not associated with acute ischemic events.

These data show that blockade of TGF-β signaling decreases the advanced plaque area and alters the balance between plaque inflammation and fibrosis. This results in an inflammatory plaque phenotype with a low extracellular matrix content. Systemic effects, as well as changes in lipid profile, were not observed.

**Discussion**

In accordance with the present results, Mallat et al.\(^20\) also reported that inhibition of TGF-β in primary atherosclerosis induces an increase in plaque inflammation and a decrease in plaque fibrosis. However, besides these similarities in outcome, there are also important differences between both studies. First of all, we used a different kind of TGF-β blockade. Whereas Mallat et al. treated the mice with an inhibiting antibody against TGF-β1, -β2, and -β3, we used a soluble recombinant TGFβRII. Second, besides the early time points that were studied by Mallat et al. (treatment weeks 6 to 15) and us (treatment weeks 5 to 17), we also studied the effects of TGF-β blockade after 29 weeks. In this delayed treatment group, we were able to show that the increase in plaque inflammation and decrease in plaque fibrosis were associated with intraplaque hemorrhages and iron and fibrin deposition.

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**Figure 2.** Histological characteristics of atherosclerotic lesions of TGFβRII:Fc-treated and control mice of the delayed treatment study. a and b, Hematoxylin and eosin (HE)-stained section of advanced atherosclerotic lesions in the aortic arch, revealing an enlarged lipid core (arrow) after TGFβRII:Fc treatment (a) compared with control treatment (b). Original magnification ×125. c and d, CD45 staining of the shoulder region of advanced atherosclerotic lesions, revealing an increased amount of CD45-positive cells (arrows) after TGFβRII:Fc treatment (c) compared with control treatment (d). Original magnification ×400. e and f, CD40L staining of advanced atherosclerotic lesions. CD40L is most abundantly present after TGFβRII:Fc treatment (e) compared with control treatment (f). Original magnification ×125. g and h, Sirius red staining of advanced atherosclerotic lesions. The amount of collagen significantly decreased after TGFβRII:Fc treatment (g) compared with control treatment (h).
deposition. Last, in contrast to the study of Mallat et al, we did not observe systemic effects after inhibition of TGF-β signaling. Besides the increase in plaque inflammation and decrease in plaque fibrosis, Mallat et al also observed adventitial inflammation (vasculitis) as well as systemic inflammation in other organs.20,28 Because vasculitis and (systemic) inflammation are associated with an acceleration of atherosclerosis,29,30 the systemic effects of the anti–TGF-β antibody might have confounded the results of Mallat et al (see Lutgens and Daemen28).

Our results are in agreement with the data earlier reported in in vitro studies. These studies show that TGF-β functions as an anti-inflammatory cytokine in cell types that are also present in atherosclerotic plaques.31–33 TGF-β is also known to be an important fibrotic cytokine that plays an important role in matrix remodeling and collagen synthesis.34

The signaling cascade by which TGF-β exerts its actions is well known. In brief, TGF-β binds via receptor III to receptor II or directly to receptor I, and this complex binds to receptor I. This induces the phosphorylation of Smad2 or Smad3. Subsequently, phospho-Smad-2 or -Smad-3 binds to Smad-4, and the resulting complex moves to the nucleus, where it interacts with various transcription factors to regulate the transcription of TGF-β–responsive genes and mediates the effects of TGF-β at the cellular level.2 In the present study, it was shown that TGFβRII:Fc treatment suppresses phosphorylation of Smad2/3, thereby inhibiting the actions of TGF-β.

The phenomenon that inhibition of inflammation causes a plaque phenotype that contains a high extracellular matrix content has been described previously. Genetic disruption of interferon-γ in apoE−/− mice has been reported to result in smaller atherosclerotic plaques with higher collagen content.35 Furthermore, we and others observed a collagen-rich phenotype after inhibition of CD40L in apoE−/− and LDL receptor–deficient mice.24,25,36 Overexpression of the anti-inflammatory cytokine interleukin-10 increased collagen content in atherosclerotic lesions, whereas inhibition of interleukin-10 decreased collagen content.37,38

Our earlier reports suggested the importance of TGF-β as a mediator of plaque fibrosis. Inhibition of CD40L signaling in atherosclerosis resulted in a lipid-poor collagen-rich plaque phenotype. Interestingly, we observed an increased immunoreactivity of TGF-β in these lesions.25,39 Additional analysis with a cDNA expression array revealed that anti-CD40L treatment induced a 2.3-fold upregulation of TGF-β mRNA. These results suggest that upregulation of TGF-β may be associated with the CD40L inhibition–induced fibrotic plaque phenotype. The observed balance between CD40L and TGF-β and the effects on plaque phenotype observed after CD40L (fibrosis-rich inflammatory-poor plaque phenotype) or TGFβRII inhibition (inflammatory-rich fibrosis-poor plaque phenotype) reflect the importance of the balance between inflammation and fibrosis on atherosclerotic plaque phenotype.

The results of the present study suggest that TGF-β may be used as a therapeutic target. However, whether TGF-β treatment would be beneficial or detrimental still needs to be determined. On the one hand, TGF-β would induce plaques with a high extracellular matrix content and a low inflammatory cell content, which may help to stabilize the plaque. On the other hand, the possible drawbacks of administration of TGF-β may be possible systemic fibrotic effects and an increase in plaque size. Targeted administration of TGF-β may solve the first problem. A role for TGF-β in atherosclerotic plaque growth has been postulated previously. Immunohistochemical analysis revealed that TGF-β and TGFβRII are most abundant in initial lesions and lesions with pathological intimal thickening and may stimulate the production of lipid-trapping proteoglycans.40–42 In other models of neo-intima formation, such as the balloon-injured rat, TGF-β also
induced neointimal growth, and inhibition of TGF-β signaling was able to significantly reduce neointima formation.  

A decrease in plaque size is beneficial in preventing clinical symptoms of atherosclerosis. However, because in humans, plaque composition is a more important predictor of the acute vascular symptoms than is plaque size,27,43 one may hypothesize that inhibition of TGF-β may increase plaque instability and evoke more clinical symptoms, even when plaque size decreases. Thus, local activation of TGF-β signaling may provide a therapeutic target in atherosclerosis. Although it may not prevent the initiation of atherosclerosis, it may prevent the transition into an unstable plaque phenotype because of its anti-inflammatory and profibrotic effects.

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References


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Online figure legends

Figure I
Figure showing no systemic effects of TGFβRII:Fc treatment in heart (A+B), spleen (C+D), liver (E+F) and kidney (G+H).

Figure II
Figure showing no effects of TGFβRII:Fc treatment on normal arteries (fig 2a and b) and the adventitia of atherosclerotic arteries fig 2 c and d. Figures e-g show the results of FACS analysis of spleen and peripheral lymph nodes. * p<0.05, A = adventitia, L=lumen.

Figure III
Immunoreactivity of p-Smad 2/3 significantly decreased after TGFβRII:Fc treatment (a and b).