Studies on the Histogenesis of Myxomatous Tissue of Human Coronary Lesions

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Abstract—Myxomatous tissue is a characteristic component of human coronary artery lesions, found more often in restenotic lesions. It represents a bulky accumulation of stellate-shaped cells of unknown histogenesis that are embedded in a loose stroma. We analyzed 64 atherectomy specimens containing substantial amounts of myxomatous tissue by using immunohistochemistry, in situ hybridization, and electron microscopy techniques. Stellate cells represented a heterogeneous population, sharing features of smooth muscle cells (SMCs), macrophages, as well as antigen-presenting dendritic cells. Like quiescent medial SMCs, the stellate cells in all specimens expressed high levels of SM α-actin message and protein and showed heterogeneity with respect to heavy-chain myosin, SM22, desmin, and vimentin. Ultrastructurally, stellate cells resembled SMCs, with some peculiarities that distinguish them from both differentiated and dedifferentiated SMCs. In contrast to quiescent SMCs, the stellate cells expressed high levels of acidic fibroblast growth factor mRNA and protein similar to cells of monocyte/macrophage lineage. However, stellate cells did not express the marker of mature macrophages, HAM56, and were heterogeneous with respect to CD68. Moreover, unlike SMCs, the stellate cells bore some of the major phenotypic markers of dendritic cells: they were S100-positive and showed various reactivity with respect to CD1a and human leukocyte antigen (HLA)-DR. Invasion of myxomatous tissue with CD45RO-positive T lymphocytes was correlated with strong expression of CD1a in these specimens. Stellate cells also expressed a pericyte marker, high-molecular-weight melanoma-associated antigen. We conclude that stellate cells of myxomatous tissue represent a specific phenotype of mesenchymal cells (possibly pericytes), which is activated to express some markers of antigen-presenting cells. These findings suggest involvement of the stellate cells in a local immune response. (Arterioscler Thromb Vasc Biol. 1999;19:83-97.)

Key Words: atherosclerosis ■ angioplasty ■ smooth muscle cell phenotype ■ dendritic cells ■ pericytes

Proliferation of smooth muscle cells (SMCs) and accumulation of extracellular matrix (ECM) are generally viewed as the main mechanisms leading to intimal hyperplasia, which plays a role in atherogenesis and even more so in restenosis after angioplasty and stenting.1 Cellular components of intimal hyperplasia include phenotypically altered SMCs and activated macrophages,1 but many cells in human lesions do not fit this simple classification. An important characteristic component of human intimal hyperplasia is the so-called myxomatous tissue, also named “loose neointima” and “fibroproliferative tissue.” It is defined as regions of moderate cellularity in which stellate-shaped cells are dispersed in a random fashion in abundant ECM, or “myxoid” stroma, composed mainly of proteoglycans and sparse, loosely arranged collagen fibrils.2–7 Myxomatous tissue comprises large parts of restenotic lesions of human coronary arteries after angioplasty, but it is also present in atherectomy specimens from primary lesions.4,5,9,9 The matrix of the myxomatous tissue is positive for the proteoglycans versican and biglycan, and its pattern of unique collagen staining suggests a collagen type I and type III fiber network.6,7,10 Because many cells in intimal hyperplasia are positive for SMC-specific α-actin, desmin, and vimentin,8,9,11 stellate cells are thought to be typical SMCs. However, despite its unique morphology, in most analyses of atherectomy specimens the myxomatous tissue is not distinguished from other types of intimal hyperplasia,4,5,8,9,12 and the nature of stellate cells remains unclear.

Besides SMCs, immunohistochemical analysis shows significant accumulation of macrophages and T lymphocytes in the regions of intimal hyperplasia.11–15 These cells are the local source of multiple cytokines, which affect SMC proliferation and ECM production in the lesion.1,16 In this respect, it is interesting that cells of stellate morphology can be found in the regions of organizing thrombus, in the peripheral vasculature, and especially in the atrium, ie, in the areas usually invaded by blood-borne and endothelial cells.2,17,18 Therefore, an alternative source of stellate cells could be macrophage/monocyte–related cells that could invade...
thrombi or inflammatory sites and acquire a stellate morphology. Obvious candidates are migrating, star-shaped dendritic cells (DCs) that arise from bone marrow progenitors and share a common ancestor with monocytes/macrophages. They are widely distributed in the skin epidermis (Langerhans cells [LCs]), the cardiovascular system (interstitial DCs), the cortical zones of lymph nodes (interdigitating follicular cells), and also in the peripheral blood. DCs express a number of unique markers that distinguish them from regular monocytes/macrophages. DCs function as antigen-presenting cells and are involved in various immune responses, such as migration to draining lymph nodes, activation of T lymphocytes, and presentation of antigens to B lymphocytes.

Another alternative source of origin of stellate cells could be mesenchymal cells with temporary differentiation as pericytes or myofibroblasts. There are reports that migrating adventitial myofibroblasts may contribute to the process of lesion formation after balloon overstretch injury in a swine model. Studies in the rat model suggest that pericytes could be a normal component of the arterial media.

