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In Situ Detection of Platelet-Derived Growth Factor-A and -B Chain mRNA in Human Coronary Arteries after Percutaneous Transluminal Coronary Angioplasty

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Experimental studies have shown that platelet-derived growth factor (PDGF) plays a role in wound-healing processes after angioplasty. In humans, after percutaneous transluminal coronary angioplasty (PTCA), this has not yet been documented. Six coronary arteries of five patients who died after PTCA were studied. The angioplasty sites were sliced serially, and the slices were studied using immunocytochemistry and* in situ *hybridization. Monoclonal antibodies were directed against muscle actin, vimentin, macrophages, and endothelium.* In situ *hybridization was performed using a synthetic oligonucleotide probe complementary to the PDGF-A and -B chain mRNAs. The identification of cells was based on a comparison with immune-stained sections. Positive autoradiographic signals for PDGF-A and -B chain mRNAs were found at the site of the PTCA injury and related to areas that contained macrophages, spindle cells, smooth muscle cells, and endothelial cells of neovascularization. In humans, both PDGF-A and -B chain mRNAs are expressed at sites of PTCA injury. The expression relates to the reparative response, and it appears that the cells involved are macrophages, spindle cells, smooth muscle cells, and endothelial cells of neovascularization. This is the first study to document the expression of PDGF-A and -B mRNAs at sites of repair in human coronary arteries after PTCA. It suggests strongly that PDGF is involved in the repair process after PTCA. (Am J Pathol 1996, 149:831–843)

Experimental work, both in animals and in *in vitro* models, suggests that platelet-derived growth factor (PDGF) plays a role in the process of wound healing after percutaneous transluminal coronary angioplasty (PTCA).[1–5] This is of interest, as in humans the site of injury after PTCA is covered almost instantly by thrombus.[6] Hence, platelet adherence at the initial stage of injury could provide the source for PDGF. However, it is known that the cells involved in the reparative response after PTCA also may produce PDGF.

Thus far, to the best of our knowledge, no studies have been reported that document the expression of PDGF-A and -B mRNA in human coronary arteries in relation to PTCA injury. The present study has been designed for this purpose.

Materials and Methods

Five hearts of patients who died after PTCA were collected for this study. In these five patients, six coronary arteries had been dilated. Each of these five patients died of causes related to ischemic heart disease. The relevant clinical data are summarized in Table 1.

All autopsies were performed within 3 hours after death. The site of angioplasty, which contained the

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Table 1. *Human Coronary Arteries after PTCA*

Patient	Age (years)	Sex	Indication for PTCA	Dilated coronary artery	Interval between PTCA and death	Cause of death
1	70	M	AMI	LAD	6 days	Cardiac rupture
2	72	M	AMI	LAD	14 days	CHF
3	67	M	AMI	RCA	28 days	CHF
4	67	M	SAP, OMI	LAD	44 days	CHF, pneumonia
				LCx	44 days	
5	58	M	AMI	LAD	56 days	CHF

M, male; AMI, acute myocardial infarction; SAP, stable angina pectoris; OMI, old myocardial infarction; LAD, left anterior descending artery; RCA, right coronary artery; LCx, left circumflex artery; CHF, congestive heart failure.

culprit lesion, was established by comparing the specimens with the angiograms after PTCA, using coronary ostia and bifurcation sites in the coronary arteries as points of reference. The coronary arteries in which the angioplasty had been performed were then dissected from the epicardial surface in the fresh state. These segments were sliced serially at approximately 1-mm intervals. Alternately, one slice was fixed in methanol-Carnoy's solution for histological and immunocytochemical investigations, a second slice was immersed in freshly prepared 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for *in situ* hybridization, and a third slice was snap-frozen. This sequence was repeated throughout the total length of the dilated arterial segment. From each slice, irrespective of its fixation, microscopic sections were cut (see below) to verify the exact location of the site of angioplasty. The identification of PTCA-related injury was based on finding a laceration that continued through several slices and remained of the same nature and stayed around the same location in the artery. Moreover, the injury extended far beyond the area that contained the culprit lesion. On that basis, we were certain that the sections selected for study contained PTCA-induced laceration. Coronary artery segments obtained from non-target sites, remote from the dilated sites, were studied as controls.

Histological and Immunocytochemical Investigation

The coronary slices fixed in methanol-Carnoy's solution were processed routinely, embedded in paraffin, and serially sectioned at 5 μm thickness. From each slice, 60 histological sections were obtained. Approximately every 10th section was stained with hematoxylin and eosin and one with Weigert's elastic van Gieson stain. The other sections were used for immunocytochemical staining. Serial frozen sections from the paraformaldehyde-fixed slices, adjacent to the sections used for *in situ* hybridization (see below), were used also for immunocytochemistry in all cases except for case 4. Monoclonal antibodies were used against muscle actin (HHF-35), smooth muscle actin (CGA-7), vimentin, macrophages (HAM-56), and von Willebrand factor (vWf). The specificities, working dilutions, and sources are summarized in Table 2. The monoclonal antibody against vimentin did not work well with paraformaldehyde-fixed sections and was used only on methanol-Carnoy's-fixed sections. The antibodies were incubated at room temperature for 1 hour. The labeled streptavidin-biotin complex system, with diaminobenzidine/nickel chloride color development or the streptavidin/alkaline phosphatase system with new fuchsin development was performed. For the former, sections were counterstained with methyl green; for the latter, sections were counterstained with hematoxylin.

In Situ *Hybridization*

The coronary slices immersed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) were fixed overnight at room temperature, transferred to the same fresh fixative for another hour, and then rinsed in phosphate-buffered 20% sucrose for 2 to 4 hours at 4°C. The tissues were then frozen and stored at −80°C. The frozen samples were serially sectioned at 10 μm thickness.

Table 2. *Monoclonal Antibodies Used in the Study*

Antibody	Specificity and reactivity	Working dilution
HHF-35*	Muscle actin	1:50
CGA-7†	Smooth muscle cell actin	1:20
Anti-vimentin*	Smooth muscle cells, fibroblasts, macrophages, and endothelial cells	1:100
HAM-56*	Macrophages and some endothelial cells	1:70
Anti-vWf*	Endothelial cells	1:50

* From Dako, Glostrup, Denmark.
† From Enzo Biochemicals, New York, NY.

