

Comparison of Apolipoprotein and Proteoglycan Deposits in Human Coronary Atherosclerotic Plaques

Colocalization of Biglycan With Apolipoproteins

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Background—Because the content of specific proteoglycans and apolipoproteins is increased in atherosclerotic plaques and in vitro studies have suggested a role for proteoglycans in mediating plaque apolipoprotein (apo) retention, immunohistochemistry was performed to systematically examine the relative locations of proteoglycans and apolipoproteins in human atherosclerosis.

Methods and Results—The spatial relationships of versican, biglycan, and apoE were compared on 68 human coronary artery segments; apoA-I and apoB also were evaluated on an additional 20 segments. Nonatherosclerotic intima contained extensive deposits of versican, whereas deposits of apoE, apoB, and apoA-I were much less prevalent. In contrast, nearly all atherosclerotic segments contained substantial deposits of biglycan, apoE, apoA-I, and apoB. There was a high degree of colocalization of apoE and biglycan deposits. ApoA-I, the major apolipoprotein of HDL, and apoB also were detected in regions with apoE and biglycan deposition. Exceptions to the localization of biglycan with apolipoproteins were found in regions that lacked intact extracellular matrix because of necrosis or dense macrophage accumulation. In vitro studies demonstrated that biglycan binds apoE-containing but not apoE-free HDL and that biglycan also binds LDL.

Conclusions—These results suggest that biglycan may bind apoE and apoB in atherosclerotic intima. They also raise the possibility that apoE may act as a “bridging” molecule that traps apoA-I-containing HDL in atherosclerotic intima. Taken together, these findings are consistent with the hypothesis that biglycan may contribute to the pathogenesis of atherosclerosis by trapping lipoproteins in the artery wall. (*Circulation*. 1998;98:519-527.)

Key Words: muscle, smooth ■ cells ■ cholesterol ■ lipoproteins ■ immunohistochemistry

In addition to the presence of smooth muscle and inflammatory cells,¹ hallmarks of the atherosclerotic plaque are accumulations of lipid and of extracellular matrix molecules such as proteoglycans (reviewed in Reference 2). In the past few years, substantial evidence has been obtained for interactions between lipoproteins and proteoglycans in the pathogenesis of atherosclerosis (reviewed in Reference 3). In vitro, chondroitin sulfate proteoglycans bind, via ionic interactions, to LDL,⁴ and lipoprotein/proteoglycan complexes are internalized and degraded by both macrophages⁵⁻⁸ and SMCs.⁹ In vivo, apoB and chondroitin sulfate have been shown to colocalize in the injured intima of rabbits,¹⁰ and apoB/chondroitin sulfate proteoglycan complexes have been eluted from human atherosclerotic lesions.¹¹

Recently, other apolipoproteins, such as apoE^{12,13} and apoA-I,¹⁴ and other proteoglycans, such as biglycan,¹⁵ a small dermatan sulfate proteoglycan, also have been detected in human atherosclerotic plaques. Several lines of evidence suggest roles for these molecules in atherogenesis. ApoE is abundant in human lesions, where it may be expressed locally

by macrophages,^{12,13} especially those that have accumulated excess lipid.¹² ApoE also has been shown to bind cell-surface heparan sulfate proteoglycans in vitro.¹⁶ However, it is not known whether apoE colocalizes with specific extracellular matrix molecules in atherosclerosis.

Also, a consistently reported change in arterial proteoglycans during the progression of atherosclerosis is an increase in dermatan sulfate proteoglycans,¹⁷⁻²⁰ including biglycan,¹⁵ a small, leucine-rich proteoglycan of unknown function. The purpose of the present investigation was to determine whether the extensive deposits of apoE found in human atherosclerotic lesions were associated with deposits of specific proteoglycans. The results demonstrate that apoE is localized to regions of the plaque enriched with biglycan and that apoA-I and apoB also are colocalized to regions with deposits of apoE and biglycan.

Methods

Human Coronary Arterial Tissue

Human coronary arterial segments were obtained from hearts removed from 24 patients at the time of cardiac transplantation. The

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Selected Abbreviations and Acronyms

apo	= apolipoprotein
GMSA	= gel mobility shift assay
HDL ₃ -E	= HDL ₃ that lacked apoE
HDL ₃ +E	= HDL ₃ that contained apoE
SMC	= smooth muscle cell
TGF	= transforming growth factor

coronary arteries were dissected from the surface of the hearts and placed in 10% neutral buffered formalin within 2 hours of organ excision, and segments of these coronary arteries then were embedded in paraffin. Collection and use of these tissues were approved by the University of Washington Human Subjects Review Committee. Coronary artery segments used in this study were classified according to morphological criteria rather than by the primary diagnosis (ie, ischemic or nonischemic cardiomyopathy) of the patients from whom the segments had been obtained. Atherosclerotic regions were defined by the presence of luminal narrowing due to regional accumulation of cholesterol, foam-cell and non-foam-cell macrophages, and the presence of fibrous caps, whereas nonatherosclerotic regions were defined by the presence of intimal thickening due to the accumulation of SMCs and matrix proteins.²¹ Intimal thickening is a characteristic morphological feature of human coronary arteries.²¹ In the first phase of the study, versican, biglycan, and apoE were examined in a total of 68 coronary arterial segments obtained from 14 patients (9 with ischemic and 5 with nonischemic cardiomyopathies), and in the second phase of the study, versican, biglycan, apoE, apoA-I, and apoB were examined in a total of 20 coronary arterial segments obtained from 8 patients (2 with ischemic and 6 with nonischemic cardiomyopathies).