In the present study, focusing on the myxomatous tissue only and applying various complementing technical approaches, we made an attempt to elucidate the origin of stellate cells in human coronary lesions. On the basis of results of immunohistochemistry, in situ hybridization, and electron microscopy, we found that stellate cells, although expressing all SM markers tested, in several respects differ from both quiescent medial SMCs and phenotypically altered SMCs of intimal hyperplasia. We demonstrate that in addition to SMC markers, they express some of the antigens that are considered typical for macrophages and DCs. Although it is difficult to unequivocally attribute stellate cells to 1 single lineage, we conclude that stellate cells represent a specific functional phenotype of mesenchymal cells that may be related to pericytes/myofibroblasts, with a possibly important function of antigen presentation.

### Methods

#### Tissue Processing and Characterization

Coronary atherectomy specimens were obtained from 64 patients (mean age, 64 ± 11 years) who underwent conventional directional coronary atherectomy at the Washington Hospital Center in 1993 to 1995. Specimens were flushed from the instrument chamber with saline solution and fixed immediately with 10% buffered formalin for 1 hour, followed by immersion in 70% ethanol. After dehydra-

#### Control Tissues

To compare stellate cells with the contractile phenotype of SMCs, we used the medial layer of human aortas (3 different individuals); ie, the tissue composed of the cells that by definition are quiescent SMCs (according to their anatomic position within the internal and external elastic laminae, spindle shape, close parallel arrangement, and separation by continuous elastic laminae and collagen fibers).

These intact comparison specimens also displayed a typical complicated atherosclerotic plaque with a sclerotic core and a fibrous cap, which was used as a known staining control for activated macrophages and foam cells. In each experiment, 1 of the 3 aortas was included as a standard tissue with the staining pattern known over several years of use. In addition, almost all atherectomy specimens had regions of normal media that could be distinguished by a dense, parallel arrangement of spindle-shaped cells after hematoxylin/eosin staining and the presence of the elastica interna as shown by Verhoeff–Van Gieson’s elastin stain. These regions served as built-in controls for quiescent SMCs.

In experiments comparing stellate cells with DCs, we used as a positive control the tissue from which Langerhans first described and...
defined the DC, ie, the suprabasal layer of skin epidermis. Because it is known that the number of LCs is increased with inflammation, we used a specimen of human skin characterized by mild inflammation with leukocyte infiltration in the dermis. This specimen was also run in each experiment as a standard for the intensity and quality of the staining known over several years of use.

Electron Microscopy
Atherectomy tissues were flushed from the atherectomy device with 0.9% saline, immediately immersion-fixed in 1% formaldehyde and 2.5% glutaraldehyde, rinsed with PBS, and stored in PBS containing 5% sucrose. The tissues were postfixed in 1% aqueous OsO₄ and stained en bloc with 0.2% uranyl acetate. After dehydration, embedding in Epon, sectioning, and staining with uranyl acetate and lead citrate, the tissues were examined with a Phillips CM12 transmission electron microscope.

Figure 1. General morphology and ECM composition of different areas of a human atherectomy specimen. A, Movat’s staining. Proteoglycan-rich ECM of myxomatous tissue is stained blue (separate piece); hypocellular, collagenous sclerotic region is stained yellow; regions containing both types of ECM molecules are stained green; a region of normal media is stained red and is separated by an internal elastic lamina stained black (arrow). B, A higher magnification of the myxomatous area. Stellate-shaped cells (red) are embedded in a loose, proteoglycan-rich ECM (blue). C, Hematoxylin and eosin staining of the same myxomatous area. Note the characteristic shape of the cells forming a network. Magnification, ×95 for A; ×190 for B and C.

Figure 2. An electron photomicrograph of stellate cells. A, Overview of a myxomatous region. Note the dendritic shape of the cells; their abundant, thin filaments; and the myxomatous spaces between the cells, containing multilayered, thick, basal membranes (BM) and proteoglycans (G). Magnification, ×1650. B, Nucleus and perinuclear cytoplasm of a typical stellate cell. Note a bizarre shape of the nucleus (N), the abundance of thin filaments (F), and the rare, synthetic organelles concentrated in the perinuclear area. V indicates vacuoles; G, proteoglycan-rich ECM; and BM, basal membrane. Magnification, ×15 000.
Immunohistochemistry

The indirect avidin-biotin horseradish peroxidase method (ABC Standard and Elite, Vector Laboratories) was used. Endogenous peroxidase activity was exhausted in methanol–0.3% H₂O₂. Antigen retrieval was performed in a microwave oven (800 W on “high”) for 15 minutes in the solution prepared as follows. Nine milliliters of 0.1 mol/L citric acid was mixed with 41 mL of 0.1 mol/L sodium citrate, the volume was brought to 500 mL, and pH was adjusted to 6.0. The source and specificity of the primary antibodies are summarized in Table 1. Mayer’s hematoxylin was used to counterstain the sections.

