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Elevated circulating TGF- β is not the cause of increased atherosclerosis development in biglycan deficient mice

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Abstract

Background and aims—Vascular biglycan contributes to atherosclerosis development and increased biglycan expression correlates with increased atherosclerosis. However, mice deficient in biglycan have either no reduction in atherosclerosis or an unexpected increase in atherosclerosis. Biglycan deficient mice have systemically elevated TGF- β , likely due to lack of sequestration of TGF- β in the extracellular matrix. The purpose of this study was to determine if prevention of TGF- β elevations in biglycan deficient mice affected atherosclerosis development.

Methods—Biglycan deficient mice were crossed to *Ldlr* deficient mice. Diabetes was induced via streptozotocin and all mice were fed a high cholesterol diet. Diabetic biglycan wild type and biglycan deficient *Ldlr* deficient mice were injected with the TGF- β neutralizing antibody 1D11 or irrelevant control antibody 13C4.

Results—Biglycan deficient mice had significantly elevated plasma TGF- β levels, which was further increased by diabetes, and significantly increased atherosclerosis. There was a significant correlation between TGF- β concentrations and atherosclerosis. However, despite nearly complete suppression of plasma TGF- β levels in mice treated with the TGF- β neutralizing antibody 1D11, there was no significant difference in atherosclerosis between mice with elevated TGF- β levels and mice with suppressed TGF- β levels.

Conclusions—The increased atherosclerosis in biglycan deficient mice does not appear to be due to elevations in TGF- β .

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Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript. There is no conflict of interest for any author.

Author contributions

LRT conceived and designed the studies, wrote the manuscript and completed all analyses. JCT, PGW, APW and AKW performed the scientific experiments, and contributed to data analysis and the writing of the manuscript.

Keywords

atherosclerosis; cholesterol; proteoglycans; extracellular matrix; diabetes; animal models

Introduction

Atherosclerosis is the leading cause of death in developed countries, and despite best current medical therapies, it continues to be a major public health issue. The response to the retention hypothesis of atherosclerosis proposes that atherosclerosis begins in the subendothelial space of the vessel wall when atherogenic lipoproteins from the circulation are retained via ionic interactions with vascular proteoglycans [1, 2]. Clinical studies demonstrate that diffuse intimal thickening with an increase in proteoglycan content occurs prior to the influx of inflammatory cells [3]. Boren's group demonstrated that mice expressing proteoglycan-binding-defective LDL had attenuated and delayed development of atherosclerosis compared to mice with normal proteoglycan-binding activity of LDL [4, 5].

There are a number of different proteoglycans present in the vascular wall. We and others previously showed that biglycan is the proteoglycan most consistently co-localized with apoB within atherosclerotic lesions in both mouse and man [6–8], suggesting that vascular biglycan content may affect atherosclerosis development. In previous studies, using a mouse model in which biglycan is overexpressed in smooth muscle cells, we found that increased vascular biglycan content leads to increased lipid retention and increased atherosclerosis [9]. In related works, we reported increased vascular biglycan content and increased atherosclerosis in mice infused with angiotensin II (angII) [6] or over-expressing serum amyloid A [10]. Vascular biglycan is increased in diabetes [11, 12]. Biglycan has pro-inflammatory activities via signaling through Toll-like receptors [13], and biglycan appears to act as a damage-associated molecular pattern (DAMP) molecule. Thus, increased vascular biglycan content appears to be proatherogenic via increased lipid retention and increased inflammation in the vascular wall.

However, biglycan deficiency does not appear to protect against atherosclerosis. Mice globally deficient in biglycan crossed with apoE deficient mice (*apoE*^{-/-}) demonstrated increased atherosclerosis, due, at least in part, to increased macrophage accumulation, increased thrombin activity, and platelet activation [14]. In another study, biglycan deficient mice crossed with LDL receptor deficient (*Ldlr*^{-/-}) mice infused with angII or saline, then fed an atherogenic Western diet, had a striking increase in thoracic and abdominal aortic aneurysms, but no differences in atherosclerosis lesion area compared to biglycan wild type mice [15]. Thus both increased vascular biglycan and deficiency of vascular biglycan led to increased atherosclerosis. One possible factor confounding these analyses is that biglycan deficient mice have elevated systemic TGF- β levels [16], likely due to lack of sequestration of TGF- β in the extracellular matrix [17].

