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Selective Deposits of Versican in the Extracellular Matrix of Restenotic Lesions from Human Peripheral Arteries

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Although a large percentage of the volume of human restenotic arterial lesions is occupied by extracellular matrix (ECM), the composition and organization of this ECM are not well characterized. In this study, restenotic segments taken from 30 human peripheral arteries by directional atherectomy at times ranging from 13 days to 36 months after angioplasty were analyzed for specific patterns of ECM composition and organization by light and electron microscopic histochemistry and immunohistochemistry. Histochemical analysis revealed the presence of distinct zones, enriched either in proteoglycans or fibrillar collagen. Most sections contained these regions juxtaposed to each other. The frequency of these two distinct ECMs did not change as a function of time after angioplasty. The collagen-rich zone usually contained elongated smooth muscle cells spaced close together except in regions resembling fibrous plaques. The proteoglycan-rich ECM contained both elongated and stellate-shaped smooth muscle cells randomly arranged and separated by wide distances. This region resembled the loose-connective-tissue-containing myxoid region typical of restenotic lesions. Immunohistochemical analysis of these regions revealed that the proteoglycan-containing zone stained intensely for versican, a large interstitial chondroitin sulfate proteoglycan, whereas the collagen-containing areas were mostly negative for versican but positive for type I collagen. The versican-positive regions also immunostained for biglycan, a small leucine-rich dermatan sulfate proteoglycan, and sparsely for elastin. However, both of these ECM molecules were present in the versican-negative type I collagen-positive regions of the lesions. These results suggest that the development of restenotic lesions involves localized deposits of specific ECM molecules that may play a role in the asymmetric renarrowing of this tissue after angioplasty. ***(Am J Pathol 1997, 151:963–973)***

Restenosis is a term used to describe the renarrowing of blood vessels after vascular lesions have been treated by reconstructive techniques such as endarterectomy, bypass grafting, percutaneous transluminal coronary angioplasty (PTCA) or intravascular stenting.[1–10] Restenosis after PTCA has been identified as a major clinical problem as gradual renarrowing of vessels takes place in the first 6 months after PTCA in 40 to 50% of the patients receiving this treatment, often resulting in multiple subsequent surgeries. Despite the fact that these lesions have been recognized for more than a decade, treatment strategies designed to limit restenosis have been largely unsuccessful.[2–4,6,9,11] These failures no doubt are due, in part, to an incomplete understanding of the biological mechanisms that underlie the formation of these lesions.[12–14]

Studies analyzing the morphology of restenotic lesions after PTCA reveal the presence of fibrocellular tissue containing stellate-shaped smooth muscle cells dispersed in a random fashion surrounded by an ECM the composition of which ranges from loosely arranged collagen fibrils and abundant proteoglycans to dense collagen fibrillar networks.[15–25] Despite the significant contribution of the ECM to restenotic lesion mass, only a few studies have addressed the specific nature and organization of ECM in PTCA-induced restenotic lesions in human peripheral arteries.[23–27]

In an attempt to further define the nature of the ECM in restenotic lesions and to determine whether there are specific compositional and organizational patterns in the ECM that characterize these lesions, we have examined the location and distribution of particular ECM components using light and electron microscopic histochemistry and immunocytochemistry. Our results show that sections from peripheral restenotic vascular lesions retrieved by directional atherectomy contain morphologically dis-

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tinct ECM regions that are juxtaposed to one another and are enriched in either specific types of proteoglycans or collagens fibrils. Such localized deposits of markedly different ECM molecules within these lesions may contribute to the asymmetric characteristic of this vascular lesion renarrowing.

Materials and Methods

Tissues

Tissue segments from 30 patients with peripheral artery disease were retrieved by directional atherectomy using the Simpson Atherocath (Devices for Vascular Intervention, Redwood City, CA) as previously described.[28,29] The tissues were retrieved from peripheral arteries previously treated by balloon angioplasty and were therefore classified as restenotic. Specimens were grouped according to time elapsed after angioplasty: 3 months or less, 6 to 12 months, more than 12 months.

Tissue Processing

For light microscopic histochemistry and immunohistochemistry, specimens were fixed immediately after retrieval for 2 hours in one of the following fixatives: ice-cold 4% paraformaldehyde, 100% methanol, or 10% formalin. Tissues were embedded in paraffin and cut into 5-μm sections. All other tissue for immunocytochemistry and electron microscopy were placed immediately in fixative consisting of 3% paraformaldehyde, 0.25 mol/L glutaraldehyde in phosphate-buffered saline (PBS) at 4°C for 3 days to 1 week.

Histochemistry and Immunohistochemistry

Light Microscopy

Representative paraffin sections were stained for collagen and proteoglycans using a modification of the Movat's stain.[30] The modification employs a saffron/alcian blue combination such that collagen-containing areas stain yellow, proteoglycan-containing areas stain blue, and regions that contain both sets of macromolecules stain green. In addition, sections were also stained with Massons trichrome,[31] which distinguishes collagen-containing areas from elastin- and proteoglycan-containing regions. In specimens embedded for electron microscopy, thick sections were cut from epoxy resin and stained with 1% toluidine blue, which is a general stain to distinguish cells from ECM. For immunocytochemistry, a variety of antisera directed against different ECM components were used. To demonstrate the distribution of

Table 1. Occurrence of Collagen and Proteoglycans in Sections Taken from Patients with Restenotic Lesions at Various Times after Angioplasty