Hybridization In Situ

Nonradioactive in situ hybridization was performed on paraffin sections with digoxigenin-labeled DNA oligoprobes (40 and 50 bp): human vascular SM α-actin, AGGCCGTGATCCACAAACAT-TCACAGTGTTCTGCTAGAG; human SM22, CATAAACCAGTT- TGGGATCTCCACGGTATGCCCATCCTC; and human CD1a, GGAAAGCCCTCGGAGTAAGGTATCTCTAAAAACGGCTT- GATGATCCATT. Human fibroblast growth factor (FGF)-1 was detected using 450-bp digoxigenin-labeled riboprobe. The oligoprobes were designed using GCG software to select the unique regions and “tailed” with digoxigenin-11-dUTP by using terminal transferase according to established procedures. Digoxigenin-labeled FGF-1 riboprobe (antisense and sense, courtesy of Dr J.A. Thompson) was synthesized in a standard in vitro transcription reaction. In some experiments, instead of enzymatic treatment of the rehydrated sections, we used heat in a variation of the antigen retrieval procedure to facilitate access of the probe to the target. The antigen retrieval solution (see the Immunohistochemistry section) was brought to boiling in a microwave oven and heated for an additional 4 minutes, and the slides were left to cool for 15 minutes. The slides were postfixed for 10 minutes in freshly prepared 4% paraformaldehyde in PBS. Prehybridization was performed at 37°C for 1 to 2 hours in a buffer containing 50% formamide, 10% dextran sulfate, 5 ¥ SSC, 5 ¥ Denhardt’s solution, and 100 µg/mL blocking DNA (all reagents purchased from Sigma Chemical Co). Hybridization buffer contained 50% formamide, 10% dextran sulfate, 5 ¥ SSC, 1 ¥ Denhardt’s solution, 100 µg/mL DNA, and 1 ng/µL of digoxigenin-labeled probe. Hybridization was performed at 37°C for oligoprobes and at 50°C for the riboprobe overnight in a moist chamber saturated with 4 ¥ SSC. To control nonspecific binding of the probes, in the negative controls we either omitted digoxigenin-labeled oligoprobes or used a sense FGF-1 riboprobe that should not hybridize with mRNA. To detect the hybridization product, sections were incubated with anti-digoxigenin antibody (Fab fragment coupled with alkaline phosphatase; Boehringer Mannheim) at a dilution of 1:500 in the blocking solution (1% blocking reagent, Boehringer Mannheim, in 0.1 mol/L Tris-HCl, pH 7.5, and 0.15 mol/L NaCl) at room temperature for 2 hours. The hybridization products were visualized in color reaction for alkaline phosphatase (45 µL nitro blue tetrazolium chloride, 35 µL 5-bromo-4-chloro-3-indolyl phosphate disodium salt, 2.4 mg levamisole in 10 mL of 0.1 mol/L Tris-HCl (pH 9.5), 0.1 mol/L NaCl, and 0.05 mol/L MgCl₂) performed in the dark from 2 hours to overnight at 4°C. The reaction was stopped by rinsing the sections in 10 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 8.0. Nuclear fast red was used to counterstain the sections.

Double Staining

To colocalize 2 markers, in several experiments we performed immunohistochemistry after in situ hybridization. The solutions of primary antibodies were applied after the color developing reaction step of the first procedure (see above). These sections were not counterstained.

Results

General Histology of Myxomatous Tissue

The myxomatous tissue retrieved by directional coronary atherectomy from lesions of human coronary arteries formed areas that could be clearly distinguished from other types of intimal constituents on hematoxylin and eosin staining by their predominant cell type. This tissue was composed of randomly oriented cells with a characteristic branching, “stellate” shape that formed a network in abundant, loosely organized ECM (Figure 1C). This morphological pattern was clearly distinct from that of densely cellular areas of intimal hyperplasia with spindle-shaped cells in a dense fibrous matrix, and it also differed clearly from the typical scierotic plaque areas with sparse, elongated cells in a matrix of very dense, tendonlike collagen. Movat’s stain of ECM components showed that stellate cells in the myxomatous regions were separated by wide, proteoglycan-rich spaces that stained intensely blue, in contrast to collagen-rich areas of fibrocellular and scierotic tissue, which stained yellow (Figure 1A and 1B). Mixtures of proteoglycan-rich and collagenous matrix were occasionally seen, which stained green; some of these areas retained multidirectional matrix architecture following the shapes of the stellate cells and thus were included in this study, whereas areas with mixed proteoglycan/collagen matrix containing spindle-shaped cells in a parallel arrangement were counted as maturing fibrocellular SMC areas and were not included. Transition between stellate and spindle cell areas was infrequent. Normal media in atherectomy specimens was recognized by the internal elastic boundary’s staining black (Figure 1A). In trichrome staining, myxomatous areas were identified by dissolution or “fuzzy” staining of the collagenous fibrous layers, which were stained blue where present (data not shown).