The procedure for *in situ* hybridization was essentially identical to that described by Bloch et al.[7] The present protocol was carried out at room temperature unless otherwise mentioned. The sections were pretreated in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for 15 minutes. The sections were rinsed in 1X standard saline citrate (SSC) solution (1X SSC contains 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate), using three changes for 5 minutes each, and then incubated in 4X SSC containing 1X Denhardt's solution for 1 hour (1X Denhardt's solution contains 0.02% bovine serum albumin, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone k-30). The sections were then dehydrated in ethanol and chloroform. The hybridization reaction was performed at 42°C for 24 hours in the following buffer containing approximately 1.6 ng/ml ^{35}S-labeled probes: 4X SSC, 50% deionized formamide, 1% *N*-lauroyl-sarcosine sodium salt, 0.12 mol/L phosphate buffer (pH 7.2), 0.5X Denhardt's solution. After hybridization, the sections were washed sequentially in 1X SSC at 55°C using four changes for 15 minutes each and in 0.2X SSC for 10 minutes. The sections were then dehydrated in a series of graded ethanol and dried. For emulsion autoradiography, the slides were dipped in autoradiographic emulsion (Ilford K-5) diluted with an equal volume of distilled water. After a 3- or 4-week period of exposure, the autoradiograms were developed in Kodak D-19 and counterstained with hematoxylin.

Synthetic Oligonucleotide Probes

Oligonucleotide probes (50-mers), complementary to nucleotide sequences of human PDGF-A and -B chains,[8,9] were synthesized on a DNA synthesizer (Applied Biosystems, Foster City, CA). These probes were analyzed using the computer-assisted homology search of the GCG program (Wisconsin University Genetics Computer Group, Madison, Wisconsin) and chosen as specific antisense sequences at nucleotide position 404 to 453 for the PDGF-A chain[8] (ATGAGGACCT TGGCTTGCCTGCTGCTCCTCGGCTGCGGATACCTCGCCCA) and at nucleotide position 983 to 1032 for the PDGF-B chain[9] (ATGAATCGCTGCTGGGCGCTCTT CCTGTCTCTCTGCTG CTACCTGCGTCT). To confirm the validity of hybridization, competition experiments were carried out by hybridizing with a 100-fold excess of unlabeled probe. Furthermore, sense sequences at the corresponding positions of the probes were used also as controls in this study. In these control experiments, no specific hybrid signals were detected on autoradiograms.

Identification of Cell Types

For the identification of cells, the *in situ* autoradiographed slides were counterstained with hematoxylin. In all cases, the cells were compared with those identified from immunohistochemically stained sections, using sections from coronary slices fixed in methanol-Carnoy's solution next to the slices used for *in situ* hybridization. In most cases, moreover, immunohistochemically stained serial frozen sections from the paraformaldehyde-fixed slices, used for *in situ* hybridization, were used also to evaluate cell types.

Results

The target site in each of the six dilated coronary artery segments contained a laceration of the intimal lesion but without involvement of the media. The *in situ* hybridization, using PDGF-A and -B chain probes, was considered positive when the grain density exceeded three times that of the mean background density.

The sites remote from the target site showed mild atherosclerotic plaques or diffuse intimal thickening without injury. *In situ* hybridization revealed no positive signals in these lesions (Figure 1).

At 6 days after PTCA, positive autoradiographic signals for PDGF-A and -B chain mRNA were found. These were confined to the lacerated area at the shoulder of an eccentric and predominantly fibrous plaque (Figure 2). The signals for PDGF-B mRNA were more prominent than those obtained for PDGF-A mRNA. Immunocytochemical studies showed this area to contain macrophages (HAM-56$^+$). The anti-vWf stain for endothelial cells at these sites was negative.

At 14 days after PTCA, there was a concentric, mainly fibrous plaque with a superficial laceration. At this site a few spindle cells were identified (vimentin$^+$), but none showed positivity for actin (HHF-35$^-$). Macrophages were not identified. *In situ* hybridization showed this area to contain an occasional cell with a weak positive signal for PDGF-B mRNA, whereas that for PDGF-A was negative (data not shown).

At 28 days after PTCA, the target site contained a concentric and lipid-rich plaque. A plaque fissure was present, with intraplaque hemorrhage and partial wash-out of atheromatous debris. The fibrous border zone of the PTCA-lacerated pre-existent plaque contained spindle cells (vimentin$^+$, HHF-35$^-$) and a few macrophages (HAM-56$^+$). At the luminal side, moreover, serial sections revealed

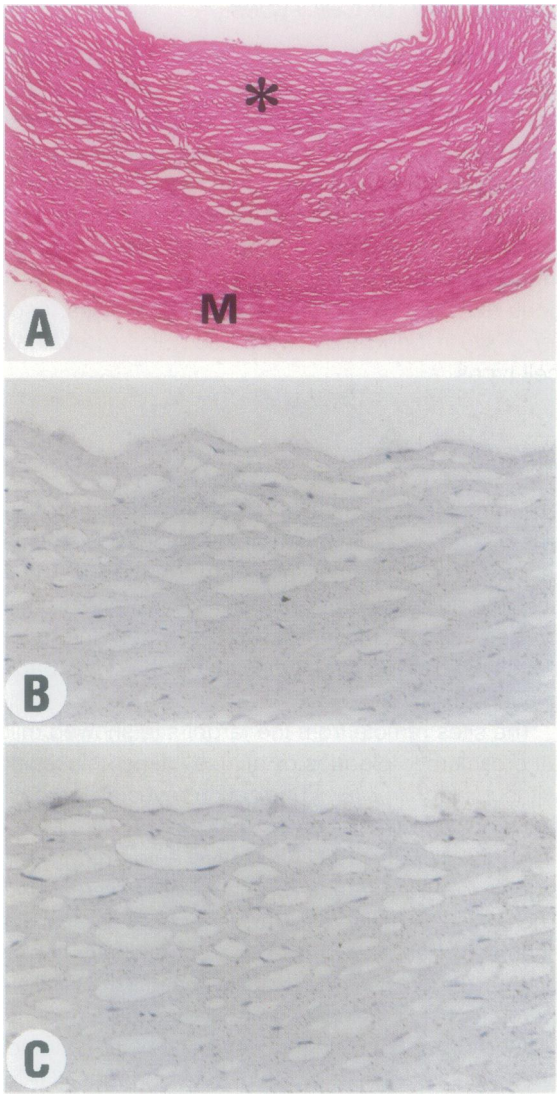

Figure 1. *A fibrous plaque remote from the target site* (**A**) *used for* in situ *hybridization for PDGF-A* (**B**) *and PDGF-B* (**C**) *mRNA. There are no positive signals. The* **asterisk** *indicates the fibrous plaque from which* **B** *and* **C** *have been taken. Magnification,* ×*33* (**A**) *and* ×*130* (**B** *and* **C**). *M, media.*

areas with a localized accumulation of cells. Immunocytochemical staining showed these areas also to contain spindle cells (vimentin$^+$, HHF-35$^-$) and macrophages (HAM-56$^+$). *In situ* hybridization revealed that a subset of the cells along the borders of the crater, but also within the adjacent fibrous tissue, contained positive signals for both PDGF-A and -B mRNA (data not shown).