Patient	Time interval	Collagen* (dense connective tissue)	Proteoglycan† (loose connective tissue)	Collagen/Proteoglycan‡
1	13 days	+	+	+
2	3 months	+	+	−
3	3 months	+	−	+
4	5 months	+	+	+
5	6 months	+	+	+
6	6 months	+	+	−
7	6 months	+	−	−
8	6 months	+	+	+
9	6 months	+	+	+
10	7 months	+	+	+
11	7 months	+	+	+
12	7 months	+	+	+
13	8 months	+	+	+
14	8 months	+	+	+
15	8 months	+	+	−
16	10 months	+	+	+
17	11 months	+	+	+
18	12 months	+	+	+
19	12 months	+	+	+
20	12 months	+	+	+
21	12 months	+	+	+
22	12 months	+	+	+
23	14 months	+	+	−
24	14 months	+	+	+
25	16 months	+	+	+
26	16 months	+	−	−
27	16 months	+	−	−
28	16 months	+	−	+
29	23 months	+	+	+
30	36 months	+	+	−

* At least one section on slide stained primarily for collagen.
† At least one section on slide stained primarily for proteoglycan.
‡ At least one section on slide contained collagen and proteoglycan region juxtaposed to each other.

versican, several different antisera were raised against different domains of human recombinant versican core protein and affinity purified on a versican fusion protein-Sepharose column.[32] The fusion proteins correspond to three different regions of the core protein: VC-1 (59 to 348), VC-E (383 to 408), and VC-3 (1815 to 2036). All of these antisera were used at a dilution of 1:800. Controls consisted of substitution of primary antiserum with PBS or with isotype-matched, irrevelant polyclonal antibodies or normal rabbit serum. To confirm the abundance of chondroitin sulfate proteoglycan in these lesions, we used a monoclonal antibody developed against vascular proteoglycans that recognizes chondroitin sulfate (CS) chains attached to versican.[33,34] This antiserum was used at a dilution of 1:200. Sections pretreated with 0.2 U/ml chondroitin ABC lyase for 1 hour before application of the antisera served as negative controls.[33] Antisera against biglycan (LF51) was the generous gift of Dr. L. Fisher (National Institute of Dental Research, Bethesda, MD). This polyclonal antisera, raised against a specific synthetic peptide in rabbits, has been previously described and shown to be monospecific for biglycan.[35] To confirm specificity of the biglycan antisera, the antisera were incubated with excess biglycan peptide used to raise the antisera[23,35] before application to the tissue sections. The antisera were used at a dilution of 1:1000. The distribution of elastin in these tissues was examined using a 1:1000 dilution of a polyclonal antisera raised against human aortic elastin (HAE-2) generously contributed by Dr. R. Mecham, Washington University, St. Louis, MO. The collagen type I antiserum was raised in the rat against human gingival type I collagen and kindly provided by Dr. Sampath Narayanan, Department of Pathology, University of Washington. This antiserum shows no cross-reactivity with types III or IV collagens and was used at a dilution of 1:200.[36] Smooth muscle actin was recognized by a monoclonal antibody clone 1A4 (Sigma Chemical Co., St. Louis, MO) at a dilution of 1:1000.

For immunocytochemistry, sections taken from either paraffin or epoxy resin blocks were used. After removal of paraffin or epoxy resin using a modification of procedures described by Mar and Wight,[37,38] the sections were treated with 0.3% hydrogen peroxide in absolute methanol to quench endogenous peroxidase, hydrated, and treated with a mixture of 10% normal goat serum and 1% bovine serum albumin (BSA) in PBS to block nonspecific interactions. Specific antibodies diluted with BSA/PBS were applied and incubated overnight at 4°C. The sections were then washed with PBS and incubated for 1 hour with a biotinylated secondary antibody, followed by streptavidin-conjugated horseradish peroxidase (Zymed, San Francisco, CA) diluted 1:200 in PBS. The color was developed with diaminobenzidine/hydrogen peroxide for 10 minutes at room temperature. The sections were then counterstained with hematoxylin and eosin and mounted for examination.

Electron Microscopy

For histochemistry, tissue was fixed in half-strength Karnovsky's fixative[39] in 0.1 mol/L sodium cacodylate in the presence of 0.2% ruthenium red (Johnson-Matthey Co., West Hill, MA), as described,[40] overnight at 4°C. After rinsing with 0.1% ruthenium red in 0.1 mol/L cacodylate buffer, the tissue was post-fixed in 1% osmium tetroxide containing 0.05% ruthenium red in cacodylate buffer and processed routinely for electron microscopy with final embedment in the epoxy resin Medcast (Ted Pella, Redding, CA) following the vendor's instructions.

Tissue for immunocytochemistry was rinsed in PBS with several changes after initial fixation, dehydrated through graded ethanol and stained *en bloc* with 3% uranyl acetate for 1 hour. Portions of each tissue were further processed in the acrylic resin LR White[41] for polymerization at 50°C. Thin sections were mounted on formvar-coated/reverse carbon-coated 200-mesh nickel grids and immunostained with primary antisera overnight at 4°C. After several rinses with PBS, the sections were immunostained with 10-nm gold-conjugated secondary antisera (Polysciences, Warrington, PA) diluted 1:50 in PBS for 2 hours at room temperature. After rinsing, the sections were fixed in 3% glutaraldehyde in PBS for 10 minutes, post-fixed with 2% osmium tetroxide vapors for 1 hour, and stained with uranyl acetate and lead citrate.