Ultrastructural Features of Myxomatous Tissue

Stellate cells of myxomatous tissue had a characteristic ultrastructural appearance: they showed a complex branching pattern of condensed cytoplasm and an exaggerated, often multilayered, basal membranes (Figure 2A). Many of the gray patches in the matrix represented detached layers of excessive basal membrane material. The punctate pattern of matrix indicated abundant proteoglycans that could also be seen in some cellular vacuoles. In contrast to typical fibrocellular lesions, mature collagen fibrils were sparse. Stellate cells invariably showed bizarre shapes of the nucleus (Figure 2B). They contained few synthetic and metabolic organelles, which were concentrated in the perinuclear area; instead, they displayed abundant actin filamentous in the cell body and in the multiple branches of the cell periphery (Figure 2B). Occlus...
Histogenesis of Myxomatous Tissue
sional “dense bodies” were present within the actin filaments, but no typical SMC adhesion plaques were observed.

SMC Markers
All stellate cells stained positive with a monoclonal antibody recognizing SM α-actin, which is considered the major phenotypic marker of SMCs.45–47 Quiescent medial cells of an aorta (Figure 3A) and occasional areas of normal media in atherectomy specimens (data not shown) that were used as positive controls also displayed strong reactivity. In situ hybridization confirmed these findings and showed that stellate cells expressed α-actin mRNA (Figure 3E) at a level comparable to or even higher than that in medial SMCs in the control aortic media tissue (Figure 3D). Stellate cells showed a heterogeneous pattern of expression (approximately half of the cells were positive) for another marker of SMCs, myosin heavy chain (Figure 3C). A similar pattern of myosin staining was observed for quiescent medial SMCs found in some atherectomy specimens (data not shown). SM22, a calponin-related protein, is specifically expressed in adult SM and is recognized as one of the markers of differentiated SMCs.42,43 In situ hybridization showed 100% expression of SM22 by quiescent medial SMCs, both from control aortas and from atherectomy specimens (Figure 3G). On the contrary, <10% of stellate cells were positive for the SM22 probe (Figure 3H); large areas of myxomatous tissue were completely negative. Among intermediate filaments, desmin-type filaments are specific for muscle cells and have not been observed in other cell types,45–47 whereas vimentin-type filaments are present in all kinds of mesenchymal cells, including vascular cells.45,46 Approximately a third of stellate cells were positive for desmin (Figure 3F), and 80% of the stellate cells were positive for vimentin (Figure 3I).

Expression of FGF-1 by Stellate Cells
In contrast to quiescent medial SMCs, all stellate cells contained high levels of FGF-1 protein (Figure 4A). Lipid-laden foam cells and monocytes/macrophages that had accumulated in inflammatory regions were strongly FGF-1-positive (Figure 4C), whereas quiescent medial SMCs (Figure 4B) as well as the spindle-shaped cells in the typically densely cellular nonmyxomatous areas of intimal hyperplasia (data not shown) were negative for FGF-1 protein. As shown by in situ hybridization with FGF-1 riboprobe, a strong hybridization signal was observed in the myxomatous areas outlining the characteristic shape of stellate cells (Figure 4D). The specificity of hybridization was confirmed by the absence of the signal with the sense probe. The macrophages in the aortic control tissue also expressed FGF-1 mRNA, whereas medial SMCs were “silent” for FGF-1 message (Figure 4E). Thus, in situ hybridization demonstrated that the stellate cells not only contained FGF-1 but also synthesized this growth factor.

Although FGF-1 is known to be produced in different cell types, our finding of FGF-1 in stellate cells is a major difference from quiescent medial SMCs and spindle-shaped intimal SMCs that were reported to express hardly detectable levels of FGF-1 mRNA or protein.45–52 The level of FGF-1 mRNA is increased in atheromas compared with normal human arteries, and in atheromas, immunoreactivity for FGF-1 is associated with areas of neovascularization (ie, endothelial cells and/or pericytes) and macrophage-rich regions.48,49,51 Stellate cells were negative with respect to CD31 antigen, a specific marker of endothelial cells, whereas capillary endothelium in the same section was strongly positive (data not shown). On the basis of these results, we ruled out a relationship between the 2 cell types. Thus, expression of FGF-1 by stellate cells could be an argument in favor of a monocyte/macrophage origin for these cells.

To test this hypothesis, we studied the expression of phenotypic markers of differentiated macrophages, HAM56 and CD68. However, the myxomatous tissue in atherectomy specimens proved to be negative for HAM56 (Figure 4G) and so were the quiescent SMCs, whereas monocytes, macrophages, and foam cells in the positive control tissue (inflammatory region of an atherectomy specimen) stained strongly (Figure 4H). Only 15% of the stellate cells expressed CD68 (Figure 4F), whereas monocytes, macrophages, and foam cell macrophages in the inflammatory areas were strongly positive (Figure 4I). Thus, by these criteria, stellate cells do not represent mature monocytes or differentiated macrophages.