At 44 days after PTCA, there were two target sites available for study. The site in the left circumflex artery showed laceration of the shoulder part of an eccentric plaque, which was partly fibrous and in part contained a large lipid pool. The latter part showed organization of an intraplaque hemorrhage with a fibrocellular tissue response at the luminal site. The tissue was composed of a large number of non-lipid-laden macrophages, capillaries, and spindle cells, which were positive for vimentin but negative for HHF-35. In addition, a few smooth muscle cells (HHF-35$^+$, CGA-7$^-$) were present (Figure 3). *In situ* hybridization revealed distinct positivity for both PDGF-A and -B mRNA (Figure 4). The signals for PDGF-B mRNA appeared more numerous than those for PDGF-A mRNA. The sites with positive signals co-localized with the site of the organizing intraplaque hemorrhage. Other sections, obtained from the same angioplasty site but approximately 2 mm remote from the area with intraplaque hemorrhage, also showed a fibrocellular response at the luminal site. The neointima was composed mainly of spindle cells (vimentin$^+$, HHF-35$^-$) and macrophages (HAM-56$^+$), with only a small number of smooth muscle cells (HHF-35$^+$, CGA-7$^-$; Figure 5). The neointima contained cells positive for PDGF-A and -B mRNA (Figure 6).

The second target site was located in the left anterior descending artery. It showed laceration of an eccentric lipid-rich plaque. At this site, remnants of an intraplaque hemorrhage were seen, accompanied by an accumulation of macrophages. Along the fibrous border of the ruptured atheroma, a cellular reaction was found, composed mainly of macrophages and spindle cells. *In situ* hybridization revealed positivity similar to that observed at 28 days.

At 56 days after PTCA, the target site showed an eccentric plaque with a laceration limited to the intima and localized at the shoulder of the plaque. The gap was filled with fibrocellular tissue. The cells adjacent to the pre-existent intima, deep inside the fibrocellular tissue, showed positivity for both PDGF-A and -B mRNA. On the other hand, at the luminal site of the fibrocellular tissue response, only a few cells were positive and only for PDGF-A mRNA (Figure 7). Immunocytochemistry showed that PDGF-A and-B mRNA expression was found in macrophage-rich regions at the site adjacent to the pre-existent intima. The luminal site, which contained cells with PDGF-A mRNA expression only, coincided with areas rich in smooth muscle cells.

Discussion

PDGF has a potent effect on migration of vascular smooth muscle cells as shown clearly both in *in vitro*[10] and in experimental animal studies after balloon-induced intimal injury.[2,3] In atherosclerotic plaques in humans, the expression of PDGF-A and