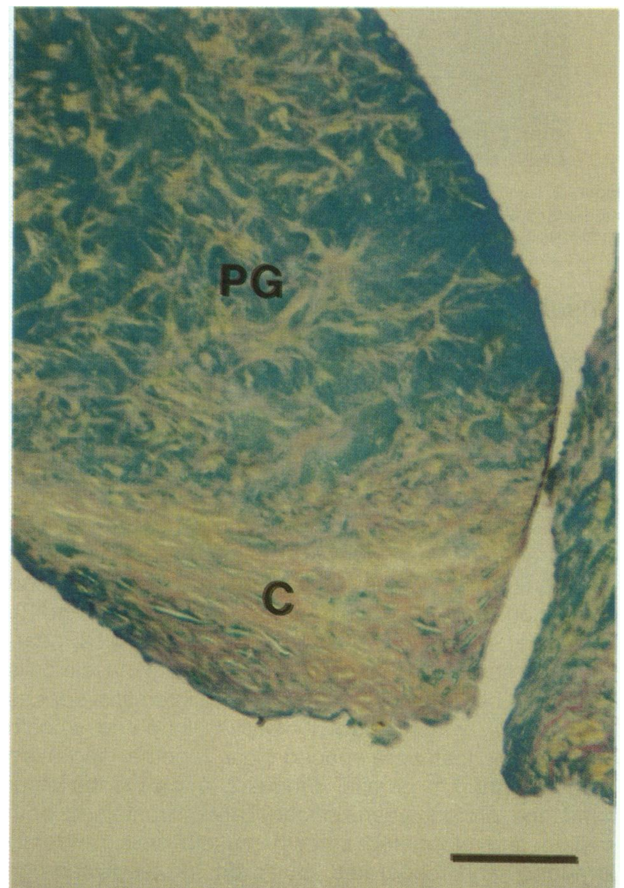

Figure 1. Light micrograph taken from a paraffin section of a human peripheral restenotic lesion stained with a modification of the Movat's stain.[30] This stain distinguishes proteoglycan-rich ECM (PG) as blue and collagen-containing ECM (C) as yellow. This section demonstrates that different regions of these lesions are composed of strikingly different ECMs juxtaposed to each other. Bar, 200 μm.

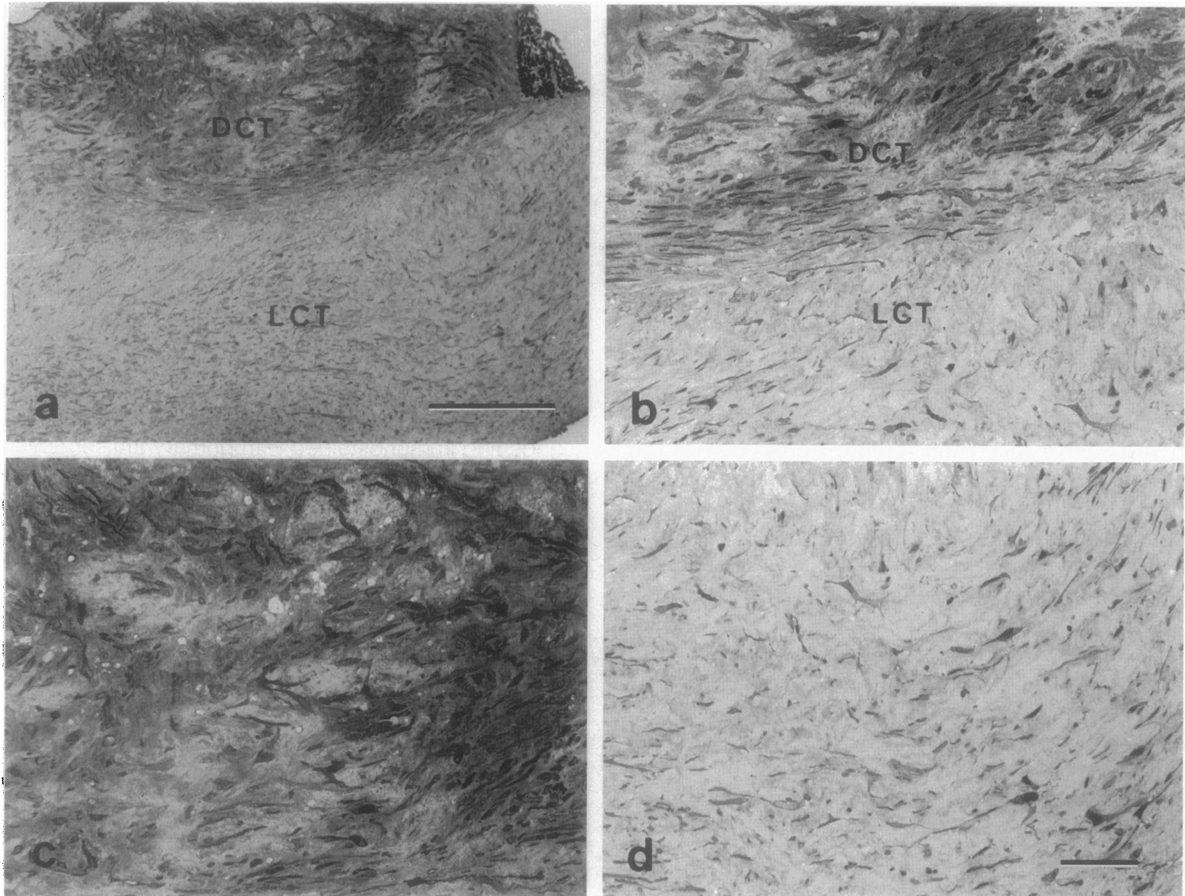

Figure 2. Light micrographs taken from 1-μm sections of human peripheral restenotic lesions at 6 months after angioplasty. **a:** Low magnification illustrating two morphologically distinct zones adjacent to one another. **b** to **d:** Higher magnifications illustrating that one zone is composed of loose connective tissue (LCT) with wide spaces between cells whereas the other zone consists of dense connective tissue (DCT) with cells closer together. Bar, 200 μm (**a**) and 50 μm (**b** to **d**).