DC Markers
We then studied expression in the myxomatous tissue of a typical member of this family, LCs. This cell family is a phenotypically and functionally diverse population of cells that actively migrate from bone marrow via the blood circulation to the peripheral organs, where they function as antigen-presenting cells responsible for induction of primary immune responses.21,25,28 A typical member of this family, LCs, are usually distributed in the suprabasal layer of the epidermis.21 These cells display major histocompatibility complex (MHC) class I and II antigens (CD1a and human leukocyte antigen [HLA]-DR), S100 antigen, and several adhesion molecules.21,24–27

Approximately 40% of the stellate cells were HLA-DR-positive (Figure 5A). In the control tissues both monocytic and foam cell macrophages expressed high levels of HLA-DR (not shown), whereas the highly organized, spindle-shaped, quiescent SMCs of a piece of normal media in the same specimen were negative (Figure 5B). In the inflamed skin used as a positive control specifically for LCs, anti–HLA-DR antibody stained these cells as well as macrophages and monocytes (Figure 5C).

Figure 4. Expression of macrophage markers by cells of human atherosclerotic lesion. Immunoperoxidase staining for FGF-1: A, myxomatous tissue from atherectomy specimen; B, atherosclerotic human aorta; and C, macrophage-rich region of an atherectomy specimen. Nonradioactive in situ hybridization with FGF-1 mRNA: D, myxomatous tissue; E, atherosclerotic human aorta. Immunoperoxidase staining for CD68: F, myxomatous tissue; I, inflammatory region of an atherectomy specimen. Immunoperoxidase staining for HAM56: G, myxomatous tissue; H, inflammatory region of an atherectomy specimen. Arrows demarcate position of the internal elastic lamina separating the media (below) from the atherosclerotic core and cap (above). Immunoperoxidase staining: positive cells are stained brown, and nuclei of negative cells are stained blue. In situ hybridization: positive cells are stained purple, with no counterstaining. Magnification, ×130.
The stellate cells uniformly and strongly expressed another phenotypic marker of LCs, S100 antigen (Figure 5D), whereas quiescent SMCs in normal media from the same specimen did not (Figure 5E). In the skin, anti-S100 antibody stained LCs as expected, as well as melanocytes and Schwann cells, but did not stain vascular SMCs, endothelial cells, leukocytes, and plasma cells (Figure 5F).

However, it is recognized that the major phenotypic marker defining LCs is CD1a antigen. We first applied the in situ hybridization technique for detection of CD1a mRNA. The oligoprobe was selected from the 5'-untranslated region specific for CD1a mRNA that is absent in CD1b or CD1c mRNA. In 60% of the specimens (22 of 36) analyzed, we detected the hybridization product in the myxomatous areas in variable numbers of cells (Figure 6D). In contrast, quiescent SMCs in the areas of normal media from the same atherectomy specimens were CD1a-negative (Figure 6E). As expected in the skin (positive control tissue), LCs in the suprabasal layer of the epidermis showed the hybridization signal (Figure 6F). This pattern was confirmed by immunohistochemistry with the anti-CD1a monoclonal antibody O10. We observed distinct cytoplasmic staining of many, though not all, stellate cells of the myxomatous regions (Figure 6A). Similarly in the skin, positive LCs scattered between keratinocytes in the epidermis could be recognized by their characteristic shape (Figure 6C). As expected, areas of normal media of the atherectomy specimens were always negative with respect to this antigen (Figure 6B).

**Double Staining**

Immunohistochemical staining of specimens for HLA-DR after in situ hybridization for CD1a revealed a small subpopulation (10% to 15%) of double-positive cells (Figure 7A).
However, for the remainder of labeled cells, these two signals did not overlap. Interestingly, staining for α-actin after CD1a in situ hybridization demonstrated coexpression of both antigens in every CD1a-positive stellate cell (Figure 7B).

If the expression of the markers of antigen-presenting LCs in stellate cells is functionally relevant, then some evidence of antigen presentation, such as an accumulation of T cells in myxomatous tissue, should be found. Indeed, staining for the T-lymphocyte marker CD45RO showed a frequent lymphocyte infiltration in myxomatous tissue, albeit not as cellular as in the macrophage-rich, inflammatory areas. Interestingly, CD45RO-positive lymphocytes were always present in the vicinity of the stellate cells, which also expressed CD1a (Figure 7C).

**Pericyte Markers**

We also investigated a possible relationship between stellate cells and pericytes. Immunohistochemical identification of pericytes is complicated owing to their phenotypic, biochemical, and physiological heterogeneity. Although a number of specific markers have been detected on pericytes, they are not continually expressed and are dependent on either a functional or metabolic state of the cells. We tested stellate cells for expression of a nonmuscle isoform of actin, which is dominant in pericytes of “true” capillaries, and melanoma-associated antigen (MAA), discovered on pericytes in tumors.