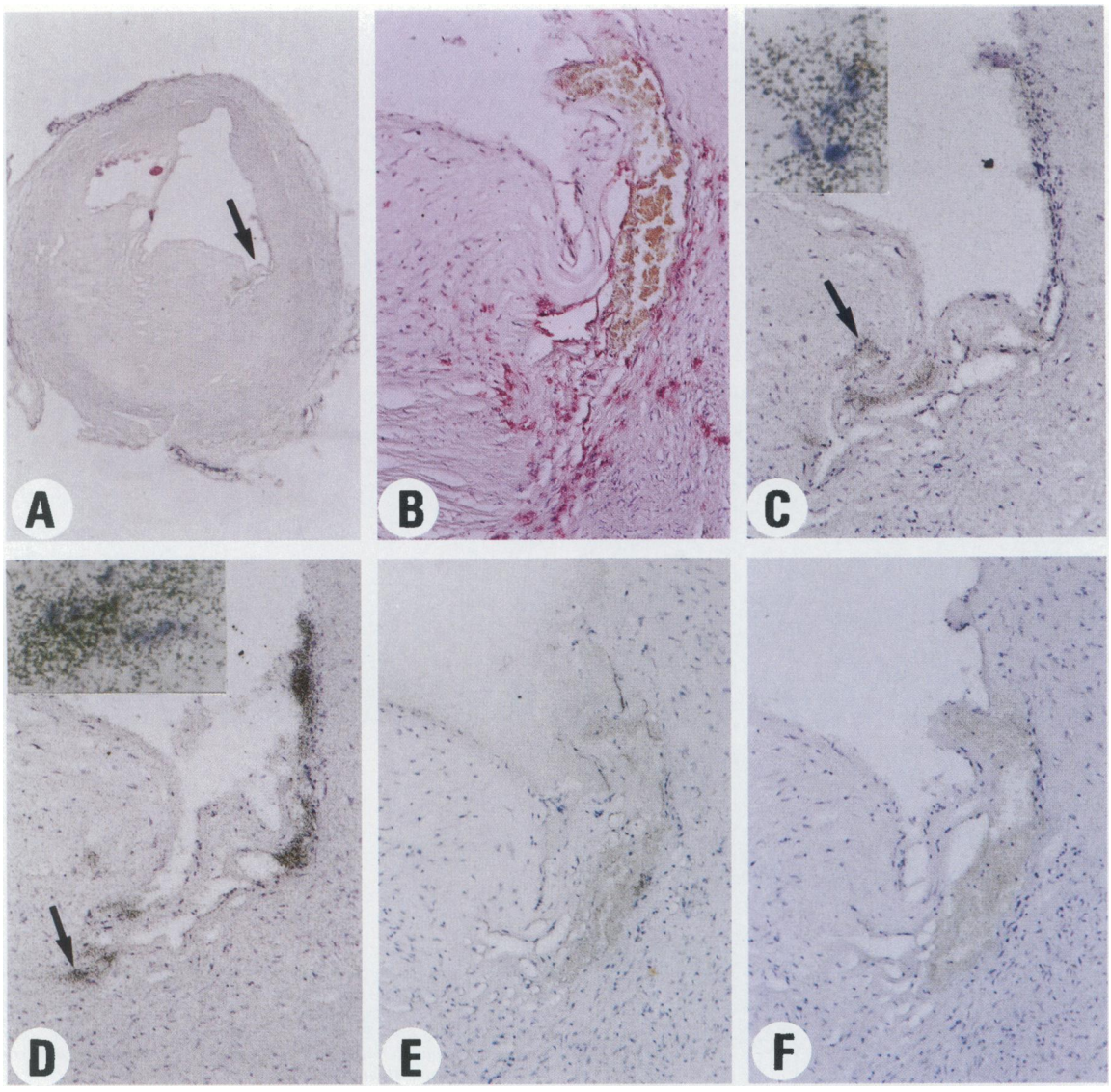

Figure 2. *Coronary artery, 6 days after PTCA.* **A:** *An eccentric and predominantly fibrous atherosclerotic plaque. Magnification, ×13. The site of PTCA injury is indicated by the* **arrow**. **B** *to* **D:** *Details of the site of injury. Magnification, ×63.* **B:** *Presence of HAM-56$^+$ macrophages at the site of injury.* **C** *and* **D:** *Results of* in situ *hybridization with the antisense PDGF-A and -B chain mRNA probes, respectively, at the site of laceration.* **Insets** *in* **C** *and* **D:** *Detail of cells with positive signals for PDGF-A and -B mRNA, respectively, taken from the area indicated by an* **arrow**, *respectively. Magnification, ×440* (**inset C**) *and ×350* (**inset D**). **E** *and* **F:** *Results of* in situ *hybridization with the sense PDGF-A and -B chain mRNA probes, respectively, which serve as controls. Magnification, ×63. There are no positive signals.*

-B mRNA has been documented using *in situ* hybridization techniques.[11] Immunohistochemical studies, moreover, have shown that both PDGF-A and -B chain protein occur in these lesions.[12,13] These observations thus provide direct evidence that PDGF plays a role in atherogenesis. However, to the best of our knowledge, the present study is the first to document the expression of PDGF-A and -B mRNA at sites of repair in human coronary arteries after PTCA. Several aspects need additional consideration.

Is PDGF Expression Part of Pre-Existent Atherosclerosis or Part of the Reparative Response?

Our observations that expression of PDGF is limited to the repair tissue itself strongly suggest that PDGF-A and -B mRNA expression is related directly to the reparative response. Indeed, the noninjured segments of these coronary arteries revealed no distinct positivity expression of PDGF mRNA, not even in segments containing athero-

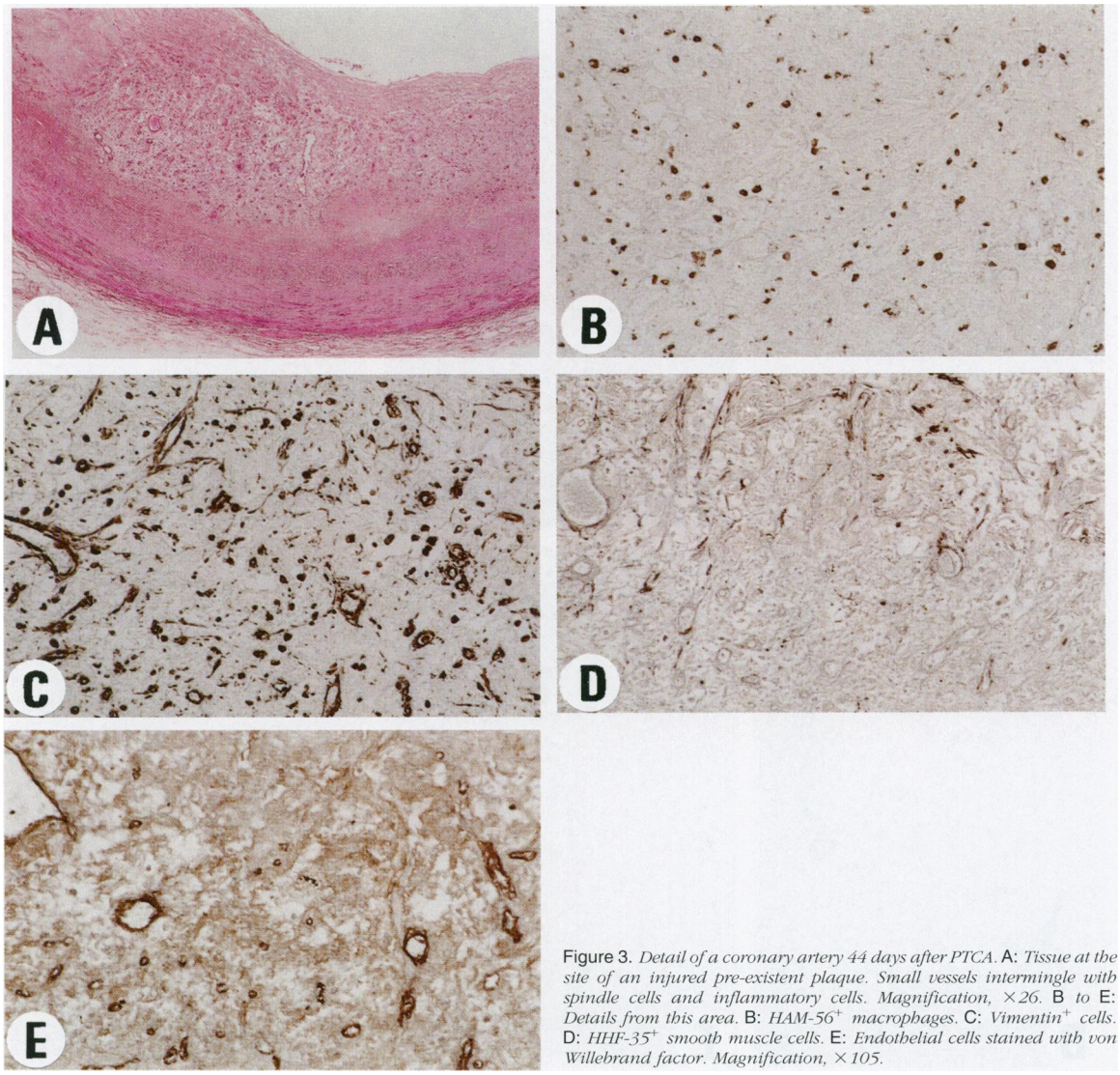

Figure 3. *Detail of a coronary artery 44 days after PTCA.* A: *Tissue at the site of an injured pre-existent plaque. Small vessels intermingle with spindle cells and inflammatory cells. Magnification, ×26.* B *to* E: *Details from this area.* B: *HAM-56$^+$ macrophages.* C: *Vimentin$^+$ cells.* D: *HHF-35$^+$ smooth muscle cells.* E: *Endothelial cells stained with von Willebrand factor. Magnification, ×105.*