The role of TGF- β in atherosclerosis development remains unclear: it may be pro-atherogenic via stimulation of extracellular matrix synthesis and increased lipid retention [18] or it may be anti-atherogenic via its anti-inflammatory effects [19]. In two distinct studies using TGF- β inhibition in *apoE*^{-/-} mice, opposite effects on atherosclerosis were

found: Mallat et al. used an antibody that neutralized TGF- β 1, β 2, and β 3 and found increased lesion area [20]; conversely, Lutgens et al. used a soluble TGF-beta receptor II to inhibit TGF- β signaling and found decreased atherosclerotic plaque area [21]. We previously reported that prevention of TGF- β elevations in angII-infused mice attenuated angII induction of atherosclerosis [22], suggesting that TGF- β is pro-atherogenic. Thus, the elevated TGF- β in biglycan deficient mice [15] could induce pro-atherogenic mechanisms and pathways, overriding any potential of biglycan deficiency to reduce atherosclerosis. We previously demonstrated elevated plasma TGF- β concentrations in diabetic mice [23], and diabetes is known to increase atherosclerosis development in *Ldlr*^{-/-} mice [24]. The purpose of this study was to determine the impact of biglycan deficiency with and without TGF- β neutralization on atherosclerosis development in diabetic mice.

Materials and methods

Animals

All studies were approved by the Animal Component of Research Protocol review by the Lexington Veterans Affairs Medical Center Institutional Animal Care and Use Committee. Biglycan deficient mice were crossed with *Ldlr*^{-/-} mice (C57BL6 background) as previously described [15]. Biglycan is X-linked; thus, only male mice were used in the present study ensuring that biglycan deficient and biglycan wild type mice were true littermates. As previously described, mice were made diabetic via streptozotocin (STZ) injections or were injected with citrate buffer without STZ for non-diabetic controls, then all mice were fed a 0.12% cholesterol diet (TD000242, Harlan Teklad, Madison, WI) for 26 weeks [23]. To test the role of TGF- β neutralization, diabetic mice were injected with TGF- β neutralizing antibody 1D11 or irrelevant control antibody 13C4, intraperitoneally, at 2 mg/kg body weight, concurrent with the first STZ injection, and plasma TGF- β levels were measured every 2–3 days for 2 weeks, then weekly. 1D11 and 13C4 injections were repeated when plasma TGF- β levels began to rise, which was every 8 weeks.

Metabolic and vascular characterization

Blood glucose was measured by tail venipuncture at least weekly, using a commercial glucose meter. Subcutaneous insulin pellets (LinShin Inc, Canada) were placed as needed (if indicated by weight loss, change in body condition score and/or blood glucose over 20.8 mmol/L). Non-fasting blood samples were collected at weeks 10, 18, and 26 of diet, for analyses of cholesterol and triglycerides, as previously described [23, 25]. Due to limitations in blood volumes collected, not every analysis was performed on every sample. Lipoprotein distribution in samples collected at week 26 was analyzed by fast performance liquid chromatography (FPLC). Cholesterol in VLDL/LDL and HDL was measured using an ELISA, according to manufacturer's instructions (Abcam ab#65390, Cambridge, MA). Fructosamine was measured using an assay kit on frozen samples, according the manufacturer's instructions (Bioo Scientific, cat#5615-01, Austin, TX). TGF- β was measured in the plasma and in homogenized heart tissue by ELISA (Promega, Madison, WI); cardiac TGF- β was normalized to tissue weights. After 26 weeks of diets, mice were euthanized by lethal anesthesia and tissues were collected. Atherosclerosis was measured on the aortic intimal surface and in oil red O stained aortic sinus as previously described [6]

(see Supplementary Materials for details). Atherosclerotic plaque composition was assessed by staining aortic root sections for collagen (Masson's trichrome), macrophages (CD68; Abcam ab#53444, Cambridge, MA) and actin (Novus Biologics, NB300-978, Littleton, CO). Quantification was performed using ImageJ software, as previously described (see Supplementary Materials for details). All quantifications were performed in duplicate by personnel blinded to the genotype and/or treatments. Primary vascular smooth muscle cells (VSMC) were isolated from 4–6 week old biglycan wild type and biglycan deficient mice, treated with TGF- β (2 ng/ml) or vehicle and 1D11 or 13C4 (10 μ g/ml) or vehicle for 24 hours, then LDL binding was assayed as previously described [10].