Stellate cells were positive for β-actin; however, the signal was comparable or weaker than that of medial SMCs (data...
not shown). With respect to MAA, in our archival atherectomy specimens the antigenic reactivity was inhibited by overfixation and the paraffin-embedding procedure. Therefore, we studied expression of this antigen in freshly frozen sections of a coronary artery of a human donor heart that had been rejected for organ transplantation. The left anterior descending coronary artery, incidentally, had a lesion containing almost exclusively stellate cells. In these frozen, left anterior descending coronary artery sections, the antibody MEL-2 against MAA reacted with pericytes of adventitial capillaries, arterioles, and postcapillary venules as expected, and we also observed a strong signal in the stellate cells. We also found abundant stellate cells in myxomatous endocardial tissues covering infarcted myocardium in an autopsy specimen (Figure 8A), which gave the same distinct cytoplasmic staining for MAA, outlining their stellate morphology (Figure 8B). The deeper layer of the thickened endocardium, mainly composed of spindle-shaped cells in more collagenous scar tissue, was negative for this marker. A similar staining pattern was observed for β-actin (Figure 8C). Thus, expression of these antigens by stellate cells may be an argument for their relationship to pericytes.

**Discussion**

Myxomatous tissue represents a subtype of intimal hyperplasia in human coronary lesions. It is composed of stellate-shaped cells randomly arranged in abundant, loosely orga-
TABLE 2. Phenotypic Profile of Various Cell Types

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<tr>
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<th>Stellate Cells</th>
<th>Medial SMCs</th>
<th>Macrophages</th>
<th>DCs (Langerhans Type)</th>
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<td>α-Actin</td>
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+ indicates positive; –, negative; +/-, heterogeneous pattern of expression.

ized, proteoglycan-rich ECM. Several reports connect this type of intimal hyperplasia with restenotic lesions.5,8,9 Our findings on 200 atherectomy specimens show that myxomatous tissue can be found in 85% of restenotic lesions versus 50% of primary lesions and that in restenotic lesions it occupies a larger cross-sectional area (C.C.H. et al, unpublished data, 1998). Cells with a similar stellate shape are occasionally observed in the subendothelium of normal and atherosclerotic intima of human aortas, suggesting that these cells may be a normal, albeit rare, component of the vessel wall.58–60 Although this peculiar stellate cell type was first described in the normal aortic intima as early as in 186661 and was found to be increased there in the inflammatory state, the nature of arterial myxomatous tissue is still poorly understood. As a component of intimal hyperplasia, stellate cells could originate from SMCs. This hypothesis is based on the observation that many cells of hyperplastic tissue are positive for the SMC markers actin, desmin, and vimentin. However, in most studies the myxomatous tissue was not studied separately from other types of hyperplasia.4,5,8,9,12

When focusing on myxomatous tissue only, our study provided many arguments that stellate cells cannot be considered typical SMCs, although they do express phenotypic SM markers (Table 2). We demonstrated that α-actin, the major phenotypic marker of quiescent SMCs,62 was indeed abundant in all stellate cells both as message and as protein. Other SM markers such as SM-specific heavy-chain myosin, SM22, desmin, and vimentin showed a heterogeneous pattern of distribution. A similar distribution of these markers was observed in quiescent SMCs of normal aortic media and media fragments in atherectomies used as positive controls and was consistent with earlier reports.44–47,63–65 However, a significant difference was observed for SM22: although this marker was strongly expressed by all medial SMCs, only approximately 10% of stellate cells were positive.

Electron microscopy analysis demonstrated that the cytoplasm of stellate cells is filled with bundles of thin filaments, showing occasional “dense bodies.” The cells had prominent basement membranes, which were often multilayered. These ultrastructural features also point to a SM nature for stellate cells. However, they clearly differ from those of the so-called “contractile” phenotype described for differentiated, quiescent SMCs of the media that also have abundant, densely packed actin filaments but are spindle-shaped and surrounded by a thin basement membrane and mature, collagenous, and elastic matrix.66,67 On the other hand, stellate cells differ ultrastructurally from SMCs with the so-called “synthetic” phenotype,66,67 which is characterized by a distinct lack of contractile filaments and abundant synthetic organelles. Thus, the pattern of expression of SMC markers in stellate cells and their ultrastructural features suggest either another (non- “contractile,” non-“synthetic”) phenotype of SMCs or a different origin for these cells.