sclerotic lesions. This is of interest as Barrett and Benditt[14,15] previously demonstrated PDGF-A and -B mRNA by Northern blot analysis of human atherosclerotic plaques removed from carotid arteries by endarterectomy. The limitation of such an analysis, however, is the lack of histological verification of the component make-up of the lesion. Wilcox and associates[11] also found expression of PDGF-A and -B mRNA in atherosclerotic plaques of carotid arteries obtained by endarterectomy and they used an *in situ* hybridization technique. The positivity that they observed was limited to cells within the intimal lesions and endothelial cells (see below). The fact that we were unable to find expression of PDGF-A and -B mRNA in the atherosclerotic lesions in coronary arteries remote from sites of PTCA injury could relate to differences in plaque morphology. Indeed, the non-target plaques in our study were mildly thickened and largely fibrous in nature, with little or no lipid pool. It could be, therefore, that PDGF was expressed at such a low level that our technique did not allow for a proper identification in the presence of the usual background scatter. Taking the above as a likely explanation for the differences between our results and those obtained by Wilcox and associates,[11] we feel confident that the expression of PDGF-A and -B mRNA at sites of repair after PTCA-induced laceration in these human coronary arteries indeed is enhanced expression.

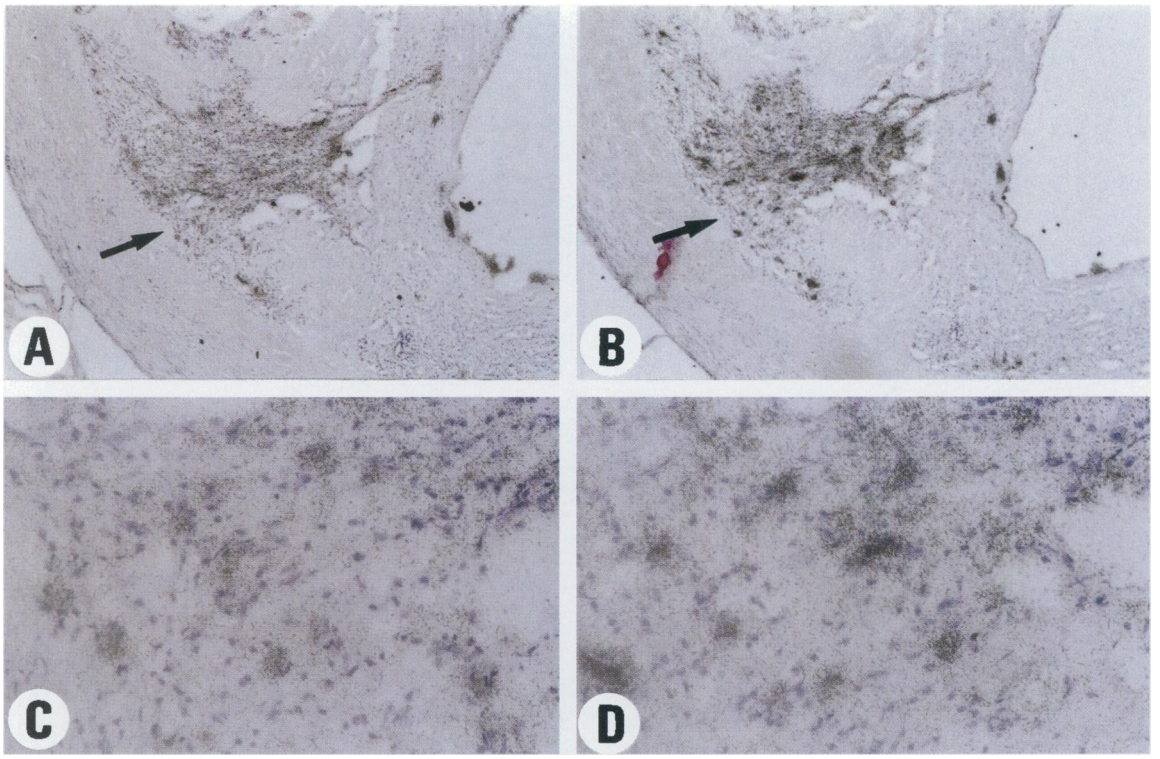

Figure 4. *Section through the neointima at the site of repair of the same case shown in Figure 3.* A *and* B: *Results of* in situ *hybridization for PDGF-A and -B chain mRNA, respectively. Magnification,* ×*26.* C: *Detail of positive signals for PDGF-A mRNA in the area indicated by an* arrow *in* A. D: *Detail of positive signals for PDGF-B mRNA in the area indicated by an* arrow *in* B. *Magnification,* ×*167.*

What Types of PDGF Are Expressed?

Another important aspect to consider is the fact that both PDGF-A and -B mRNA are expressed in human coronary arteries after PTCA. This observation is at variance with that reported by Majesky et al.[1] They studied PDGF gene expression with *in situ* hybridization techniques in rat carotid arteries 2 weeks after balloon angioplasty injury. They found strong expression for PDGF-A mRNA in a subpopulation of neointimal smooth muscle cells, localized at or near the luminal surface, but no expression for PDGF-B mRNA. The differences between our observations and those obtained in rats could be due to species differences. However, one has to consider also that the mechanisms responsible for the response to injury in normal tissues are different from those triggered in atherosclerotic lesions. The neointimal thickening that occurs after balloon injury of normal rat carotid arteries, for instance, consists almost entirely of smooth muscle cells with very few, if any, macrophages. In contrast, the underlying atherosclerotic lesion in human coronary arteries often contains large amounts of macrophages, known to contain large quantities of PDGF-B chain protein.[12] Moreover, the repair tissue induced in human coronary arteries after PTCA certainly is not uniform. Indeed, we have shown previously that the type of response depends mainly on the nature of the tissue injured.[6,16] Laceration of the media or the adjacent superficial musculoelastic layers in human coronary arteries induces a rapid proliferative fibrocellular tissue response, composed predominantly of smooth muscle cells, whereas injury to a lipid-rich atherosclerotic plaque may cause plaque thrombosis and intraplaque hemorrhage, with or without wash-out of the atheromatous debris, and a retarded proliferative response dominated by neovascularization, spindle cells, and macrophages.[6,16–18] In the present study, all target sites contained PTCA lacerations limited to the atherosclerotic plaque, without injury of the media. In view of the above, therefore, it would be of interest to be able to study the expression of PDGF-A and -B mRNA in human coronary arteries at sites of medial laceration. At this stage, we have to conclude that both in rats and in humans PDGF appears to play a role in the response to injury after angioplasty, but it remains to be settled whether the subtle differences observed between our study and that of Majesky and co-workers[1] relates to differences in species, to differences in the time interval between

