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Increased extracellular matrix synthesis by smooth-muscle cells obtained from in vivo restenotic lesions by directional coronary atherectomy

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Coronary angioplasty continues to be limited by a 30% restenosis rate within 6 months. Postmortem histopathologic studies suggest that the intimally directed migration and proliferation of vascular smooth-muscle cells (VSMC) may play a critical role in this problem. These studies, however, can provide only limited static information regarding a specific moment in the disease process. To overcome this limitation and to look at the underlying mechanisms involved, we used directional coronary atherectomy to percutaneously retrieve tissue from patients with clinically and angiographically documented coronary artery disease and examined the hypothesis that VSMC cultured from restenotic lesions differ in extracellular matrix synthesis from primary atherosclerotic cells. Our experimental strategy was first to culture the tissue segment and confirm the identity of the explant cells and then to compare extracellular matrix synthesis between primary and restenotic cells.

Methods. A total of 98 in vivo plaque specimens were obtained from 98 patients undergoing directional coronary atherectomy with a previously described standard technique.[1] Tissue specimens were cultured with standard explant techniques. Smooth-muscle cell outgrowth was assessed by light microscopy with morphologic criteria reinforced by electron microscopy and positive immunostaining against specific smooth-muscle cell α-actin. Extracellular matrix synthesis was assessed by studying the synthesis of collagen and sulfated aminoglycans, the principal components of the extracellular matrix. To determine collagen synthesis growth, arrested cells of passage 2 to 4 were incubated for 48 hours at 37° C in culture medium containing 2 μmol/L ascorbic acid and 10 $\mu Ci.ml^{-1}$ ^3H-proline (specific activity 23 $mCi.mg^{-1}$). To determine sulfated glycosaminoglycans synthesis growth, arrested cells were incubated for 48 hours at 37° C in culture medium containing 0.5 ml ^{35}S-sulfate (specific activity 20 $mCi.mg^{-1}$). Subsequent analyses were performed with previously described techniques.[1] Measurements are expressed as mean ± SEM. Mean values were compared by the Student's t test for unpaired data. Data were considered significant if $p < 0.05$.

Results. Initial cell outgrowth and successful secondary culture (up to seven serial passages) was achieved in 11 patients, 7 with primary and 4 with restenotic lesions. Cells started to grow out from explants after 4 to 8 days (Fig. 1), and confluent multilayer primary cultures from the 11 patients were established after 4 to 6 weeks. Subconfluent cultures took the form of a network of multilayered elongated cells, whereas in confluent multilayer structures the cells appeared as whorls, producing the "hill and valley" pattern typical of vascular smooth-muscle cells in culture (Fig. 1). Immunostaining of the cells for α-actin was positive and confirmed them to be of smooth-muscle origin. Electron microscopy revealed features consistent with the synthetic phenotype. Collagen synthesis, reflected by the incorporation of ^3H-proline, was significantly greater for cells of restenotic than primary origin (0.034 ± 0.002 vs 0.024 ± 0.001 nmol [^3H]-proline · μg total cell $protein^{-1}$, $n = 11$, $p < 0.05$) (Fig. 1). Similarly, production of sulfated aminoglycans as assessed by the incorporation of ^{35}S-sulfate was significantly greater for restenotic than primary cells (7.49 ± 0.58 vs 5.11 ± 0.48 nmol [^3H]-sulphate · μg total cell $protein^{-1}$, $n = 11$, $p < 0.05$) (Fig. 1).

Comments. Our study has demonstrated that restenotic cells synthesize significantly more collagen and sulfated aminoglycans than do primary cells, suggesting that extracellular matrix synthesis may play a significant role in postangioplasty restenosis. This finding is in contrast to our previous study,[1] in which we found that restenotic and primary cells synthesized more extracellular matrix pro-