Immunohistochemistry**Antibodies and Antisera**

Rabbit antisera directed against the core proteins of biglycan and versican were used on sections pretreated with chondroitinase ABC (Sigma Chemical Co) to remove chondroitin and dermatan sulfate chains.²² For biglycan, a rabbit polyclonal anti-biglycan antiserum (a kind gift of Dr Larry Fisher, NIH) was used at a titer of 1:600 for immunohistochemistry. Specificity of the antiserum for immunohistochemistry was confirmed by (1) the presence of a single band at the molecular weight of biglycan on Western blot of human aortic atherosclerotic plaque and (2) abolition of positive staining by preabsorption of the antiserum with biglycan. For versican, a polyclonal anti-versican antiserum had been generated previously by immunization of rabbits with recombinant human versican and was affinity-purified by absorption to a column containing synthetic peptides corresponding to the VC-E region of the human versican core protein.²³ The affinity-purified anti-versican antiserum was used for immunohistochemistry at a titer of 1:800. This antiserum was a kind gift of Drs Richard LeBaron (University of Texas at San Antonio) and Erkki Ruoslahti (La Jolla Cancer Research Center, La Jolla, Calif). ApoB was detected with a mouse monoclonal antibody (MB-47, titer=1:500), and apoE was detected with a goat polyclonal antiserum (titer=1:3000) as described previously.^{12,24} ApoA-I was detected with a goat polyclonal anti-apoA-I antiserum (titer=1:10 000, kind gift of Dr John F. Oram, University of Washington) that had been characterized previously as monospecific for apoA-I compared with apoB, E, A-II, and A-IV by Western blot.²⁵ Mouse monoclonal antibodies used for cell-type identification included (1) anti-smooth muscle α -actin (Dako Corp), used at a titer of 1:500 to identify SMCs,²⁶ and (2) HAM-56 (a kind gift of Dr Allen Gown, University of Washington), used at a titer of 1:10 to identify macrophages.²⁷

Immunohistochemistry

Immunohistochemistry was performed as described previously.^{12,28} 3,3'-Diaminobenzidine with nickel chloride was used as the perox-

idase substrate, yielding a black reaction product. Slides were counterstained with methyl green. Negative controls included substitution of primary antiserum or antibody with PBS, isotype-matched irrelevant monoclonal antibodies, or normal rabbit serum.

Lipoprotein and Proteoglycan Isolation and GMSA**Lipoproteins**

HDL₃ ($d=1.125$ to 1.210) was isolated by sequential density-gradient ultracentrifugation from plasma obtained from a pool of 6 normal human volunteers, as described previously,²⁹ dialyzed extensively against 50 mmol/L imidazole buffer (pH 6.7) at 4°C, and concentrated (Centriprep 100, Amicon). To remove apoE-containing particles, the HDL₃ was passed through a 50 mmol/L imidazole buffer-equilibrated heparin-Sepharose column. HDL₃+E was eluted with 1.0 mol/L NaCl. HDL₃+E and HDL₃-E preparations were dialyzed extensively at 4°C against 150 mmol/L NaCl and 1 mmol/L EDTA (pH 7.4) and stored under nitrogen at 4°C in the dark.

LDL ($d=1.019$ to 1.063) was isolated by preparative ultracentrifugation of plasma obtained from a pool of 6 normal human volunteers, as described previously.³⁰ LDL was dialyzed extensively at 4°C in the dark against 150 mmol/L NaCl and 1 mmol/L EDTA (pH 7.40) before its use in the lipoprotein-proteoglycan binding assay described later. The presence or absence of apoB and apoE in the HDL₃ preparations was confirmed by SDS-PAGE and Western blotting using either the mouse anti-apoB antibody (MB-47; titer=1:2000) or the goat anti-apoE antiserum (titer=1:1000).

Biglycan Isolation

Biglycan was prepared from cultured human arterial SMCs metabolically labeled with ³⁵S-labeled Na₂SO₄, as described previously.³¹ Briefly, cell medium was concentrated on DEAE-Sephacel minicolumns equilibrated in 8 mol/L urea, 0.25 mol/L NaCl, and 0.5% CHAPS. The ³⁵S-labeled proteoglycans were eluted with 8 mol/L urea, 3 mol/L NaCl, and 0.5% CHAPS and applied to a Sepharose CL-2B column equilibrated in 8 mol/L urea and 0.5% CHAPS. Radiolabeled proteoglycans eluting at K_{av} 0.55 to 0.8 were pooled for biglycan. Purity of the biglycan was assessed by SDS-PAGE and Western blotting.

Gel Mobility Shift Assay

The interaction between biglycan and either HDL₃+E, HDL₃-E, or LDL was investigated by a GMSA.⁴ Before the assay, the ³⁵S-labeled biglycan and lipoprotein preparations were dialyzed extensively at 4°C against 10 mmol/L HEPES, 150 mmol/L NaCl, 5 mmol/L CaCl₂, 2 mmol/L MgCl₂ (pH 7.4; buffer A), and the protein concentrations were determined (BioRad Laboratories) with bovine γ -globulin as the standard.

Increasing concentrations of lipoprotein were incubated with ≈ 2000 dpm of ³⁵S-labeled biglycan ($\approx 0.4 \mu\text{g}$ glycosaminoglycan) for 1 hour at 37°C in a total volume of 20 μL of buffer A. It should be noted that buffer A, used both for dialysis and for incubation of biglycan and lipoproteins, was adjusted to physiological pH and contains physiological concentrations of salt. Three microliters of bromphenol blue:glycerol (1:1 vol/vol) was added to the samples, and 20 μL was loaded into wells of 0.7% NuSieve (FMC BioProducts) agarose gels, which were prepared on Gel-Bond film (FMC BioProducts). Electrophoresis was run at 60 V for 3 hours in a cold room with recirculation of buffer (10 mmol/L HEPES, 2 mmol/L CaCl₂, 4 mmol/L MgCl₂, pH 7.20). Gels were fixed in 0.1% cetylpyridinium chloride in 70% ethanol for 90 minutes, air-dried, and exposed to Hyper Film-MP (Amersham Life Sciences) at -70°C .