Statistics

Data are presented as mean $\pm$ SEM. Data was analyzed by 2-way ANOVA for genotype and diabetic status, or genotype and antibody treatment as the two factors. If significant interactions between factors were detected, pairwise comparisons were performed by the Holm-Sidak method. Statistical significance was defined by a p -value less than 0.05.

Results

Effect of biglycan deficiency on TGF- β levels

There was no effect of biglycan deficiency on susceptibility to STZ-induced diabetes and all STZ-treated mice remained hyperglycemic throughout the study duration (glucose levels ranged between 13–17 mmol/L, not shown). All citrate-injected mice remained normoglycemic (glucose levels remained 5–6.6 mmol/L, not shown). Fructosamine levels were significantly higher in STZ-treated mice compared to citrate-injected mice ($p<0.001$) but did not differ between genotypes (not shown). Mice received insulin pellets per protocol for poor body condition score, weight loss, or glucose > 20.8 mmol/L. Despite use of insulin, some mice were euthanized per study protocol for poor body condition score or weight loss $> 10\%$ in one week. In total, four biglycan deficient diabetic mice died (all between weeks 2–8), one biglycan wild type diabetic mouse died (at week 2), and one non-diabetic biglycan wild type mouse died (at week 13). All mice were fed a high (0.12%) cholesterol diet throughout the study. As previously reported [23], plasma cholesterol levels increased over time in all groups, but did not differ significantly between groups (Fig. 1A). Plasma triglycerides (Fig. 1B), cholesterol in VLDL/LDL (Fig. 1C) or HDL (Fig. 1D), and lipoprotein distribution (Fig. 1E) did not differ significantly between groups. After 26 weeks of diabetes and diets, plasma TGF- β was increased by biglycan deficiency and by diabetes ($p<0.001$ for interaction between diabetes and biglycan genotype; $p<0.001$ for within-genotype comparisons and $p<0.001$ for diabetic status comparisons; Fig. 1F).

Effect of biglycan deficiency on atherosclerosis

Atherosclerosis was quantified on the aortic intimal surface and aortic sinus. There was a surprising range of atherosclerosis lesion development, with some mice seemingly resistant and others developing large atherosclerotic lesions (Figs 2A and B). Interestingly, different effects were found at the different sites. On the aortic intimal surface, atherosclerosis was significantly increased in biglycan deficient compared to biglycan wild type mice ($p=0.002$; Fig 2A), but there was no significant effect of diabetes status. Conversely, in the aortic sinus,

atherosclerosis was significantly increased in diabetic compared to control mice ($p=0.012$; Fig. 2B), but there was no effect of biglycan genotype. The biglycan deficient mice had both the highest TGF- β levels (Fig. 1D) and the most atherosclerosis (Fig. 2A and B); correlation analyses revealed significant correlations between TGF- β levels and atherosclerosis ($r=0.28$, $p<0.001$ for aortic root atherosclerosis and $r=0.17$, $p<0.001$ for aortic intimal surface). Analysis of plaque composition showed no differences in collagen content (Supplementary Fig. 2A) but biglycan deficient mice had significantly higher plaque macrophage content ($p=0.049$; Fig. 2C) and significantly lower plaque actin ($p=0.006$; Fig. 2D). There was no effect of diabetes status on plaque collagen, macrophage or actin content.