Results

Light Microscopy

Tissue sections taken from 30 patients at different times after angioplasty were assessed for connective tissue involvement utilizing histochemical stains that distinguished collagen-containing areas from proteoglycan-rich areas. The results indicate that most of the sections contained regions enriched in either proteoglycans or collagen (Table 1). Many of the sections contained these two regions juxtaposed to one another (Figures 1 and 2). The collagen-containing areas either had the appearance of a fibrous cap with elongated smooth muscle cells separated by wide collagen-containing interstitial spaces (Figure 1)[42] or contained numerous elongated smooth muscle cells that were spaced close together and more or less aligned in parallel (Figure 2, a–c). On the other hand, the proteoglycan-enriched areas contained both stellate and elongated smooth muscle cells randomly arranged and separated by wide proteoglycan-rich spaces (Figures 1 and 2, a, b, and d).[43] There was no difference in the incidence of these two regions as a function of time after angioplasty (Table 1).

Immunohistochemical analyses of these sections with various antibodies to different ECM components revealed distinct patterns of ECM organization. Some entire sections or parts of sections stained intensely for versican. Versican-positive areas were consistently present in the areas surrounding smooth muscle cells (Figure 3, a and b). Immunostaining of versican in these areas revealed intense staining throughout the ECM. Similar results were found using VC-E, VC-1, and VC-3 antisera, which recognize different epitopes within the versican molecule.[32] To confirm the CS-rich nature of the versican-rich region, we used another antisera against CS chains and found this region to strongly stain with this antisera as well (Figure 3c). Predigestion of the sections with chondroitin ABC lyase before antisera incubation completely abolished staining, indicating the specifity of the CS antisera (Figure 3d). In addition, the versican-positive areas stained in a filamentous pattern for biglycan (Figure 3e), another proteoglycan, and sparsely for elastin (Figure 3f).

A consistent feature of these specimens was the presence of a region that did not immunostain with versican antisera adjacent to the versican-positive regions (Figure 3a, arrow). The versican-negative region, however, immunostained for biglycan and elastin in a pattern similar to that observed for these ECM macromolecules in the versican-positive zones (Figure 3, e and f). Thus, biglycan and elastin also appeared to be present in both