Statistical Analysis

Categorical data were analyzed by use of χ^2 analyses with Yates' correction when cell sizes were <5 . Analyses were performed with Epi Info version 6.02 (Centers for Disease Control and Prevention). The level of significance was set at $P<0.05$.

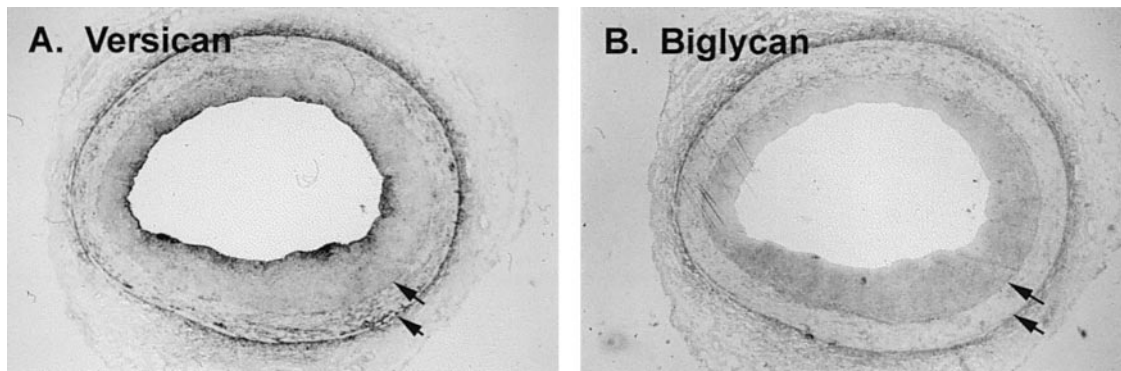


Figure 1. Proteoglycans in nonatherosclerotic coronary intima. Sections are shown of a coronary arterial segment immunostained for versican (A) or biglycan (B). Locations of internal and external elastic laminae are indicated by arrowheads. Deposits of versican are present in both adventitia and media, but most prominent staining for versican is an intense band of immunostaining immediately adjacent to endothelial cells at arterial lumen (A). Deposits of biglycan are detected primarily in adventitia, immediately adjacent to external elastic lamina, and scanty deposits also are present in media (B). Magnification $\times 40$; methyl green counterstain.

Results

Localization of Proteoglycans in Nonatherosclerotic Regions

In nonatherosclerotic arteries (Figure 1), scattered deposits of versican were detected in the adventitia and media (Figure 1A). The most prominent versican staining was seen in the intima, immediately adjacent to the arterial lumen (1A). In contrast, distinct biglycan immunostaining was detected primarily in the adventitia, immediately adjacent to the external elastic lamina (1B). Biglycan deposits also were detected occasionally in the media and intima of nonatherosclerotic segments. Apolipoproteins were not detected in this normal

arterial segment (data not shown) and were rarely detected in the nonatherosclerotic segments.

Biglycan, Versican, and ApoE Deposits in Atherosclerotic Plaques

In atherosclerotic plaques, there was striking colocalization of deposits of biglycan (Figure 2A) and apoE (2B). Biglycan deposits were most prominent in areas with matrix characterized on hematoxylin-eosin staining by a pale, homogeneous appearance in which the intimal staining patterns for biglycan and apoE appeared to be nearly identical.

Versican staining also was increased in amount in atherosclerotic plaques (Figure 2C). In contrast to the findings for