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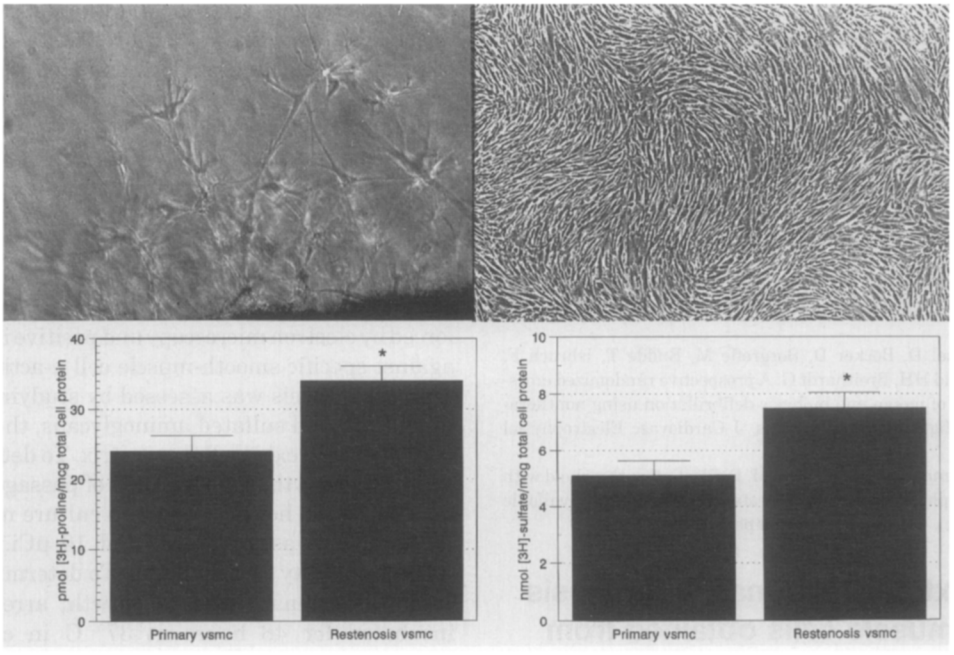

Fig. 1. *Upper left panel*, Fragment of retrieved atherectomy tissue in culture showing dense population of VSMC migrating out of explant (Original magnification ×60). *Upper right panel*, "Hill and valley" pattern typical of vascular smooth-muscle cells in culture. *Lower left panel*, Production of extracellular matrix collagen. *Lower right panel*, Production of extracellular matrix sulfated aminoglycans. $*p < 0.05$.

tein than did human umbilical vein cells, but no differences were seen between the two. This finding is likely to have occurred because of the significantly higher proliferation rate in restenotic cells during those experiments. In this study, in which we looked for differences in extracellular matrix synthesis during growth arrest, we found that restenotic cells synthesize significantly more extracellular matrix than do primary cells.

Because the difference in extracellular matrix synthesis between primary and restenotic cell lines occurred under identical culture conditions, we believe that the secretory behavior of the restenotic cells in vitro reflects a previous phenomenon of phenotypic modulation and selection in vivo rather than some effect of the culture process itself.[2] In support of this belief are histopathologic studies that suggest that smooth-muscle cells in both primary atherosclerotic and restenotic lesions are frequently of the synthetic phenotype. Additionally, coronary smooth-muscle cells in tissue excised at atherectomy have been recently shown to express messenger RNA for nonmuscle myosin heavy chain, which is associated with the synthetic smooth-muscle cell phenotype.[3]

Our data suggest that restenotic cells are fundamentally different from primary atherosclerotic cells in the production of extracellular matrix, in keeping with previous studies suggesting that restenotic cells differ from primary cells in terms of their migratory activity,[4] growth regulation,[1] and motility.[5] This finding may be a reflection of a more specialized function of the VSMC situated in the restenotic lesion, which may have been activated as a result of the angioplasty procedure itself in the response to injury hypothesis. Alternatively, it may signify a selected population of already activated VSMC in lesions that have subsequently undergone restenosis. The small numbers involved preclude us from commenting on which of the two mechanisms is most likely, but both are likely to involve the induction of growth factors such as transforming growth factor-β, the expression of which has been shown to be increased in restenotic lesions.[6] This finding is in keeping with our data and would suggest that the increased expression of transforming growth factor-β may result in increased production of extracellular matrix. This study has demonstrated that coronary smooth-muscle cells obtained from in vivo restenotic lesions synthesize significantly more extracellular matrix than do cells from primary lesions, suggesting that increased extracellular matrix production may play a fundamental role in restenosis after angioplasty.