Effect of TGF- β Inhibition on atherosclerosis development

The studies were repeated using the TGF- β neutralizing antibody 1D11 to suppress TGF- β . Mice were injected with 1D11 or irrelevant control antibody 13C4 concurrent with the first injection of STZ. To minimize animal use, only diabetic mice were included. Plasma TGF- β levels were measured every 2–3 days for 2 weeks, then weekly. 1D11 and 13C4 injections were repeated when plasma TGF- β levels began to rise, which was every 8 weeks (not shown). There was no effect of either 1D11 or 13C4 on susceptibility to STZ-induced diabetes or insulin requirements and all mice remained hyperglycemic throughout the study duration (glucose levels ranged 13–17 mmol/L; not shown). Fructosamine levels did not differ between genotypes or with antibody treatment (not shown). All mice in this study were diabetic; however, no mouse treated with 1D11 died; whereas 3 of 17 biglycan wild type (weeks 3, 7 and 21) and 4 of 27 biglycan deficient mice (weeks 7, 15, 20, 21) from 13C4-treated groups died ($p=0.01$ for antibody effect; not shown). All mice were fed the 0.12% cholesterol diet for 26 weeks. Cholesterol levels increased over time in all groups, but did not differ significantly between groups (Fig. 3A). Surprisingly, triglycerides were higher in 1D11-treated mice compared to 13C4 treated mice, regardless of genotype ($p=0.019$ for antibody effect, Fig. 3B), although no difference in triglyceride amount or distribution was found in FPLC profiles (Supplementary Fig. 1). This finding appears to be non-reproducible, as no effect of 1D11 on triglycerides has been found in previous studies [22, 26]. The amount of cholesterol in VLDL/LDL (Fig. 3C) or HDL (Fig. 3D), did not differ between groups. Mice treated with 1D11 showed similar FPLC lipoprotein distribution profiles regardless of biglycan genotype, but had increased LDL and HDL compared to 13C4-treated mice. 13C4-injected biglycan wild type mice had a more prominent LDL shoulder than did 13C4-injected biglycan deficient mice (Fig. 3E). As expected, 13C4 injections had no effect on either plasma (Fig. 3F) or cardiac TGF- β (Fig. 3G). Biglycan deficient mice had higher plasma TGF- β levels than biglycan wild type mice. 1D11 treatment suppressed plasma TGF- β levels in both genotypes to < 500 pg/ml ($p<0.001$ for interaction between biglycan genotype and antibody treatment; $p<0.001$ for within-genotype comparisons and $p<0.001$ for antibody treatment comparisons (Fig. 3F)). 1D11 treatment suppressed cardiac TGF- β levels in both genotypes ($p=0.03$), but there was no effect of 13C4 (Fig. 3G).

Despite the effective suppression of plasma TGF- β concentrations in 1D11-treated groups, there was no significant effect of TGF- β inhibition on atherosclerosis (Fig. 4AB). On the aorta intimal surface atherosclerosis was significantly increased in biglycan deficient compared to biglycan wild type mice ($p<0.001$; Fig 4A) and there was no significant effect

of 1D11 treatment. In the aortic sinus, there were no significant differences between groups. Analysis of plaque composition showed no differences between any groups on plaque collagen (Supplementary Fig. S2B), macrophage (Fig. 4C) or actin content (Fig. 4D). However, treatment of primary VSMC with 1D11 *in vitro* significantly decreased lipoprotein binding in both biglycan wild type and biglycan deficient mice (Fig. 5).

Discussion

In summary, our data demonstrates that biglycan deficient *Ldlr*^{-/-} mice have increased atherosclerosis compared to biglycan wild type *Ldlr*^{-/-} mice when fed a high cholesterol diet. Diabetes increased atherosclerosis at the aortic sinus but not the aortic intimal surface. These differences in atherosclerosis occurred despite no significant differences in cholesterol or triglyceride levels or lipoprotein distribution between biglycan deficient and biglycan wild type mice. The biglycan deficient mice had significantly elevated plasma TGF- β levels, which was further increased by diabetes, and there was a significant correlation between TGF- β concentrations and atherosclerosis. However, despite nearly complete suppression of both plasma and cardiac TGF- β levels in mice treated with the TGF- β neutralizing antibody 1D11, there was no significant difference in atherosclerosis between mice with elevated TGF- β levels (those that received the control antibody 13C4) and mice with suppressed TGF- β levels (1D11 treated mice). The increase in atherosclerosis between biglycan deficient and biglycan wild type mice was not affected by inhibition of TGF- β . Thus, elevated TGF- β does not contribute to increased atherosclerosis in biglycan deficient mice.

The role of TGF- β in atherosclerosis is controversial, and likely depends on context/dose and timing of exposure. In early stages of atherosclerosis, TGF- β may be protective due to its anti-inflammatory effects and anti-proliferative effects, whereas later in atherosclerosis, TGF- β may promote plaque progression (for review see [27–29]). TGF- β 1 is predominantly found as a large latent complex. In this form it is inactive; activation occurs by release of TGF- β 1 by acidic conditions, plasmin, thrombospondin or other factors. The latent form of TGF- β is frequently localized within the extracellular matrix, in part due to binding by proteoglycans such as biglycan or decorin [30]. Matrix binding is thought to sequester TGF- β in a particular region, and TGF- β activity and downstream events occur upon its release from this extracellular matrix “sink”. Both biglycan and decorin deficient mice have elevated systemic TGF- β [16, 31], likely due to decreased matrix sequestration. TGF- β 1 circulates in plasma and is increased in diabetes [32]; some authors have proposed that elevations in TGF- β in diabetes may contribute to the increased cardiovascular disease associated with diabetes. Broadly speaking, the pro-fibrotic activities of TGF- β appear to increase atherosclerosis development, whereas its anti-inflammatory activities appear to protect against lesion size [20, 21]. Some studies have found that the beneficial effects of some antidiabetic drugs (e.g. pioglitazone) are mediated via influencing TGF- β levels [33, 34]. In a prior work, we demonstrated that preventing the angII-mediated induction of TGF- β by 1D11 attenuated the angII-mediated increase in vascular biglycan and atherosclerosis; however, there was no significant effect of 1D11 in saline treated control mice [22]. Thus, neutralization of TGF- β only reduced atherosclerosis in the setting (angII) where TGF- β was further elevated; but there was no effect in baseline conditions. In the present study, TGF- β was elevated (as expected) in diabetic mice compared to non-diabetic mice, and even