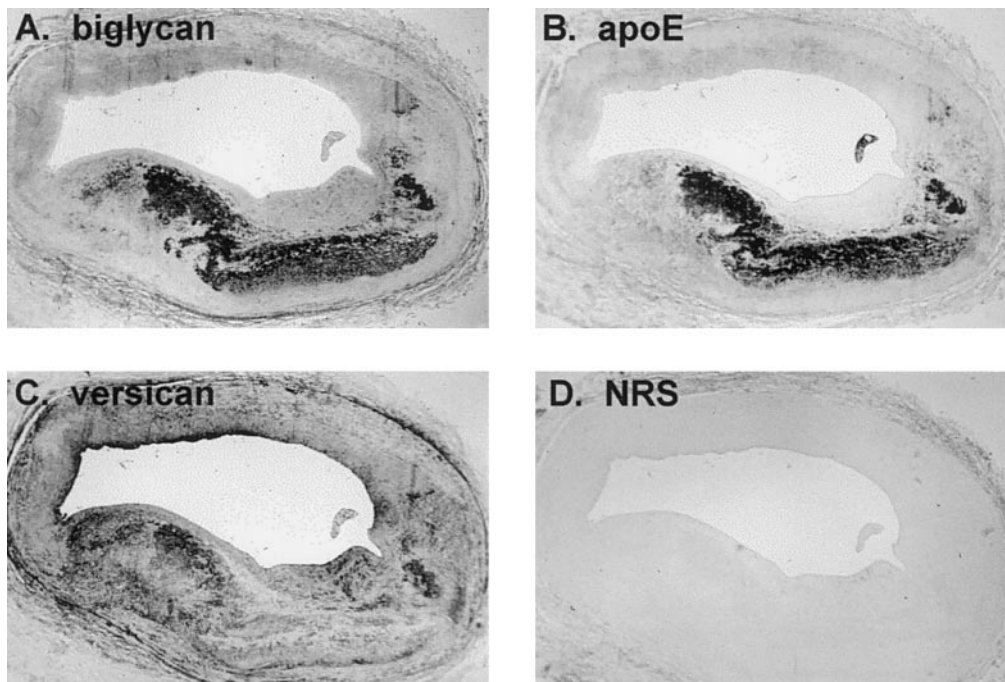


Figure 2. Colocalization of apoE with biglycan in atherosclerotic coronary intima. In atherosclerotic intima (A through D, bottom), extensive deposits of biglycan (A), apoE (B), and versican (C) are present. Immunostaining for biglycan (A) and apoE (B) reveals striking colocalization of these molecules. In contrast, although occasional areas have colocalized apoE and versican (B and C, right), for the most part, distributions of apoE and versican are different. Also, top portions show a nonatherosclerotic region that does not contain deposits of either biglycan (A) or apoE (B) but that does have a subendothelial accumulation of versican (C). Immunohistochemistry with control, normal rabbit serum (D) shows no specific immunostaining. Magnification $\times 40$; methyl green counterstain.

biglycan, only occasional areas were found in which versican colocalized with apoE, and more typically, versican and apoE had different distributions (Figure 2B versus 2C). Nonintimal proteoglycan distributions were similar in atherosclerotic and nonatherosclerotic arteries, with biglycan present in the adventitia immediately adjacent to the external elastic lamina and versican present in both the media and adventitia.

ApoA-I and ApoB Also Are Present in Biglycan-Containing Regions

The source of apoE present in plaques could be plaque macrophages, which previously have been shown to synthesize apoE.^{12,13} However, several lipoproteins contain apoE, including VLDL and their remnants, as well as a subset of HDL. In addition, *in vitro* studies have demonstrated that dermatan sulfate proteoglycans bind apoB.^{32,33} Thus, to determine whether apolipoproteins other than apoE might also colocalize with biglycan and/or versican deposits, a second series of 20 arterial segments obtained from 8 patients was examined with antibodies or antisera to apoE, apoB, apoA-I, biglycan, and versican.

Although both biglycan and versican were present in atherosclerotic intima, they very often were present in distinctly different distributions (Figure 3C and 3D), which allowed more definitive characterization of the relationships of these proteoglycans to apolipoproteins. Accumulations of biglycan frequently were present in locations devoid of versican, and not only apoE but also apoA-I and apoB often colocalized with biglycan (Figure 3). Occasional areas with apoE and apoA-I accumulation lacked detectable apoB (Figure 3G, top and right), but this finding could be due to several factors, including (1) lower sensitivity for epitope detection of the apoB antibody compared with the apoE and apoA-I antisera, (2) degradation of the epitope of the apoB antibody on trapped LDL, and/or (3) displacement of apoB from intimal LPL by apoE.³⁴

Characterization of Proteoglycans and Apolipoproteins in Areas of Macrophage Infiltration and Necrotic Cores

Areas of necrosis and dense macrophage infiltration contained apolipoproteins but not biglycan and versican, which are components of intact extracellular matrix (Figure 4). However, biglycan immunostaining was particularly prominent in the extracellular matrix immediately adjacent to areas of macrophage infiltration. Versican immunostaining often was present in these regions but was not as extensive as that for biglycan. ApoE, A-I, and B colocalized with biglycan in regions with intact extracellular matrix proteins (as identified by Gomori's trichrome staining), but the apolipoproteins also were present in regions with necrosis and in association with macrophages (Figure 4). The proximity of biglycan and versican to macrophage infiltrates raises the possibility that macrophage-derived cytokines might act in a paracrine fashion to stimulate biglycan and/or versican production by adjacent SMCs.

Distributions of Proteoglycans and Apolipoproteins in Nonatherosclerotic and Atherosclerotic Coronary Segments

To characterize the distribution of proteoglycans and apoE in nonatherosclerotic and atherosclerotic segments, each of the

first set of 68 segments was divided into 4 quadrants.²⁸ Of 256 quadrants suitable for analysis, 98 were classified as nonatherosclerotic and 158 were classified as atherosclerotic.²⁸ Versican deposits were detected in the intima of all nonatherosclerotic quadrants, whereas the prevalences of biglycan and apoE deposits in nonatherosclerotic quadrants were <50% (Figure 5A). In contrast, the vast majority of atherosclerotic quadrants contained deposits of versican, biglycan, and apoE. This analysis suggests that the deposits of biglycan and of apoE are characteristic of atherosclerotic compared with nonatherosclerotic intima. A similar analysis was performed for proteoglycan and apolipoprotein distributions in the second set of 20 atherosclerotic segments. Of 80 quadrants suitable for analysis, 30 were classified as nonatherosclerotic and 50 were classified as atherosclerotic.²⁸ Again, versican deposits were detected in all nonatherosclerotic quadrants, whereas the prevalences of deposits of biglycan and of all 3 apolipoproteins were <50% (Figure 5B). In contrast, the vast majority of atherosclerotic quadrants contained deposits of both proteoglycans and of all 3 apolipoproteins, again suggesting that deposits of biglycan, apoE, apoA-I, and apoB are characteristic of atherosclerotic compared with nonatherosclerotic intima.

In Vitro Analysis of ApoE, ApoA-I, and ApoB Interactions With Biglycan

Binding of HDL to Biglycan Is Mediated by ApoE Rather Than by ApoA-I

The immunohistochemical colocalization of apoE and apoA-I in atherosclerotic plaques could result from high-affinity binding to biglycan of apoE, apoA-I, or both. However, because all HDLs contain apoA-I but only a subset of HDLs also contain apoE, the interaction of apoA-I compared with apoE with biglycan was investigated *in vitro* by use of either HDL₃+E or HDL₃-E in a GMSA.⁴ In the GMSA, proteoglycans that are bound to lipoproteins remain at the origin of the gel, whereas unbound proteoglycans migrate into the gel.³⁵ ³⁵S-labeled biglycan bound to HDL₃+E, as demonstrated by the increasing intensity of the band at the origin of the gel with increasing concentrations of HDL₃+E (Figure 6A). There was no formation of biglycan complexes with HDL₃-E, as demonstrated by migration of all of the radio-labeled biglycan into the gel, despite increasing concentrations of HDL₃-E (Figure 6B). These *in vitro* results indicate that the interaction of biglycan with HDL is mediated by apoE rather than by either apoA-I or apoA-II.