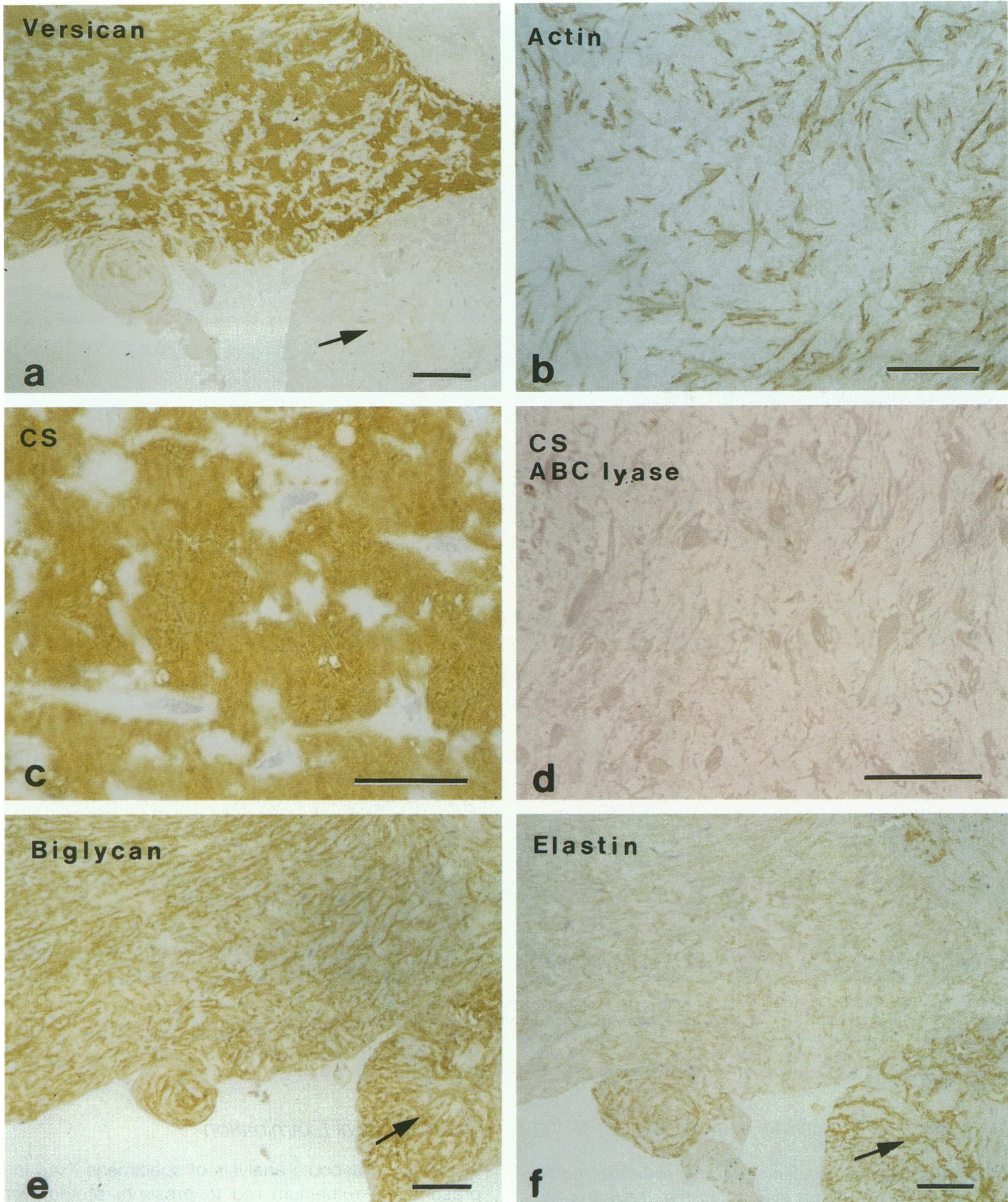

Figure 3. Immunohistochemical analysis of sections taken from human restenotic peripheral arteries. **a:** This section was immunostained with antisera to versican demonstrating that parts of the lesions are enriched in versican whereas other parts (**arrow**) do not immunostain with this antiserum. Bar, 50 μm. **b:** This section was taken from the versican-positive region of the lesion and immunostained with antisera to smooth muscle cell actin. This result demonstrates that the versican-positive zones are enriched in smooth muscle cells that are randomly arranged and separated by wide extracellular spaces. Bar, 50 μm. **c:** A section from the smooth-muscle-cell-enriched portion of the lesion immunostained with a monoclonal antibody to chondroitin sulfate glycosaminoglycan chains confirming the chondroitin sulfate (CS)-rich nature of this part of the lesion. Bar, 25 μm. **d:** A similar section as shown in **c** except that the section was pretreated with chondroitin ABC lyase before immunostaining with the chondroitin sulfate antisera. Absence of immunoreactivity indicates specificity of the chondroitin sulfate antisera. Bar, 25 μm. **e:** This is a section adjacent to the section shown in **a** immunostained with antisera to biglycan. Note the filamentous pattern of staining and the positive staining in both the versican-positive and -negative regions (**arrow**) of the lesion. Bar, 50 μm. **f:** Another adjacent section to those shown in **a** and **e** immunostained with antisera to elastin. There is sparse staining for elastin in the versican-positive regions and more intense elastin staining in the versican-negative regions. Bar, 50 μm.

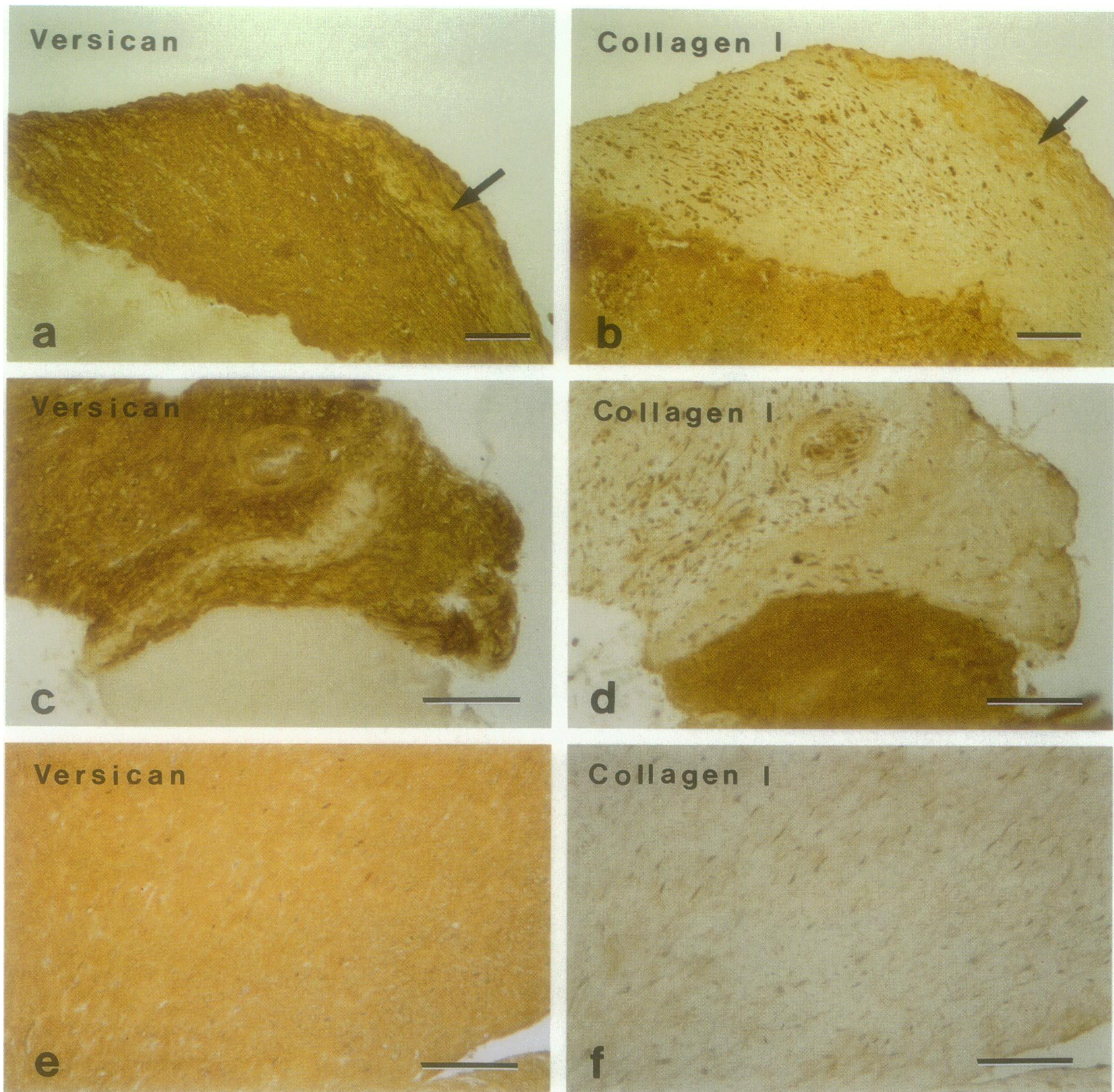

Figure 4. Immunohistochemical analysis of sections from human peripheral restenotic arteries treated with antisera to versican (**a**, **c**, and **e**) and type I collagen (**b**, **d**, and **f**). **a** and **b** Adjacent sections illustrating the reciprocal pattern of staining with these two antisera. The **arrow** denotes a region of the lesion that immunostained partially with both antisera. **c** and **d**: Another region of the lesion in which one portion immunostains strongly for versican and faintly for type I collagen whereas an adjacent portion of the lesion immunostains in a reverse pattern with these two antisera. **e** and **f**: Sections taken from the myxoid regions of the lesions indicating that these regions are enriched in versican but not in type I collagen. All bars, 100 μm.

