Fibrocellular Tissue Response After Percutaneous Transluminal Coronary Angioplasty
An Immunocytochemical Analysis of the Cellular Composition

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**Background.** Restenosis after initial, successful percutaneous transluminal coronary angioplasty (PTCA) is due to fibrocellular proliferation.

**Methods and Results.** The present study focused on the nature of fibrocellular tissue in humans by use of immunocytochemical techniques. Four hearts (five coronary arteries) were investigated; time lapse between PTCA and death varied between 20 days (two arteries) and 1 year 7 months. Proliferating cells stained positive with smooth muscle cell–specific monoclonal antibodies. Cells from early proliferative lesions (20 days) have a phenotypic expression different from cells in “old” lesions. Proliferating cells stained positive with vimentin but were negative with desmin, irrespective of the lesion’s age.

**Conclusions.** The findings indicate a change in actin isoform expression of smooth muscle cells while adapting to a pathological state. (Circulation 1991;83:1327–1332)

Restenosis after successful percutaneous transluminal coronary angioplasty (PTCA) is due to fibrocellular tissue proliferation. The cells involved are generally considered to be of vascular smooth muscle cell origin, although most of the evidence is based on findings in experimental animals. In humans, ultrastructural evidence has been produced, and one study has applied immunocytochemical techniques, using monoclonal antibodies to actin and myosin.

The present study was devoted specifically to the immunophenotypic identification of the cellular components responsible for the fibrocellular proliferation after an initial, successful PTCA.

**Methods**

Hearts were obtained from four patients who had undergone an initial, successful angioplasty but who subsequently died. In these four patients, five coronary arteries had been dilated. In one patient, a second angioplasty was performed in the same segment because of restenosis. The relevant clinical data are summarized in Table 1.

All hearts were fixed in methanol–Carnoy’s fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid). This method has the advantage that the immunocytochemical methods, necessary for this study, can be performed on paraffin-embedded sections. Thus, histological features are preserved better than those in frozen sections, allowing for better comparisons with conventional morphological studies.

The coronary arteries were removed from the epicardial surface.

The localization of the angioplasty site was decided by precise measurements of the distances in the angiograms at PTCA, using coronary ostia and bifurcation sites. The angioplasty sites of the arteries were sectioned serially at 1-mm intervals. Each 1-mm coronary segment was routinely processed and embedded in paraffin. A total of 112 segments from five coronary arteries were examined. Thirty serial sections from each segment were cut at a 5-μm thickness. Every eighth and ninth section was stained with hematoxylin and eosin and with Weigert’s elastic van Gieson’s stain, respectively, and the other sections were used for immunocytochemical staining.
TABLE 1. Relevant Clinical Data

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Reason for PTCA</th>
<th>Site of PTCA</th>
<th>PTCA (n)</th>
<th>Interval PTCA/death</th>
<th>PTCA artery narrowing (% DR)</th>
<th>Maximal atm at PTCA</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82</td>
<td>F</td>
<td>AMI</td>
<td>LAD</td>
<td>1</td>
<td>20 day</td>
<td>Pre 80, Post 30</td>
<td>12</td>
<td>AMI and pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>F</td>
<td>AP, SMI</td>
<td>LAD</td>
<td>2</td>
<td>10 mo</td>
<td>Pre 100, Post 25</td>
<td>7</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>M</td>
<td>AP</td>
<td>LAD</td>
<td>1</td>
<td>1 yr 5 mo</td>
<td>Pre 85, Post 40</td>
<td>4</td>
<td>Unknown</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>F</td>
<td>AMI</td>
<td>LAD</td>
<td>1</td>
<td>1 yr 7 mo</td>
<td>Pre 100, Post 50</td>
<td>4</td>
<td>Renal failure</td>
</tr>
</tbody>
</table>

PTCA, percutaneous transluminal coronary angioplasty; DR, diameter reduction; AMI, acute myocardial infarction; AP, angina pectoris; SMI, scarred myocardial infarction; LAD, left anterior descending coronary artery; LCx, left circumflex artery.

Immunocytochemistry

From each segment, sections were mounted for immunohistochemical staining.