Binding of LDL to Biglycan

The ability of apoB to interact directly with biglycan *in vitro* was investigated to determine whether the colocalization of apoB with biglycan seen in immunohistochemical studies might be due to retention of LDL on biglycan, which would require a direct interaction of apoB and biglycan, or to retention of apoB- and apoE-containing remnant lipoproteins.^{35,36} In the latter case, interaction of remnant lipoproteins with biglycan might be mediated by apoE and not require a specific interaction with apoB. However, ³⁵S-biglycan also bound to LDL (Figure 7). These *in vitro* results confirm that biglycan can interact with apoE-free lipoproteins that contain

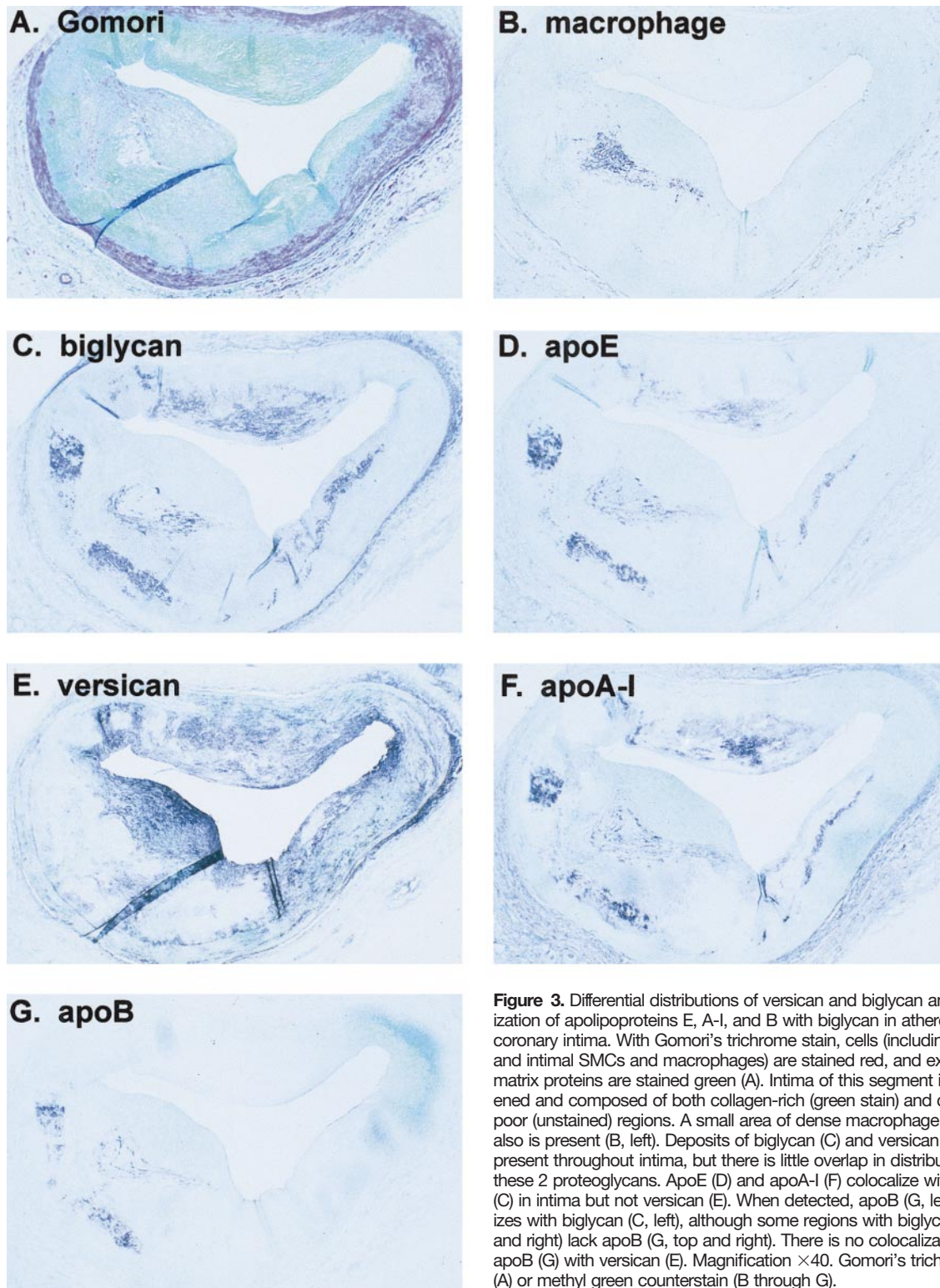


Figure 3. Differential distributions of versican and biglycan and colocalization of apolipoproteins E, A-I, and B with biglycan in atherosclerotic coronary intima. With Gomori's trichrome stain, cells (including medial and intimal SMCs and macrophages) are stained red, and extracellular matrix proteins are stained green (A). Intima of this segment is thickened and composed of both collagen-rich (green stain) and collagen-poor (unstained) regions. A small area of dense macrophage infiltration also is present (B, left). Deposits of biglycan (C) and versican (E) are present throughout intima, but there is little overlap in distributions of these 2 proteoglycans. ApoE (D) and apoA-I (F) colocalize with biglycan (C) in intima but not versican (E). When detected, apoB (G, left) colocalizes with biglycan (C, left), although some regions with biglycan (C, top and right) lack apoB (G, top and right). There is no colocalization of apoB (G) with versican (E). Magnification $\times 40$. Gomori's trichrome stain (A) or methyl green counterstain (B through G).

apoB, ie, LDL. Furthermore, the apoB concentrations in serum and peripheral lymph of normolipidemic controls range from 0.75 to 1.0 mg/dL and 0.06 to 0.08 mg/dL, respectively.³⁷ Thus, the lipoprotein concentrations used in these GMSAs probably can be achieved in interstitial tissues such as the artery wall.