versican-positive and versican-negative regions of the lesions.

Unlike biglycan and elastin, immunostaining for collagen type I revealed strong staining in the versican-negative region and little to no staining in the versican-positive region of the sections (Figure 4, a–f). In fact, there appeared to be reciprocal patterns in the staining for these two ECM macromolecules. The one exception to this reciprocal relationship was in those regions of the sections resembling fibrous caps, which tended to immunostain for both collagen I and versican (Figure 4, a and b, arrow). The myxoid regions of the lesions stained intensely for versican with little to no staining for collagen type I (Figure 4, e and f).

Ultrastructural Examination

Electron microscopic analysis of specimens fixed in the presence of ruthenium red to preserve proteoglycans revealed that some regions of the ECM consisted mainly of wavy and interdispersed collagen fibrils, few elastin fragments, and few if any ruthenium-red-positive granules (Figures 5a and 6a). When present, small ruthenium red granules were present only on the collagen fibrils (Figure 6a). Small electron-dense inclusions, some resembling matrix vesicles, were present in these regions as well as were occasional calcium appetite crystals (Figures 5a and 6a). This organization typified the dense connective tissue zones found within these lesions. On

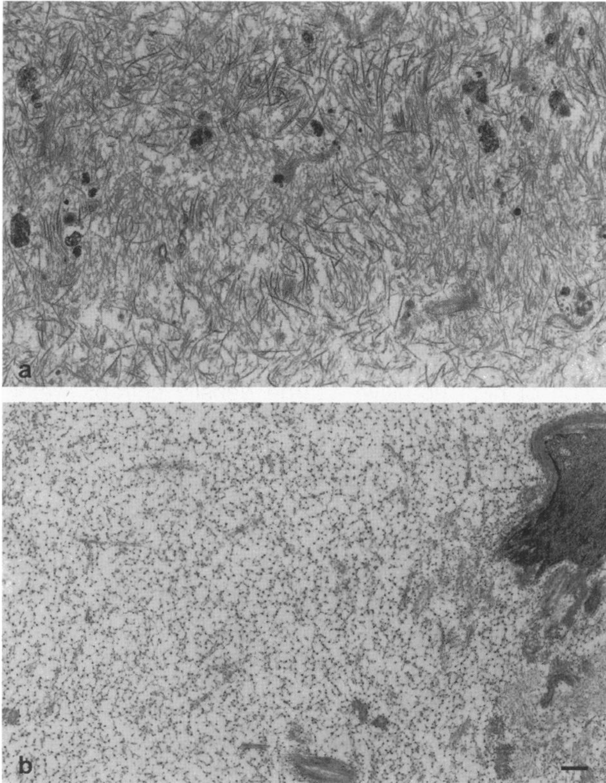

Figure 5. Electron micrographs of equal magnification of the ECM found in the two morphologically distinct regions of the peripheral lesions. Abundant but randomly organized collagen fibrils fill one region of the ECM (**a**) whereas ruthenium-red-positive proteoglycan-containing granules were numerous throughout other regions of the ECM (**b**). Bar, 0.5 μm.

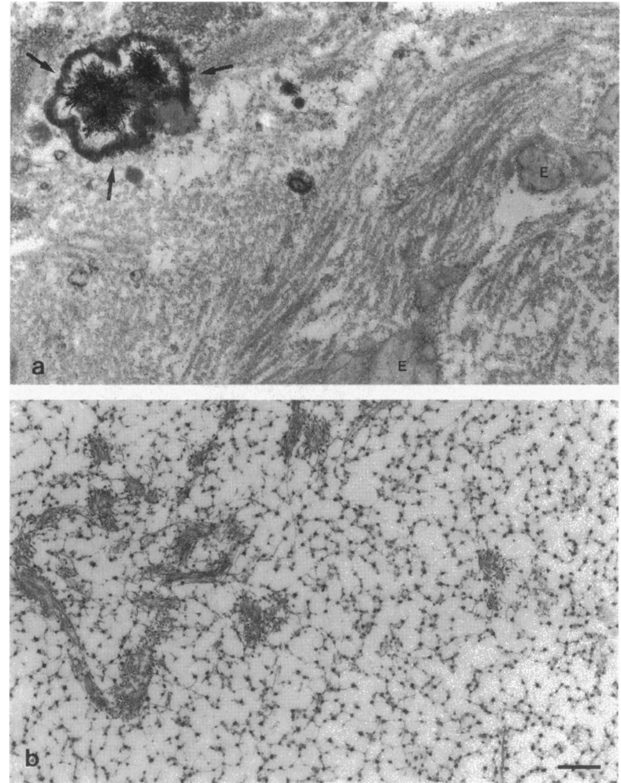

Figure 6. High-magnification electron micrographs of equal magnification of these two ECM zones. Frequently, calcium deposits (**arrow**) are present in the dense zones closely associated with collagen fibrils whereas ruthenium-red-positive granules linked together by fine filamentous threads and segments of immature elastic fibers dominate the loose ECM zone. Bar, 0.4 μm.

the other hand, the other areas of the ECM contained numerous ruthenium-red-positive granules, often connected by thin filamentous threads, occasional immature elastic fiber-forming networks, and few if any collagen fibrils (Figures 5b and 6b). The ruthenium-red-positive granular network clearly was the predominant structural feature of the wide extracellular spaces characteristic of the myxoid and loose connective tissue regions in these lesions.