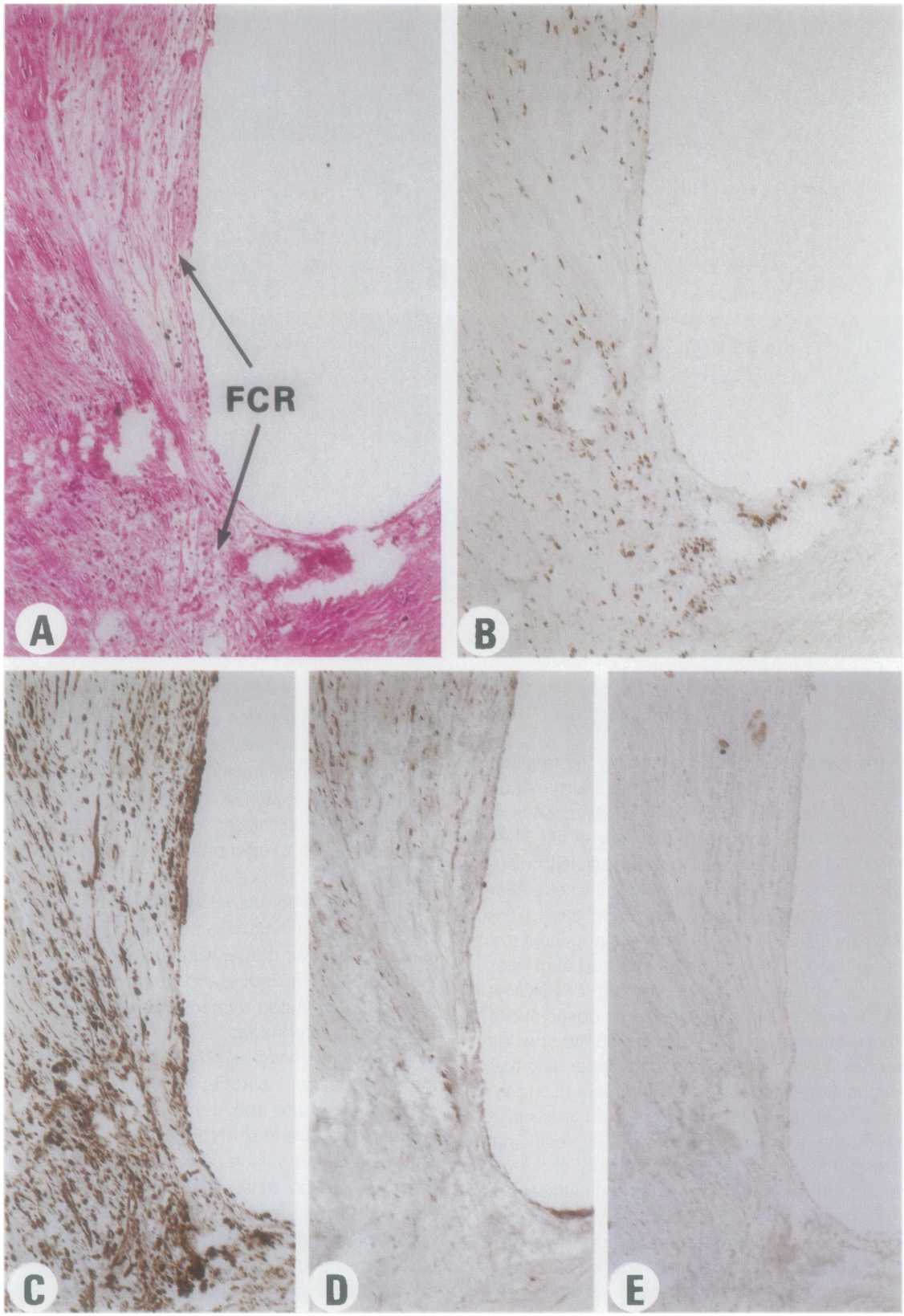

Figure 5. *Detail of the luminal surface of a coronary artery, 44 days after PTCA.* A: *An area with fibrocellular response (FCR) that consists of spindle cells and inflammatory cells.* B *to* D: *The same area as shown in* A. B: *HAM-56$^+$ macrophages.* C: *Vimentin$^+$ cells.* D: *HHF-35$^+$ smooth muscle cells.* E: *CGA-7 smooth muscle cells are not stained. Magnification, ×76.*