One of the striking peculiarities of stellate cells that distinguishes them from typical SMCs is constantly high levels of FGF-1 message and protein (Table 2). FGF-1 is expressed by several cell types involved in atherogenesis. Although vascular SMCs can produce and respond to FGF-1 in vitro,68 the most abundant growth factor of SMCs is FGF-2 but not FGF-1.1,48–51 Freshly isolated rat SMCs express a very low level of FGF-1;52 FGF-1 mRNA is hardly detectable in SMC cultures from adult rat aortas, whereas in newborn SMC cultures, FGF-1 mRNA is expressed to a much greater degree.53 There is a differential pattern of FGF-1 expression in human atherosclerotic and normal arteries. In particular, the level of FGF-1 mRNA is increased in human atheroma compared with nonatherosclerotic arteries.48,49,53 In normal human arteries, FGF-1 immunoreactivity was reported predominantly for adventitial fibroblasts but not SMCs, whereas in atheromas FGF-1 protein is associated with plaque microvessels (ie, endothelial cells) and macrophage-rich regions.49,53 Indeed, in our study, neither medial SMCs, nor α-actin–positive, spindle-shaped cells of nonmyxomatous intimal hyperplasia in the atherectomy specimens were FGF-1–positive at the threshold that could be detected with our antibody. At the same time, with the strong expression of FGF-1 in stellate cells, we detected high levels of FGF-1 in built-in control areas of monocyctic cells within macrophage-rich, inflammatory regions of lesions as well as in lipid-laden, “foamy” macrophages. This finding is a strong argument that stellate cells could be related to cells of monocyte/macrophage lineage. However, a classic marker of human tissue macrophages, HAM56, was not present in the stellate cells, and CD68 was seen in only a few stellate cells that made a clear distinction of stellate cells from mature macrophages. We also excluded a possible relationship of stellate cells to endothelial cells because the former were not reactive to anti-CD31 antibody,54 which specifically recognizes endothelial cells.

Next, we compared stellate cells of human atherectomies with DCs, which comprise a diverse family of bone marrow–derived cells able to migrate via the blood circulation to many peripheral locations of the body.20–22,24 Morphologically, DCs strikingly resemble stellate cells of myxomatous tissue: they are irregularly shaped, have long, cytoplasmic processes, and possess a lobulated nucleus. The earliest progenitor of DCs is a CD34–positive pluripotent stem cell of the bone marrow; the more mature intermediate cell precursors found in the peripheral blood give rise to either DCs or monocytes.72,27 The hypothesis that stellate cells of myxomatous tissue could represent DCs is especially attractive because vascular DCs...
were described in the human aortic intima: in normal aorta, they are mainly localized in the subendothelial layer, whereas in atheromas these cells are distributed throughout the lesion.23,69–71

We analyzed several of the most frequently expressed phenotypic markers of DCs: CD1a, HLA-DR, and S100. We found expression of these antigens in all or in a subpopulation of arterial stellate cells in our atherectomy specimens (Table 2). One of the classic markers of DCs, S100 antigen, was uniformly expressed by all cells of myxomatous tissue and was present in neither quiescent medial SMCs nor in monocytes/macrophages. Besides within LCs, S100 is found in a number of cell types of the nervous system, melanocytes, and in various tumor cells, including SM tumors.72 Two other antigens, CD1a and HLA-DR, displayed a heterogeneous pattern of expression ranging from completely negative areas to predominantly positive regions of myxomatous tissue. While expression of HLA-DR is normally restricted to cells of the immune system, such as macrophages and T lymphocytes, it can also be induced in other cell types, such as SMCs, endothelial cells, fibroblasts, and various tumor cells.73–75 CD1a belongs to the CD1 family of proteins that are primarily expressed on the surface of cortical thymocytes and some T-cell leukemias.76,77 Extrathymically, CD1a has been observed on DCs, mainly LCs.78

Thus, we found a striking antigenic similarity between stellate cells in human atherosclerotic lesions and DCs in their expression of functionally relevant molecules, including CD1a and HLA-DR (Table 2). However, for several reasons we believe that stellate cells rather belong to a mesenchymal cell lineage characterized by expression of the markers typical for macrophages and antigen-presenting DCs. First, they express most SM markers tested, such as α-actin, myosin, desmin, and vimentin, except SM22, for which only 10% of stellate cells were positive. There are no reports in the literature that blood-borne cells, such as macrophages, or other nonmuscle cells can express the SM markers α-actin and desmin even under pathological conditions or in culture.35,46,63 We did not observe a signal for α-actin in skin LCs. Second, ultrastructural features of stellate cells most closely resemble those of SMCs. Indeed, the ultrastructural appearance of these cells is very different from that of macrophages, DCs, or endothelial cells. Obviously, the ability of stellate cells to synthesize basement membranes and ECM is more typical for SMCs than for monocytic cells or DCs, which are characterized by a thin ring of cytoplasm and lack dense bodies and a basement membrane.20,71 We did not find Birbeck granules, which are a characteristic ultrastructural feature of LCs, although at certain stages of maturation DCs also do not have these structures.21,22,27,70 Third, Hansson et al80 and Jonasson et al91 reported that SMCs of atherosclerotic lesions can express HLA-DR antigen and that this expression can be induced in vitro by interferon-γ from T lymphocytes.73 The finding of S100 antigen in stellate cells also does not contradict the hypothesis of their mesenchymal origin: besides in LCs, expression of this antigen was reported for various tumor cells, including SM tumors.72 Last, in our study, double staining for CD1a and α-actin confirmed that all CD1a-positive stellate cells were also positive for α-actin, arguing against a bone marrow origin, at least for this part of the stellate cell population. In atherosclerotic lesions, vascular DCs are reported to be mainly accumulated near the neovascularure or within inflammatory infiltrates, establishing contacts with each other or with macrophages, foam cells, endothelial cells, or T lymphocytes.82,83 Indeed, in inflammatory areas of our specimens as well as in regions of angiogenesis, we could confirm the presence of CD1a-positive cells. However, unlike scattered DCs, the stellate cells of our study formed a tissue that was clearly distinct from areas of both inflammatory infiltrates and neovascularization.