Immunoelectron microscopy using antisera to versican revealed positive staining of short filamentous structures and granules throughout the ECM in regions separated by wide intercellular distances (Figure 7). Immunogold labeling using antisera against elastin revealed that there were few immature elastin fragments in the versican-enriched ECMs (Figure 8).

Discussion

The results of this study have identified topographically distinct ECM-containing regions within segments of human peripheral restenotic lesions taken from patients at various time points up to 36 months after surgery. These juxtaposed regions are distinguished by either their collagen or proteoglycan content as well as the patterns of smooth muscle organization and phenotype. Abundant collagen fibrils fill the ECM surrounding elongated smooth muscle cells in some regions in a pattern reminiscent of those cells found in the fibrous caps of advanced primary atherosclerotic lesions.[42] On the other hand, the proteoglycan-rich region contained randomly arranged, stellate-shaped smooth muscle cells separated by wide ECM spaces. We found that these cells possessed the synthetic phenotype (data not shown)[44] in agreement with recent studies by Bauriedel et al.[45] It may be that regions of the lesion that contain this type of matrix represent regions of actively proliferating smooth muscle cells.[43] The preponderance of proteoglycans and the paucity of collagen and elastic fibers in this region creates a loosely formed and open matrix and the myxoid appearance that is characteristic of human restenotic lesions.[17,19,22,23] These areas resemble also the proteoglycan-rich regions of intimal hyperplasias that characterize early experimental lesions in animals and diffuse intimal thickenings in humans. It may be that the proteoglycan-rich ECM represents a newly formed lesion on the top of an older primary lesion enriched in type I collagen.

The presence of morphologically distinct ECM-containing zones was a consistent finding in sections taken from all 30 patients, and their occurrence did not vary significantly with time after angioplasty. These data suggest that the time course for the casade of biological events that culminates in flow-limiting luminal renarrowing after angioplasty varies on an individual basis. Previous studies[46] have failed to correlate gene expression with the time interval between original intervention and clinical

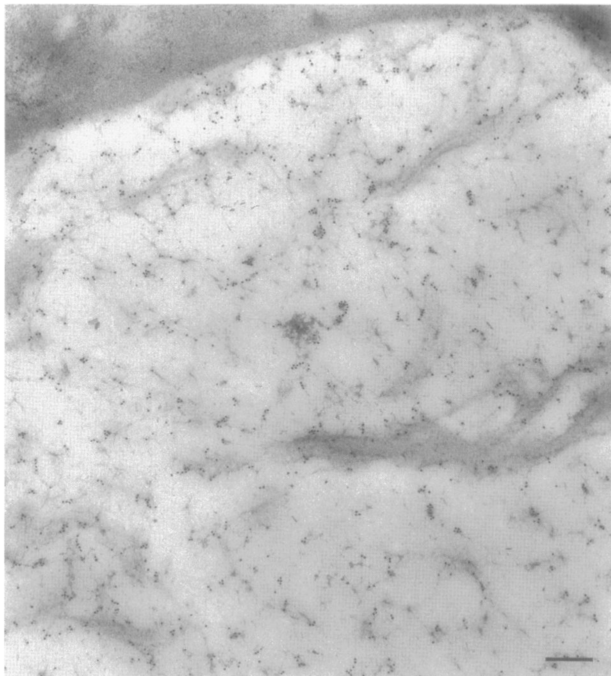

Figure 7. An electron micrograph of a section immunostained with antisera to versican and a secondary antisera conjugated with colloidal gold. Colloidal gold-positive immunoreactivity is present throughout the loose myxoid ECM. A portion of a smooth muscle cell is present in the upper left hand corner and top of the micrograph. Bar, 0.3 μm.

evidence of human restenosis. The experimental animal model in which tissue can be arbitrarily retrieved at predetermined time points after angioplasty would be expected to define the time course of gene or protein expression for cytokines and provisional matrix proteins. Such studies indicate that proteoglycans accumulate early in intimal lesions followed by gradual increases in collagen composition.[25] In contrast, histological studies of restenosis in patients is typically limited to a single time point, namely, when clinical symptoms redevelop, thus signaling maximal lesion growth. Specimens obtained from a number of individuals at 1, 3, 6, 12, 18, and 24 months are not, therefore, representative of evolutionary time points in lesion development but instead simply depict lesion composition at the endpoint of restenosis for each individual patient.

Restenotic lesions differ from primary atherosclerotic lesions in that they contain predominantly smooth muscle cells, few macrophages or other inflammatory cells, and generally no foam cells or lipid debris.[47] Additional differences between these two lesions can be found in the composition and organization of the ECM. Whereas primary advanced atherosclerotic plaques contain dense ECMs composed primarily of type I collagen (for review see Ref. 25), restenotic lesions contain ECMs that vary from regions of sparse type I and type III collagens to areas with densely packed type I collagen.[2,4,19,23,24,26,27,48] There are also differences in specific proteoglycans of the ECM between the two types of lesions. Decorin, a small leucine-rich proteoglycan, is absent from the loose myxoid ECM regions of restenotic lesions but present throughout regions of primary atherosclerotic plaques associated with large collagen deposits.[23,49] On the other hand, the closely related proteoglycan biglycan is present throughout the ECM of both types of lesions.