Discussion

Atherosclerotic intima of pigs,^{38,39} pigeons,⁴⁰ and humans^{19,20,41} is enriched in chondroitin sulfate and dermatan sulfate proteoglycans. This study demonstrates that, in human atherosclerotic plaques, regions enriched in the dermatan sulfate proteoglycan biglycan contain apolipoproteins E, A-I, and B.

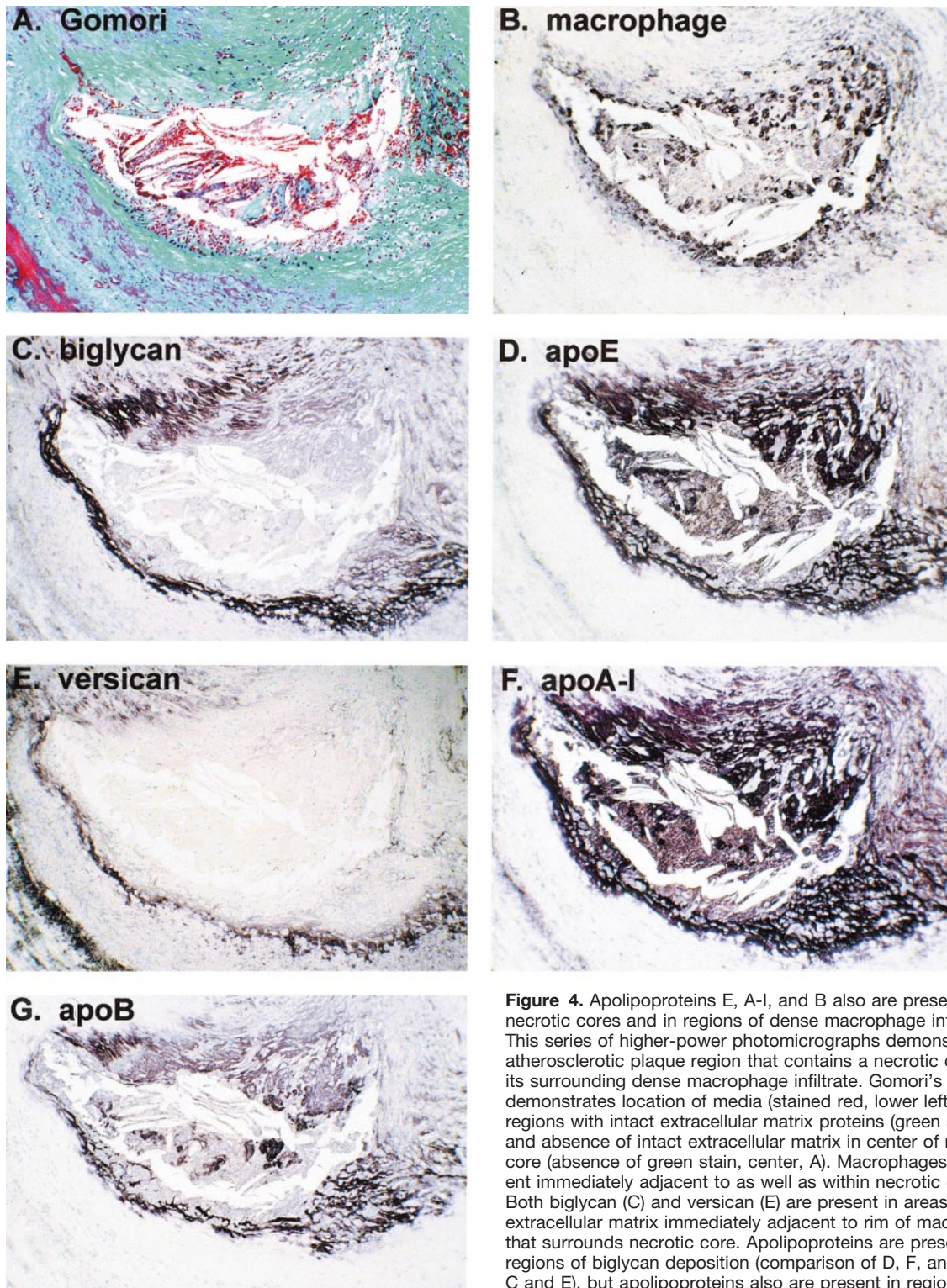


Figure 4. Apolipoproteins E, A-I, and B also are present in necrotic cores and in regions of dense macrophage infiltration. This series of higher-power photomicrographs demonstrates an atherosclerotic plaque region that contains a necrotic core and its surrounding dense macrophage infiltrate. Gomori's stain (A) demonstrates location of media (stained red, lower left, A), regions with intact extracellular matrix proteins (green stain, A), and absence of intact extracellular matrix in center of necrotic core (absence of green stain, center, A). Macrophages are present immediately adjacent to as well as within necrotic core (B). Both biglycan (C) and versican (E) are present in areas of intact extracellular matrix immediately adjacent to rim of macrophages that surrounds necrotic core. Apolipoproteins are present in regions of biglycan deposition (comparison of D, F, and G with C and E), but apolipoproteins also are present in regions that contain biglycan but lack versican (compare upper left and lower right portions of D, F, and G with same regions in C and E). Apolipoproteins (D, F, and G) also are present in regions of frank necrosis (A, center) and dense macrophage infiltration (B), which lack an intact extracellular matrix and therefore lack biglycan (C) and versican (E). Magnification $\times 100$. Gomori's trichrome stain (A) or methyl green counterstain (B through G).