Thus, by the ultrastructural peculiarities of stellate cells and their expression of SMC markers simultaneously with expression of several antigens that are not typical for SMCs, we consider stellate cells to be either an atypical (non-“contractile” and non-“synthetic”) phenotype of SMCs or to belong to another, SM-like mesenchymal cell lineage. In this respect, an intriguing possibility for the origin of stellate cells is that they could be pericytes. Pericytes, or myofibroblasts, are pluripotent mesenchymal cells that can differentiate into adipocytes, osteoblasts, and phagocytes; it is also suggested that pericytes can be specific precursors of SMCs.85,86 Pericytes are morphologically, biochemically, and physiologically heterogeneous, and the only reliable criterion for their identification is localization next to a capillary.74,75 However, these cells share many features with SMCs, such as expression of actin, desmin, and vimentin and synthesis of collagens and proteoglycans.34,44 Pericytes contain both SM and non-SM isoforms of actin and myosin.56,85 On the other hand, it has been shown that brain pericytes can express some of the markers of macrophages.86 There are reports indicating the possible presence of pericytes within arteries.32,33 It has been suggested that migrating adventitial myofibroblasts may contribute to the process of lesion formation.29–31 Morphologically, typical pericytes have a stellate shape, although they can assume a fibroblast-like shape.74,87 Pericytes synthesize and release structural constituents of the basement membrane and ECM.34 In our study, we observed a heterogeneous staining of stellate cells for the nonmuscle isoform of actin that is supposed to be one of the markers of pericytes. This finding does not contradict earlier data: pericytes from “true” capillaries mainly have nonmuscle actin and myosin isoforms, whereas pericytes from postcapillary venules contain mainly SM isoforms.34,85 The stellate cells of human endocardium were shown to express MAA that is considered another surface marker of pericytes. Taken together, our findings, such as heterogeneous antigenic pattern and peculiar ultrastructural features of stellate cells, make a strong argument for a pericyte origin for myxomatous tissue.

Despite the arguments against a bone marrow origin, finding of HLA-DR and CD1a expression in stellate cells implies that functionally, they could be compared with DCs. Both molecules are known to be directly related to the antigen-presenting function of DCs: they capture antigenic peptides, and such antigen–MHC molecule complexes can be specifically recognized by T lymphocytes via T-cell receptors.20,24–26,82 We hypothesize that in atherosclerotic lesions, stellate cells might be involved in a local immune response, where they function as antigen-presenting cells. It has been shown that endothelial cells expressing HLA-DR are capable of presenting foreign antigens to T cells.74,89,90 The same ability has been proposed for HLA-DR–bearing SMCs.91 Interestingly, the CD1 family of molecules has been proposed
as an important mechanism for the presentation of lipid antigens to T lymphocytes. On the other hand, it is known that activated memory T lymphocytes, to which the antigen is presented, invade atherosclerotic lesions in high numbers. Indeed, in most of our specimens we detected massive infiltration of CD45RO-positive lymphocytes. The highest cellularity was detected in inflammatory areas densely populated by macrophages, which are “professional” antigen-presenting cells. It is noteworthy that the myxomatous tissue was the second tissue type with frequent CD45RO-positive T-lymphocyte infiltration, whereas in all other arterial tissue types, normal and atherosclerotic, only rare CD45RO-positive T lymphocytes could be found. Although this observation does not directly prove that T lymphocytes are the inducers of CD1a and HLA-DR expression by stellate cells nor does it prove that stellate cells attract T cells, it suggests an interaction between these two cell types in the atherosclerotic/restenotic lesions. Further investigations are necessary to elucidate what the local immune response specifically implies and what the consequences are. One might speculate that the normal self-limitation of vascular wound healing may be affected by such interactions, leading to accumulation of myxomatous tissue with abnormal matrix in these foci.

Thus, on the basis of results of the present study, we propose that stellate cells of myxomatous tissue from human coronary lesions represent a specific phenotype of mesenchymal cells, which could originate from pericytes. Stellate cells acquire some features of antigen-presenting cells such as DCs and macrophages and could be involved in local immune reactions in atherosclerotic lesions. This type of activation may be an important mechanism of atherogenesis and restenosis.

Note added in proof. Randolph et al have recently reported that “DCs arise from monocytes that migrate across endothelium in an abuminal-to-luminal direction, whereas macrophages develop from monocytes that remain in the subendothelial matrix.” These findings emphasize the existence of a common precursor for DCs and macrophages and the importance of local vascular wall interactions for their differentiation pathways. (Science. 1998;282:480–483.)

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Studies on the Histogenesis of Myxomatous Tissue of Human Coronary Lesions
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