Although differences exist in the presence of decorin and biglycan between the two types of lesions, versican is present in both primary[49–51] and restenotic lesions but confined to specific topographical regions. These regions are characterized by abundant but randomly arranged smooth muscle cells and a loose and myxoid-appearing ECM. Versican is a large macromolecule that contains a core glycoprotein ranging in size from 400 to 500 kd to which are covalently attached 15 to 17 anionic CS glycosaminoglycan chains.[52] This structural organization of high negative charge density entraps water, thus creating large hydrodynamic domains and a swelling pressure that is offset by the inextensible collagen fibrillar network that, under normal circumstances, forms the fabric of the ECM.[52] However, if collagen fibrils are absent, reduced, or randomly organized as observed in the myxoid regions of the restenotic lesions, this region may be prone to swelling due to the proteoglycan-rich, negative charged nature of the ECM. In fact, a number of studies have shown that the extent of tissue swelling directly depends on glycosaminoglycan concentration.[53–55] In addition, versican interacts with hyaluronan, a large polyanionic, flexible, glycosaminoglycan polymer that also entraps water[56] and is found in high amounts in human restenotic lesions.[24] Thus, the interaction of these two molecules may fill large volumes of watery space in regions of the restenotic lesions. These findings suggest that at least a portion of the restenotic lesion volume may indeed be due to water. For example, tissues undergoing

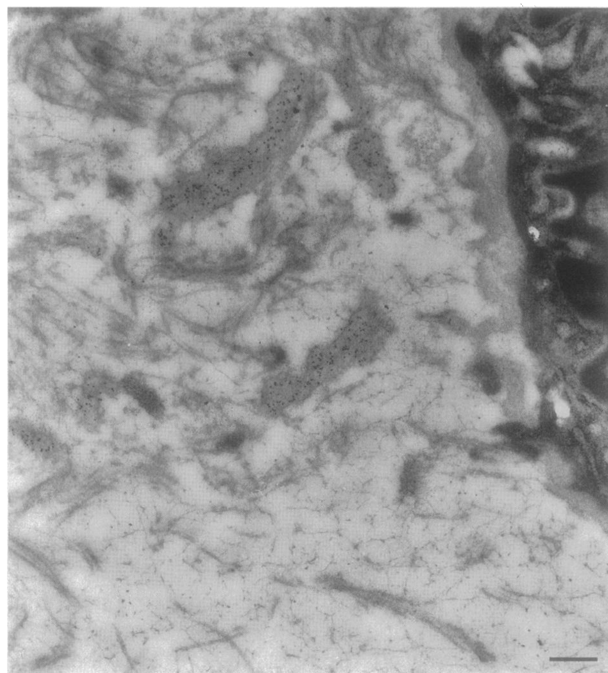

Figure 8. An electron micrograph of a section similar to that shown in Figure 7 immunostained with antisera to elastin and secondary antisera conjugated with colloidal gold. Positive immunogold staining was confined to immature elastic fibers that are frequently present in low abundance in the versican-positive region of the ECM. Bar, 0.4 μm.

rapid expansion due to water influx such as occurs during development[57] and follicular expansion in oogenesis[58] are enriched in hyaluronan.

A major source of the proteoglycans and other ECM molecules in restenotic lesions appears to be the smooth muscle cell. *In vitro* studies indicate that the synthesis of proteoglycans by arterial smooth muscle cells are differentially regulated by cytokines and growth factors (reviewed in Ref. 25). For example, transforming growth factor (TGF)-β1 increases versican and biglycan synthesis by cultured smooth muscle cells.[59–61] TGF-β1 expression is elevated in human restenotic lesions compared with primary lesions[62] and increased after experimental balloon angioplasty injury in animals.[63] Furthermore, antibodies to TGF-β1 block versican accumulation in injury-induced neointimas and reduce intimal thickenings in experimental animals, emphasizing the importance of this ECM component in lesion development.[64]

The large amount of versican/hyaluronan in restenotic lesions suggests that the appearance of these macromolecules may be an early response to the injury created by the trauma of PTCA. For example, the accumulation of hyaluronan and proteoglycans in the ECM is an early response in dermal wounding.[65,66] Proteoglycans and hyaluronan are thought to provide a provisional matrix into which cells will migrate and proliferate to heal the wound. Proteoglycans/hyaluronan are well known for their ability to promote cell migration and proliferation.[67] Wounds heal by replacement of this proteoglycan/hyaluronan matrix by a more dense and more highly cross-linked ECM characterized by increased collagen deposits and other fibrous proteins that operate in wound closure. The finding of large areas in restenotic lesions occupied by provisional matrix components with little to no collagen involvement suggests that these regions are not remodeled and resemble a wound matrix that does not heal.[68,69] Such regions would be prone to swell and could lead to lesion expansion and lumen occlusion. However, it may be that these regions represent foci of increased cellular proliferative activity whereas other areas, once enriched in versican and hyaluronan, have been converted to a scar. This conversion may involve the waterlogged ECM becoming a cicatrix that shrinks and contracts the arterial wall causing loss of lumen diameter. Whether restenotic lesions are wounds that do not heal or wounds in different phases of healing remains to be determined.

In summary, there have been a number of different strategies developed over the years for preventing accelerated atherosclerosis associated with PCTA, most of them focusing on limiting the vascular injury and reducing thrombosis and the proliferative cellular response. Surprisingly, these strategies have failed to account for the massive increase in specific components of the ECM that results from the injury created by the surgery. The results of the present study suggest that specific proteoglycans of the ECM may be partially responsible for restenotic lesion progression.

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