The study also demonstrates, using the *in vitro* GMSA, that biglycan interacts with both apoE and apoB but not with apoA-I or apoA-II. These observations raise the possibility that specific proteoglycans that accumulate in atherosclerosis, especially biglycan, might bind apoE and thereby be partially

responsible for the abundant extracellular apoE deposits also characteristic of this disease.^{12,13} These findings also raise the possibility that apoE acts as a bridging molecule between biglycan and HDL particles, thereby mediating retention of a subset of HDL in plaques. Furthermore, the localization of

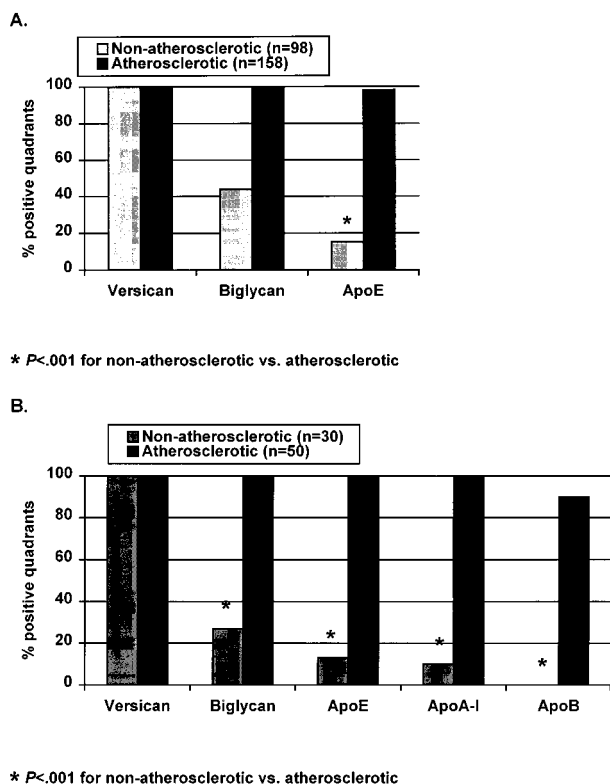


Figure 5. Prevalences of proteoglycan and apolipoprotein deposits in 2 sets of nonatherosclerotic and atherosclerotic plaque quadrants. A, Percentages (from first group of 68 segments) of nonatherosclerotic and atherosclerotic quadrants that contained deposits of versican, biglycan, and apoE. Versican deposits were detected in all nonatherosclerotic and atherosclerotic quadrants. Statistically significant differences in prevalences between nonatherosclerotic and atherosclerotic quadrants were found for biglycan ($\chi^2=105.75$, $P<0.001$) and apoE ($\chi^2=132.88$, $P<0.001$). B, Percentages (from second group of 20 segments) of nonatherosclerotic and atherosclerotic quadrants that contained deposits of versican, biglycan, apoE, apoA-I, and apoB. Versican deposits were detected in all nonatherosclerotic and atherosclerotic quadrants. Significant differences were found between nonatherosclerotic and atherosclerotic quadrants in prevalences of biglycan ($\chi^2=46.96$, $P<0.001$), apoE ($\chi^2=60.31$, $P<0.001$), apoA-I ($\chi^2=63.96$, $P<0.001$), and apoB ($\chi^2=58.11$, $P<0.001$).

apoB along with apoE to biglycan-enriched regions raises the possibility that biglycan also may mediate accumulation of remnant lipoproteins and LDL.

Biglycan is one of several proteoglycans found in human atherosclerotic plaques.^{15,42} Biglycan consists of a 38-kDa core protein with 2 dermatan sulfate side chains.⁴³ An increased content of dermatan sulfate has been demonstrated in both primary and restenotic human atherosclerotic lesions,^{15,18–20} and dermatan sulfate binds avidly to lipoproteins.^{32,33} This interaction is believed to be mediated by electrostatic interaction of the negatively charged dermatan sulfate with the positively charged amino acids of apolipoproteins^{44,45} and is one mechanism by which biglycan and apoE might colocalize in plaques.

Biglycan is synthesized by arterial SMCs,^{46–48} in which exposure to the growth factor TGF- β_1 ^{49,50} leads to upregulation of the synthesis of biglycan mRNA and protein.⁴⁸ Exposure to either TGF- β_1 or PDGF induces elongation of the glycosaminoglycan