The following monoclonal antibodies were used. Antibody HHF-35, a monoclonal anti-actin antibody against muscle-specific actin, was used. It recognizes α- and γ-actin isoforms common to all muscle cells, including smooth muscle cells. A second monoclonal antibody, antibody CGA-7, was used. It reacts with specific α- and γ-actin isoforms specific to smooth muscle cells. The specificity of these antibodies has been reported previously.4–7

Monoclonal antibodies to factor VIII–related antigen, to vimentin, and to desmin were obtained from Dako Laboratories (catalog Nos. M616, M725, and M760, respectively). The source, specification, and working dilutions of the antibodies used are summarized in Table 2.

The immunoperoxidase avidin–biotin complex system with nickel chloride color modification was performed in all instances.8,9 Sections were counterstained with hematoxylin.

Results

All the dilated arterial segments revealed lacerations extending into the media. Each of these segments, moreover, contained a distinct fibrocellular proliferation at the site of injury. The results of the immunohistochemical studies are summarized in Table 3.

In case 1 (20 days after PTCA), the cells within the reactive fibrocellular tissue, in both arteries, were stained positive with HHF-35 but negative with CGA-7. The cells, moreover, were stained positive with vimentin but negative with desmin. No positivity with the antibody against factor VIII–related antigen was found within the reactive fibrocellular tissue or at the luminal surface (Figures 1A–1F).

In the remaining three arteries (cases 2–4), the reactive tissue was of a distinct older age, that is, being less cellular and more fibrotic. Most cells, nevertheless, were stained positive with both HHF-35 and CGA-7 (Figures 2A and 2B). These cells were generally stained negative with desmin but, again, were positive with vimentin. There was a gradient in the staining reactivity of the CGA-7 anti-actin antibody that appeared to be weaker in the subendothelial layers, which was in contrast to the staining intensity of HHF-35 anti-actin antibody and anti-vimentin antibody. Factor VIII–positive cells, covering the fibrocellular tissue, were observed in cases 3 (Figure 2C) and 4. In case 2, factor VIII–positive cells were lining the surface at some sites but were absent at others.

In the left anterior descending coronary artery of case 2, the fibrocellular tissue consisted of two distinct layers, suggesting an earlier and older response, in keeping with previous observations of repeated PTCA.10 There was a difference in the number of cells between these two layers but no differences in reactivity with HHF-35 and CGA-7 anti-actin, with anti-desmin, and with anti-vimentin antibodies.

Discussion

The cellular component causing restenosis after successful PTCA is widely considered to be the

TABLE 2. Source, Specification, and Working Dilutions of the Antibodies

<table>
<thead>
<tr>
<th>Designation</th>
<th>Specificity</th>
<th>Cell identified</th>
<th>Reference/source</th>
<th>Working dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHF-35</td>
<td>Muscle actin</td>
<td>SMC</td>
<td>Reference 4</td>
<td>1:3,200†</td>
</tr>
<tr>
<td>CGA-7</td>
<td>SMC actin</td>
<td>SMC</td>
<td>Reference 2</td>
<td>1:400*</td>
</tr>
<tr>
<td></td>
<td>Desmin</td>
<td>SMC</td>
<td>Dako Laboratories</td>
<td>1:100†</td>
</tr>
<tr>
<td></td>
<td>Vimentin</td>
<td>Fibroblasts</td>
<td>Dako Laboratories</td>
<td>1:100†</td>
</tr>
<tr>
<td>Factor VIII-Rag</td>
<td>Macrophages</td>
<td>Endothelial cells</td>
<td>Dako Laboratories</td>
<td>1:20†</td>
</tr>
</tbody>
</table>

SMC, smooth muscle cells.

*As ascites fluid; †as supernatant fluid.
vascular smooth muscle cell; thus far, though, in humans, this conclusion is largely inferred from findings in experimental animals.

Using arterial segments after PTCA obtained at necropsy and using electron microscopy, Ohara et al concluded that the proliferating cells, which appeared to migrate from the media, were smooth muscle cells that changed from the contractile to the synthetic phenotype. At late stages of the response (in their series from 52 days onward), the smooth muscle cells had returned to the contractile phenotype. Gravanis and Roubin recently reported the results of an immunocytochemical study, using monoclonal antibodies directed against actin and myosin on “intimal myoproliferative” lesions at sites of previous coronary artery dilations. They concluded that the vast majority of cells were of smooth muscle cell origin.