more elevated in biglycan deficient compared to biglycan wild type mice (presumably due to loss of sequestration). However, there was no significant effect of 1D11 on atherosclerosis in diabetic mice. We did not study 1D11 in non-diabetic mice, but these results demonstrate that increased circulating TGF- β (presumably due to loss of sequestration) is not causal of the increased atherosclerosis observed in biglycan deficiency. There was no effect of 1D11 on atherosclerotic plaque composition as assessed by collagen, macrophage and actin content. However, as expected from our prior work [10], 1D11 treatment decreased LDL binding to both biglycan wild type and biglycan deficient primary VSMC *in vitro*. This finding suggests that factors beyond lipid retention contribute to atherosclerosis development in this model.

The mechanisms by which biglycan is atheroprotective have been explored recently. In biglycan deficient mice crossed with *apoE*^{-/-}, biglycan was found to play a protective role against atherosclerosis progression by decreased macrophage accumulation, inhibition of thrombin activity, and decreased platelet activation [14]. Although we have previously shown that increased biglycan content leads to increased lipid retention and increased atherosclerosis [9], all proteoglycans are capable of binding to lipoproteins, and we previously demonstrated that other proteoglycans, such as perlecan, could compensate for biglycan deficiency [15]. However, until now, it was not clear if the elevated TGF- β seen in biglycan deficiency had a role in the development of atherosclerosis. The results of the present study suggest that the increase in plasma TGF- β in biglycan deficient mice is coincidental to the increased atherosclerosis, and not causal.

In summary, the data presented here demonstrate that elevated TGF- β in biglycan deficient mice is not a cause of the increased atherosclerosis found in this model. In addition, our data suggests that elevated TGF- β may contribute to increased atherosclerosis in diabetes. A limitation of this study is that we only evaluated the effect of TGF- β neutralization in diabetic mice. However, the extent of atherosclerosis found in 1D11-treated (diabetic) mice was similar to that found in non-diabetic mice at least within aortic sinus lesions (compare Figs. 2B with 4B). Strikingly, no animals treated with 1D11 died although all were diabetic, whereas the mortality of all other diabetic groups ranged between 10–20%, which is similar to our prior studies [23, 25]. The reason for the reduced mortality in 1D11-treated mice is unclear, but does not appear to be due to an effect of 1D11 treatment on diabetes susceptibility (based on glucose or fructosamine levels). TGF- β inhibitors are being studied in clinical trials for several diseases including cancers and fibrotic diseases; it will be of great interest if there is an improvement in mortality seen. The results of this study suggest that TGF- β inhibition will not have adverse effects on atherosclerosis, but likely will not have beneficial effects either. In summary, biglycan deficiency causes striking elevations of TGF- β but neutralization of TGF- β has no effect on atherosclerosis development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

Increased vascular biglycan leads to increased atherosclerosis

However, biglycan deficiency does not appear to lead to decreased atherosclerosis

Biglycan deficiency leads to increased circulating TGF- β

However, neutralization of TGF- β has no effect on atherosclerosis development

Figure 1.

Biglycan deficiency increases TGF-β.