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Recurrent non–Q-wave myocardial infarction associated with toluene abuse

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Toluene, a common substance of solvent abuse in the United States, is associated with significant cardiac morbidity and mortality after inhalation. Several reports have described cardiac complications of acute (arrhythmia, myocardial infarction[1]) and chronic intoxication (dilated cardiomyopathy[2]). We report the case of a 55-year-old man with toluene-related recurrent non–Q-wave myocardial infarction.

The patient is a 55-year-old Hawaiian man who first came to medical attention in March 1992 when he arrived with dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and vague chest discomfort. He gave a history of alcohol abuse but no tobacco or illicit drug use. On physical examination he was noted to have a blood pressure of 204/136, bibasilar rales, an elevated jugular venous pressure, and mild peripheral edema. An electrocardiogram demonstrated normal sinus rhythm and left ventricular hypertrophy with repolarization abnormalities. Serial creatinine kinase determinations revealed a peak level of 94 IU/L with an elevated MB fraction. A chest x-ray examination showed cardiomegaly, and an echocardiogram demonstrated a left ventricular ejection fraction of 45% with mild global hypokinesis and mild concentric left ventricular hypertrophy. A thallium exercise treadmill test demonstrated an inferior wall defect at exercise, but the patient did not return for a rest study. Cardiac catheterization revealed normal coronary arteries. Results of a complete blood count, electrolytes, and liver function tests were within normal limits, and a urinalysis was notable for mild proteinuria (30 mg/dl). The patient was successfully treated with a combination of nitrates, diuretics, calcium channel blocker, and an angiotensin-converting enzyme inhibitor. However, he was frequently noted to be noncompliant with his medications.

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Since that initial visit the patient has had 11 admissions for non-Q-wave myocardial infarction over an 18-month period. His presentations to the emergency department were for acute episodes of severe substernal chest pain with radiation to the left arm; these episodes lasted more than 30 minutes, often occurred at rest and were associated with nausea and diaphoresis. Admitting blood pressure varied from 135 to 200 mm Hg, and mild congestive heart failure was frequently noted on examination and chest radiography. Occasional transient electrocardiographic changes showed pseudonormalization of the T-wave abnormalities or ST depression. Over time echocardiographic studies showed decreased left ventricular ejection fraction from 45% to 33%. He underwent two additional cardiac catheterizations, again revealing normal coronary arteries. An ergonovine challenge test or myocardial biopsy was not performed. Throughout this time the results of his complete blood count, electrolytes, and liver function tests were within normal limits except for a trend toward hypokalemia and mild proteinuria. His toluene abuse was detected during his tenth admission for non–Q-wave myocardial infarction, when he was found saturating paper towels with industrial strength toluene in preparation for "sniffing" in the hospital. When confronted, the patient admitted to inhaling toluene in a similar manner one to two times per week since 1968, often immediately before chest pain developed. On the last recorded admission for non–Q-wave myocardial infarction, his electrolytes were notable for an anion gap acidosis with hypokalemia (Na 138, K 2.8, Cl 106, CO2 13). A urinalysis showed worsening proteinuria (300 mg/dl) and numerous hyaline casts. The electrolyte abnormalities returned to normal at discharge.

The cardiac toxicity of toluene most frequently described in the literature is arrhythmia, specifically ventricular tachycardia, ventricular fibrillation, and sinus bradycardia, often leading to sudden death.[3] Possible mechanisms of action include oversensitization of the heart to endogenous catecholamines and direct slowing of the sinoatrial node.[4] Coexisting electrolyte abnormalities caused by toluene's renal toxicity may also contribute to the development of these fatal arrhythmias.[5] Less commonly reported is the presence of dilated cardiomyopathy with toluene abuse.[2]

Acute myocardial infarction has rarely been reported in the setting of solvent abuse. One case report describes a 16-year-old boy who had ventricular fibrillation and an anterior myocardial infarction shortly after prolonged inhalation of an adhesive containing toluene.[1] We believe our case to be the first linking toluene abuse to recurrent myocardial infarction. We postulate that acute exposure to toluene led to coronary vasospasm caused by increased sensitivity to catecholamines,[4] with the subsequent development of non–Q-wave myocardial infarction. The patient admitted to toluene use before the onset of the acute symptoms, linking the two processes temporally. Moreover the development of both an anion gap acidosis with hypokalemia and proteinuria is consistent with distal tubular acidosis, which is a well-known phenomenon in toluene abuse.[6]