Work in experimental animals has shown unequivocally that the phenotypic expression of vascular smooth muscle cells, in many ways, adapts to pathological conditions. Chamley-Campbell et al described “diminished” myosin immunostaining in the smooth muscle cells of monkey intimal plaques. Gown and associates reported a markedly reduced reactivity with the CGA-7 anti-actin antibody in proliferating rat smooth muscle cells after experimentally induced wall injury. Gabbiani and his group reported that proliferating rat aortic smooth muscle cells, after endothelial cell injury, showed a decrease in actin and desmin in a switch in the actin isotype expression from a predominant α-actin isotype of smooth muscle cells to a predominant β-actin isotype. These changes in the cytoskeleton of the intimal smooth muscle cells have also been documented at the level of messenger RNA. Fifteen days after balloon injury to the rat aorta, at a stage of active smooth muscle cell replication, the cells of the neointima showed decreases in α-actin messenger RNA levels in smooth muscle cells and α-actin smooth muscle cell synthesis, both of which reverted to normal 60 days after injury when the aorta was reendothelialized. These investigators suggest a relation between cytoskeletal changes and the presence or absence of endothelial cells. Moreover, their results also indicate that rat aortic smooth muscle cells, involved in a reparative response, may return to “normal” morphological and biochemical states once their task has been completed.

The present study, using immunohistochemical techniques and based on human arterial segments obtained at necropsy, after an initial, successful PTCA, provides further scientific basis for the contention that the cell response leading to restenosis is one of vascular smooth muscle cells. The conclusion is based on the positive staining with monoclonal antibodies specific to smooth muscle cells. In addition to the previous study of Gravanis and Roubin, the present work adds to the understanding of biological processes operative in these proliferative lesions by the use of different anti-actin muscle antibodies and by the use of vimentin and desmin stains. Several points of additional interest arise.

First, a difference in the staining capacity with the two actin-related monoclonals was observed. In the early stage of response, the proliferating cells stained positive with HHF-35, but no reactivity was obtained with CGA-7. However, in more advanced (older) lesions, the latter monoclonal did react with the proliferating cells. Apparently, the epitope for α- and γ-actin isotypes of smooth muscle cells is masked or modified during the early stage of the proliferative response. This is of interest because it is assumed that the fibrocellular response at sites of PTCA laceration is “mature” at approximately 2–3 months. Because case 1 (20 days after PTCA) still contained actively proliferating cells, whereas the lesions in the other cases were of a much older age, one may argue that the phenotypic expression, as revealed by the two monoclonals used, is different from early to mature stages. These results thus endorse the experimental data obtained by Gown and associates, using the same (CGA-7) anti-actin antibody in rat and in human arterial smooth muscle cells in culture. This strongly suggests that vascular smooth muscle cells in humans also exhibit differentiations in the expression of different isotypes of actin in relation to the stage of the proliferative response. It remains a matter of further study to what extent this finding relates to the changes from a “contractile” to a “synthetic” vascular smooth muscle cell. In fact, it is still controversial whether or not prolifer-

### Table 3. Results of the Immunohistochemical Studies

<table>
<thead>
<tr>
<th>Case</th>
<th>Site of PTCA</th>
<th>Interval PTCA/death</th>
<th>Immunohistochemical studies on fibrocellular tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LAD</td>
<td>20 days</td>
<td>HHF-35: ++, CGA-7: −, Desmin: −, Vimentin: +++, Factor VIII: −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>LAD</td>
<td>10 months</td>
<td>HHF-35: ++, CGA-7: +*, Desmin: −, Vimentin: +, Factor VIII: +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 months</td>
<td>HHF-35: ++, CGA-7: +*, Desmin: −, Vimentin: +, Factor VIII: +</td>
</tr>
<tr>
<td>3</td>
<td>LAD</td>
<td>1 year/5 months</td>
<td>HHF-35: ++, CGA-7: +*, Desmin: −, Vimentin: +, Factor VIII: +</td>
</tr>
<tr>
<td>4</td>
<td>LAD</td>
<td>1 year/7 months</td>
<td>HHF-35: +++, CGA-7: +*, Desmin: −, Vimentin: +, Factor VIII: +</td>
</tr>
</tbody>
</table>

PTCA, percutaneous transluminal coronary angioplasty; LAD, left anterior descending coronary artery, LCx, left circumflex artery; +++, almost all cells positive; +*, approximately half of all cells positive; −, negative.