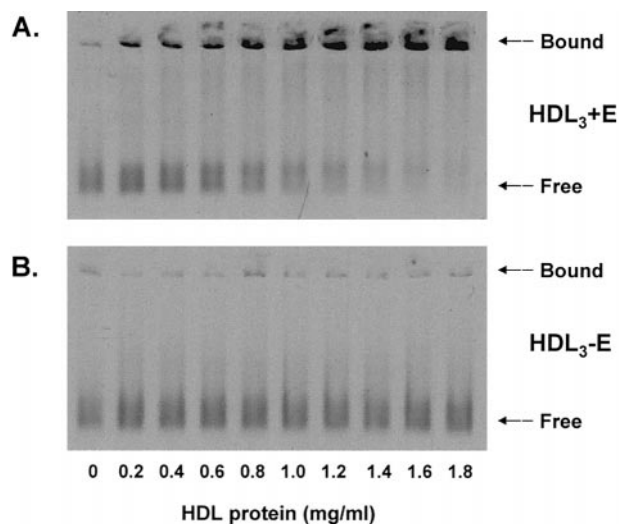


Figure 6. ApoE is required for interaction of biglycan and HDL₃. Autoradiograph of an agarose gel from a GMSA, in which ³⁵S-labeled biglycan (2×10^3 dpm) had been incubated with increasing concentrations (as mg/mL of protein) of either human HDL₃+E (A) or HDL₃-E (B) for 1 hour at 37°C before electrophoresis. Radiolabeled biglycan complexed with HDL₃ is retained at origin. In the absence of an interaction with HDL₃, unbound biglycan migrates into gel and is detected as a band at solvent front. In the presence of increasing concentrations of HDL₃+E, progressively greater amounts of radiolabeled biglycan are retained at origin of gel, indicating formation of complexes between HDL₃+E and biglycan (A). In contrast, despite addition of progressively increasing concentrations of HDL₃-E, biglycan remains free to enter gel (Figure 6B), demonstrating that interaction of HDL₃ with biglycan requires apoE.

chains of biglycan.⁴⁸ Increased glycosaminoglycan chain length is found in proteoglycans extracted from atherosclerosis-susceptible arteries, and increased glycosaminoglycan chain length correlates positively with lipoprotein binding capacity.⁴ Therefore, plaque-

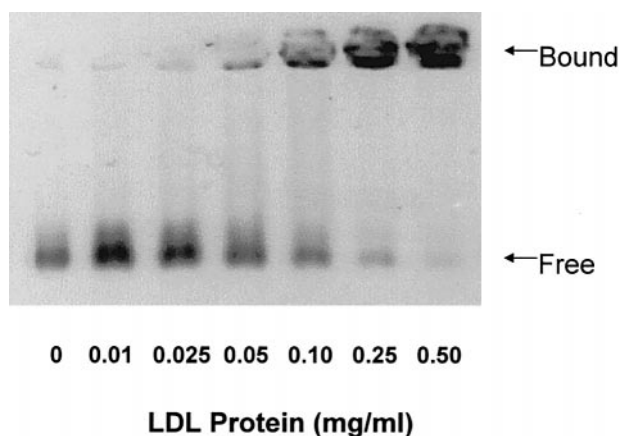


Figure 7. Biglycan interacts with LDL. Autoradiograph of agarose gel from a GMSA, in which ³⁵S-labeled biglycan (2×10^3 dpm) had been incubated with increasing concentrations (as mg/mL of protein) of human LDL for 1 hour at 37°C before electrophoresis. Radiolabeled biglycan that has formed a complex with LDL is retained at origin. In the absence of an interaction between LDL and biglycan, unbound biglycan migrates into gel and is detected as a band at solvent front. In the presence of increasing concentrations of LDL, progressively greater amounts of radiolabeled biglycan are retained at origin of gel, indicating formation of complexes between LDL and biglycan.

associated cytokines such as TGF- β_1 ^{51,52} might facilitate these associations by virtue of increasing the amount of biglycan core protein produced and/or increasing glycosaminoglycan chain length. It also is noteworthy that TGF- β_1 has been shown to stimulate secretion of apoE by macrophages.⁵³ Thus, biglycan and apoE might associate as a consequence of close temporal and spatial secretion by SMCs and macrophages, respectively, in response to a common stimulus (eg, TGF- β_1).

The finding that the chondroitin sulfate proteoglycan versican only occasionally colocalizes with apoE and apoB suggests a degree of specificity for biglycan in mediating apolipoprotein retention. Versican, a large, interstitial chondroitin sulfate proteoglycan synthesized by SMCs,⁵⁴ is present in vascular tissue and is enriched in human atherosclerotic lesions. Recently, a study of balloon-injured rabbit arteries found immunohistochemical colocalization of apoB and chondroitin sulfate in intima immediately below regenerated endothelium but not in noninjured intima.¹⁰ However, the present study demonstrates that apoB localizes predominantly to regions with biglycan rather than versican, suggesting that biglycan may play a major role in the accumulation of apoB in atherosclerotic lesions.

Another surprising observation of this study is that extensive regions of apoA-I immunostaining in atherosclerotic plaques frequently localize to regions of biglycan deposition and apoE accumulation. The observation that apoA-I is present in atherosclerotic plaques confirms the findings of another study¹⁴ and has important implications. First, it demonstrates that lipoprotein retention³ in atherosclerotic extracellular matrix includes not only "atherogenic" lipoproteins, such as LDL and VLDL remnants, but also HDL. Second, it raises the question of how apoA-I retention might be mediated. One possibility is that apoA-I might interact directly with biglycan. However, *in vitro* studies using the GMSA failed to demonstrate an interaction between biglycan and apoE-free HDL, arguing against a direct interaction between apoA-I and biglycan. Instead, these results are more consistent with an interaction between apoE and biglycan, which results in the apparent colocalization of apoA-I through either or both of the following mechanisms: (1) apoE-containing HDL particles interacting with biglycan or (2) non-apoE-containing HDL particles associating with apoE that has been produced locally, eg, by macrophages, and then interacting with biglycan.

Finally, VLDL remnants, which contain both apoE and apoB, have been detected in atherosclerotic plaques.^{35,36} Thus, VLDL remnants are a potential source of some plaque-associated apoE, because this study found that apoB often localized to apoE-containing regions.

In conclusion, this study documents colocalization of biglycan with apoE, apoA-I, and apoB deposits in atherosclerotic plaques. The study also demonstrates that apoA-I and apoB colocalize with biglycan in atherosclerosis. These results suggest that biglycan may trap not only apoE and its associated lipid but also VLDL remnants, LDL, and HDL in atherosclerotic intima.

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