Biglycan wild type (*bgn*^{+/+}, solid symbols) and biglycan deficient (*bgn*^{-/-}, open symbols) *Ldlr*^{-/-} mice were injected with STZ to induce diabetes (triangles) or with citrate vehicle (squares), then fed a 0.12% cholesterol diet for 26 weeks. (A) Cholesterol was measured at baseline, and weeks 10, 18 and 26. To convert to SI units divide by 38.5. (B) Triglycerides were measured at week 26; mean±SEM for N=8–31 mice/group is shown. To convert to SI units divide by 88.5. (C) VLDL/LDL cholesterol was measured at week 26; mean±SEM for N=4–5 mice/group is shown. To convert to SI units divide by 38.5. (D) HDL cholesterol was measured at week 26; mean±SEM for N=4–5 mice/group is shown. To convert to SI units divide by 38.5. (E) Lipoprotein distribution was assessed by FPLC; mean±SEM for N=3–4 mice/group is shown. (F) TGF-β was measured at week 26; mean±SEM for N=14–26 mice/group is shown. Groups without the same letter differ from each other ($p < 0.05$).

Figure 2.

Biglycan deficiency increases atherosclerosis.

Biglycan wild type (*bgn*^{+/+}, solid symbols) and biglycan deficient (*bgn*^{-/-}, open symbols) *Ldlr*^{-/-} mice were injected with STZ to induce diabetes (triangles) or with citrate vehicle (squares), then fed a 0.12% cholesterol diet for 26 weeks. Atherosclerosis area was determined on the aortic intimal surface (A) and the aortic sinus (B). Atherosclerosis plaque composition was assessed by staining for macrophages (CD68, C) and actin (D). Each symbol represents an individual mouse; the horizontal line indicates the group mean. Representative images from each group are shown next to each analysis. Scale bar in the *en face* images: 1.0 mm; scale bars in all other images: 250 μm . $*p=0.012$ for effect of diabetic status within each genotype.

Figure 3.

Effect of TGF-β inhibition.

Biglycan wild type (*bgn*^{+/+}, solid symbols) and biglycan deficient (*bgn*^{-/-}, open symbols) *Ldlr*^{-/-} mice were injected with STZ to induce diabetes, and with TGF-β neutralizing antibody 1D11 (triangles) or irrelevant control antibody 13C4 (squares), every 8 weeks, while fed a 0.12% cholesterol diet for 26 weeks. (A) Cholesterol was measured at baseline, and weeks 10, 18 and 26. To convert to SI units divide by 38.5. (B) Triglycerides were measured at week 26; mean±SEM for N=17–27 mice/group is shown. To convert to SI units divide by 88.5. (C) VLDL/LDL cholesterol was measured at week 26; mean±SEM for N=5 mice/group is shown. To convert to SI units divide by 38.5. (D) HDL cholesterol was measured at week 26; mean±SEM for N=5 mice/group is shown. To convert to SI units divide by 38.5. (E) Lipoprotein distribution was assessed by FPLC; mean±SEM for N=3–4 mice/group is shown. (F) Plasma TGF-β was measured at week 26; mean±SEM for N=14–26 mice/group is shown. (G) Cardiac TGF-β was measured at week 26; mean±SEM for N=5 mice/group is shown. Groups without the same letter differ from each other ($p < 0.05$).

Figure 4.

Effect of TGF- β inhibition on atherosclerosis.

Biglycan wild type (*bgn*^{+/+}, solid symbols) and biglycan deficient (*bgn*^{-/-}, open symbols) *Ldlr*^{-/-} mice were injected with STZ to induce diabetes and with TGF- β neutralizing antibody 1D11 (triangles) or irrelevant control antibody 13C4 (squares), every 8 weeks, while fed a 0.12% cholesterol diet for 26 weeks. Atherosclerosis area was determined on the aortic intimal surface (A) and the aortic sinus (B). Atherosclerosis plaque composition was assessed by staining for macrophages (CD68, C) and actin (D). Each symbol represents an individual mouse; the horizontal line indicates the group mean. Representative images from each group are shown next to each analysis. Scale bar in the *en face* images: 1.0 mm; scale bars in all other images: 250 μm .

Figure 5.

Effect of TGF- β inhibition on lipoprotein binding.

VSMC were isolated from 6–8 week old biglycan wild type (*bgn*^{+/+}, solid bars) and biglycan deficient (*bgn*^{-/-}, open bars) *Ldlr*^{-/-} mice, then were treated for 24 hours with TGF- β (2 ng/ml) or vehicle, and/or 13C4 or 1D11 (10 μ g/ml) or vehicle, then incubated with Alexa-fluor labeled LDL for 4 hours. (A) Mean $\pm$ SEM for Alexa-fluor staining normalized to DAPI area for n=5. (B) 20 $\times$ representative images of the binding assay. Scale bars: 100 μ m.