*There is a gradient of antibody reactivity: The closer to the preexistent media, the stronger the reactivity.
Figure 1. Micrographs of a segment of the left anterior descending coronary artery in case 1 (20 days after percutaneous transluminal coronary angioplasty), revealing immunocytochemical characteristics of proliferating cells at the site of laceration. Panels depict serial sections. Panels A and B: Laceration of the preexistent (plaque-free) wall with the damage affecting the musculoelastic intimal layer and the media. (Panel A, elastic tissue stain; original magnification, ×90; panel B, hematoxylin-eosin stain; original magnification, ×140). Panel C: Adjacent section stained with the anti-actin monoclonal antibody HHF-35. Proliferating cells show distinct stain positivity (as do preexistent smooth muscle cells of the media). Adjacent preexistent musculoelastic intimal layer shows absence of staining at sites with little cellularity. Panel D: Adjacent section stained with the anti-actin monoclonal antibody CGA-7. With this monoclonal, there is weak staining of preexistent cells of the media but no reactivity of proliferating cells at the site of laceration. Panel E: Staining characteristics after the use of an anti-desmin monoclonal antibody: absence of staining in proliferating cells but distinct positivity in smooth muscle cells of the preexistent media. Panel F: Application of the monoclonal antibody to factor VIII-related antigen shows absence of staining at the site of the lesion; at one site, adjacent to the proliferative response, the preexistent endothelial cell layer shows positive staining. (Panels C–F: original magnification, ×90.)
ating cells at sites of PTCA are of the “synthetic” type. One ultrastructural study provides evidence for such a change, but other studies describe “contractile” smooth muscle cells in those lesions. The aforementioned observation could well indicate that the differences expressed in the literature reflect differences in the stage of the response rather than a fundamental difference. In this context, it is of major interest that despite a change in the phenotypic expression of actin, the cells were all stained positive with vimentin and negative with desmin. Because the media of these arterial segments stains positive with desmin, a change in the expression of cytoskeletal proteins should have occurred if the cells are assumed to derive from the preexistent medial smooth muscle cells.

The present study, moreover, shows that once antibody reactivity with the CGA-7 anti-actin antibody is present, a gradient in staining intensity occurs. The monoclonal antibody stained weakest in the immediate subintimal layers and strongest deep in the media. Gown et al described a similar gradient of antibody reactivity with this monoclonal antibody (CGA-7) in human arteries, albeit at sites of a fibrous atherosclerotic plaque. The interpretation of this phenomenon remains to be elucidated but could relate to aspects of cell adaptation as previously discussed.

The present study also documents reendothelialization at the site of a lesion. To the best of our knowledge, only Gravanis and associates have reported immunohistochemical data regarding the restoration of endothelial cells at sites of PTCA repair. They conclude that complete restoration of the endothelial cell lining is accomplished usually within 1 month from the time of dilation. In our study, the 20-day-old lesions showed no positivity for factor VIII-related antigen. Case 3 showed an “incomplete” lining with regard to staining characteristics. Of possible relevance in this context is that this particular artery had undergone repeated PTCA at 10 and 5 months before our study. The other two arteries, both with aged fibrocellular tissue reactions, showed endothelial cells uniformly present and covering the site of the lesions. Of interest also is that the reappearance of endothelial cells coincides with the change in staining characteristics with the anti-actin monoclonal CGA-7, although this finding may be criticized because of the rather large differences in the “age” of the lesions in case 1 and in cases 2–4. Nevertheless, it brings to mind the experimental findings by Gabbiani’s group, which suggest that a direct relation exists between cytoskeletal differentiations and the state of the endothelial cell lining.

The present study thus provides data that the proliferative response in humans, after PTCA, is indeed one of vascular smooth muscle cells. What is more, these observations in humans seem to endorse data obtained from experimental animals, indicating that smooth muscle cell adaptation to a pathological
condition, such as lacerations produced by PTCA, is related to a change in the actin isoform expression. Hence, these findings add new information to the nature of the cellular mechanisms that underlie restenosis in humans and, for that matter, to the concepts of intervention.

References


KEY WORDS • percutaneous transluminal coronary angioplasty • restenosis • vascular smooth muscle cells • actin isoforms
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