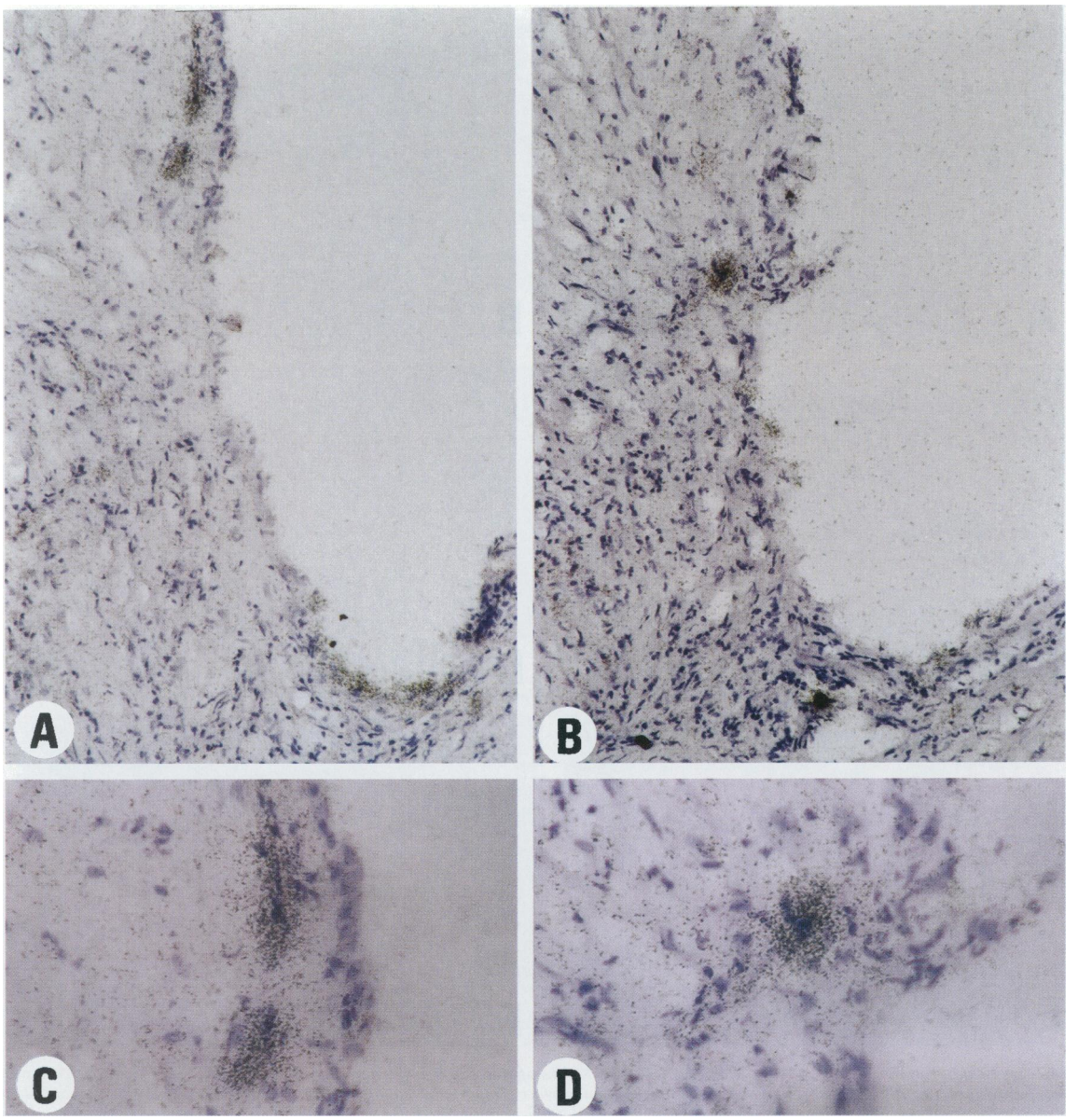

Figure 6. *Same case as shown in Figure 5.* **A** *and* **B:** *Results after* in situ *hybridization for PDGF-A and -B chain mRNA, respectively. Magnification, ×122.* **C:** *Cells with positive signals for PDGF-A mRNA at higher magnification.* **D:** *Cells with positive signals for PDGF-B mRNA at higher magnification. Magnification, ×260* (**C** *and* **D**).

the procedure and the sampling of tissue, or to differences in the tissue exposed, such as the presence of PDGF-B-rich macrophages in humans, which could account for the pronounced expression of PDGF-A mRNA in rats compared with man.

Are Cells Involved in the Expression of PDGF after PTCA and, if so, What Cells?

In vitro experiments have revealed that macrophages,[19–21] endothelial cells,[22–24] and smooth muscle cells[25–27] can produce PDGF. In human atherosclerotic lesions, Ross and associates[12] demonstrated that PDGF-B chain protein (in the form of PDGF-BB or PDGF-AB) is expressed predominantly by macrophages. *In situ* hybridization studies by Wilcox and associates,[11] on the other hand, led to the conclusion that the predominant cell types involved in the expression of PDGF-A and -B chain mRNA are mesenchymal-appearing intimal cells and endothelial cells, respectively, with little or no expression in macrophages.

The present study revealed a rather heterogeneous population of cells that co-localized with the

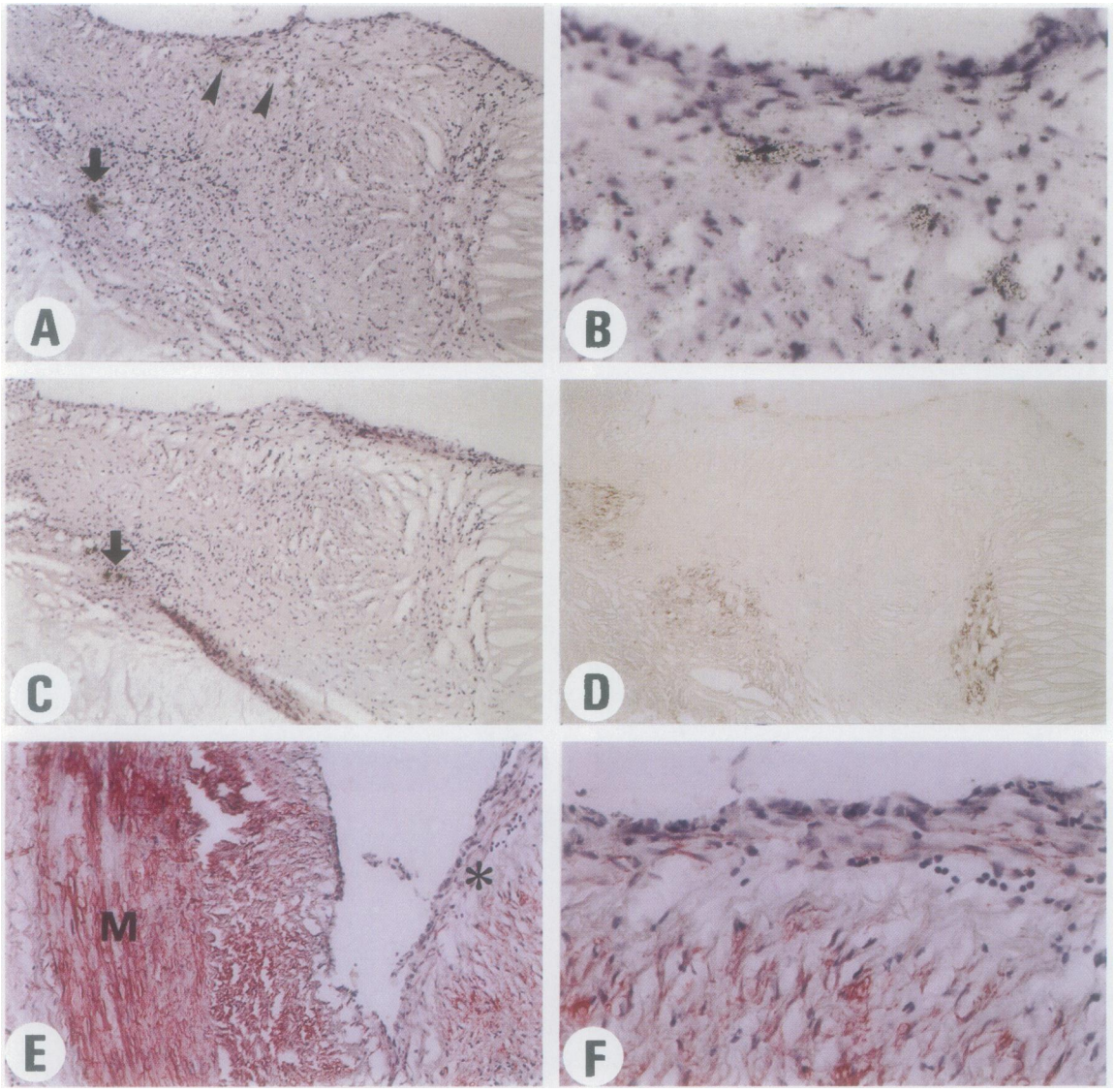

Figure 7. *Segment of coronary artery, 56 days after PTCA.* **A** *to* **C**: *Results after* in situ *hybridization for PDGF-A and -B chain mRNA, respectively.* **A**: *Some positive cells* (**arrowheads**) *accumulated close to the luminal surface. This area is magnified in* **B** (×*212*). **A** *and* **C**: *Positivity is also shown in the deeper zone* (**arrows**), *adjacent to the pre-existent wall tissues. Magnification,* ×*52.* **D**: *HAM-56*$^{+}$ *macrophage-rich regions in the deeper layers, which correspond with the areas of positivity for PDGF-A and -B mRNA. Magnification,* ×*52.* **E**: *HHF-35*$^{+}$ *smooth muscle cells in the superficial layers* (**asterisk**). *Smooth muscle cells of the plaque-free media* (*M*) *in the same artery stain positive and serve as an internal control. Magnification,* ×*83.* **F**: *Enlargement of the area indicated by the* **asterisk** *in* **E**. *Magnification,* ×*210. Note the HHF-35*$^{+}$ *smooth muscle cells.*

cells that express PDGF-A or PDGF-B mRNA. The cells that co-localized were macrophages, spindle cells (cells negative with actin markers, such as HHF-35 and CGA-7, but positive with anti-vimentin), smooth muscle cells (cells positive with HHF-35, but negative with CGA-7; see also Ref. 17), and endothelial cells at sites of neovascularization. The precise origin of the spindle cells remains to be elucidated. Experimental studies[28,29] and our own observations in human repair tissues in coronary arteries after PTCA[30] strongly suggest that these cells represent de-differentiated smooth muscle cells. They may represent the mesenchymal-appearing intimal cells described by Wilcox and co-workers,[11] reported to express PDGF-A and -B chain mRNA. This is of interest as the expression of PDGF mRNAs relates to the degree of differentiation of smooth muscle cells.[4,31] Majesky and co-workers,[31] for instance, showed that cultured smooth muscle cells isolated from young rat aortas expressed both PDGF-A and -B genes, whereas those isolated from adult rat aortas expressed the PDGF-A gene only. Furthermore, these investigators[4] recently demonstrated

also that smooth muscle cells cultured from the neointima of injured rat arteries expressed both PDGF-A and -B genes, whereas those from the media of non-injured rat arteries expressed PDGF-A but little or no PDGF-B mRNA. Our observations in human coronary arteries after PTCA, therefore, endorse the experimental data and seem to indicate that the de-differentiated smooth muscle cell, which appears as the initial response to injury, is capable of expressing both PDGF-A and -B genes.

At the latest stage in this series (56 days after PTCA), it appeared that the expression of PDGF-A and -B mRNA was much reduced compared with earlier stages. In addition, smooth muscle cells, identified as such because of their positive staining with HHF-35, also co-localized with PDGF-A mRNA but not with PDGF-B mRNA. At this stage, spindle cells as defined above were rarely identified in the neointima. This observation further suggests that a shift had occurred from the synthetic phenotype toward a more contractile phenotype of smooth muscle cells, as previously reported in human coronary arteries after PTCA.[17,18,30] Hence, phenotypic modulation of smooth muscle cells may relate to the expression of PDGF.

What Role Can Be Attributed to the Expression of PDGF-A and -B mRNA?

Recently, Siegbahn and co-workers[32] and Koyama and co-workers[33] have demonstrated that PDGF-AB and -BB are potent chemotactic factors for fibroblasts, monocytes, and smooth muscle cells *in vitro*. Moreover, it has been suggested recently that PDGF-BB also acts as an extremely potent and efficacious repressor of the expression of multiple smooth-muscle-specific proteins, including smooth muscle α-actin, smooth muscle myosin heavy chain, and smooth muscle α-tropomyosin.[34–36] Recent *in vitro* experiments, moreover, have shown that PDGF-AA inhibits smooth muscle cell migration[33] and that repression of smooth muscle cell α-actin by PDGF is not dependent on the PDGF-A chain.[35] These experimental data support the concept that PDGF gene expression is important not only for the regulation of chemotaxis but also for the phenotypic cytoskeletal modulation of smooth muscle cells as part of the formation of neointima after vessel wall injury. Our observations suggest that a similar mechanism is in operation in human coronary arteries after PTCA injury. In fact, our findings may well provide the background for the favorable results of a clinical trial, recently reported by Maresta et al,[37] demonstrating that trapidil (triazolopyrimidine), a PDGF antagonist, reduces restenosis after PTCA.

Limitations of the Study

The main limitation is the small number of cases studied. The reason for that is obvious, but it should not detract from the fact that the observations may be different once a large cohort of patients is studied. For instance, all post-PTCA lacerations encountered in this series were limited to the intima. It could well be that cases with medial injury may give a different pattern of PDGF expression. Moreover, as only six points in time were available, the findings do not necessarily represent a reliable time course.

A second drawback relates to the fact that we have not been able to co-localize cell types with *in situ* hybridization. Being unsuccessful initially, we ran out of sections covering the target sites. Hence, the co-localization as referred to in this work is based on comparisons between adjacent sections, so that strictly speaking the identification of the cell types involved has not been demonstrated unequivocally.

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