Induction of Acidic Fibroblast Growth Factor and Full-Length Platelet-Derived Growth Factor Expression in Human Cardiac Allografts

Analysis by PCR, In Situ Hybridization, and Immunohistochemistry

Xiao-Ming Zhao, MD; Tiong-Keat Yeoh, MD; William H. Frist, MD; Diane L. Porterfield, BS; Geraldine G. Miller, MD

Background Further understanding of cardiac allograft vasculopathy (CAV) is needed to improve long-term survival after cardiac transplantation. The diffuse hyperplasia of coronary intima characteristic of CAV suggests that growth factors may play a role in the development of CAV. Fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) are potent mitogens for smooth muscle cells (SMCs), and PDGF is an important cofactor in the pathogenesis of native coronary atherosclerosis.

Methods and Results Reverse transcriptase/polymerase chain reaction (RT/PCR), in situ hybridization, and immunohistochemistry were used to determine whether transplantation results in increased cardiac expression of acidic (a)FGF, basic (b)FGF, and PDGF-A and -B chains. Sixty-eight myocardial biopsies from 36 heart transplant recipients and 7 normal hearts were analyzed by PCR. aFGF mRNA was present in 54 of 61 allograft biopsies and was not found in any normal heart. In situ hybridization and immunohistochemistry demonstrated diffuse, intense expression of aFGF mRNA and protein in allograft biopsies, predominantly in myocytes and vascular walls. Only scattered aFGF expression was observed in normal hearts. mRNA for the full-length isoform of PDGF-A chain was found in 43 of 61 allograft biopsies and was not detected in any normal heart. In situ hybridization and immunohistochemistry confirmed that full-length PDGF-A chain mRNA and PDGF protein were present in myocytes and vascular walls.

Conclusions Expression of aFGF and PDGF-A chain is significantly increased in cardiac allografts. Cardiac myocytes and vascular walls are the predominant sources of aFGF and PDGF. Diffuse expression of these growth factors in cardiac allografts may be important in the pathogenesis of CAV.

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Key Words • vessels • polymerase chain reaction • RNA • growth substances

Cardiac allograft vasculopathy (CAV) is a major cause of late death in heart transplant recipients.1,2 CAV occurs at an annual incidence of 15% each year and is angiographically detectable in up to 45% of heart transplant recipients at 4 years,3 although its true prevalence may be higher.4 Immunological mechanisms are suspected to underlie the pathogenesis of CAV, and increasing evidence, although controversial, supports an association between CAV and rejection.1,2,5-10

CAV is a diffuse and rapidly developing process characterized histologically by smooth muscle cell (SMC) hyperplasia and endothelial cell (EC) proliferation.4 Several growth factors have been identified that promote vascular cell proliferation in vitro. Acidic fibroblast growth factor (aFGF) (also known as heparin-binding growth factor-1 and EC growth factor) and basic FGF (bFGF) are potent mitogens for SMCs and ECs,11-15 whereas platelet-derived growth factor (PDGF) stimulates growth of SMCs and is associated with atherosclerosis of native coronary arteries.16-19 Studies on humans and animals have demonstrated enhanced expression of bFGF and PDGF receptors in heart and kidney allografts.20-24 Upregulation of PDGF-A chain but not -B chain is noted in vascular grafts.25 Our previous studies on small numbers of human cardiac allograft biopsies and T-cell clones found aFGF gene expression in the majority of cardiac allografts and several T-cell clones,26 leading to the hypothesis that T cells present in allografts might be a source of this growth factor. The present studies were performed to confirm that expression of aFGF increases with transplantation, to determine whether upregulation of other growth factors occurs in cardiac allografts, and to identify the cellular sources of these growth factors in allografts.

Methods

Isolation of RNA From Myocardial Biopsy Specimens

Sixty-eight myocardial biopsies from 36 heart transplant recipients (mean age, 42 ± 12 years; mean follow-up, 22 ± 20 months; range, 1 week to 72 months) and 7 normal donor
hearts before transplantation were analyzed in this study. All patients participating in this study received standard immunosuppression (cyclosporine, azathioprine, and prednisone). The studies were approved by the Institutional Review Board. Myocardial allograft (posttransplant heart) specimens were collected at the time of regular surveillance biopsy. Biopsy specimens were read for histopathology and classified according to ISHLT (International Society for Heart Transplantation) criteria by staff pathologists who were unaware of the study results. Myocardium from pretransplant normal donor hearts was obtained from the right ventricle (endomyocardium) immediately after organ excision. Individual biopsies for reverse transcriptase polymerase chain reaction (RT/PCR) were placed in sterile freezer vials (Nunc) and stored at −70°C until used for RNA preparation. Total cytoplasmic RNA was isolated from myocardial biopsy specimens by the guanidinium thiocyanate–phenol chloroform method in 500 μL homogenization buffer and used for RT (reverse transcription).

**RT/PCR**

Total RNA from myocardial biopsies and cultured human kidney ECs (kindly provided by Dr T.O. Daniel, Vanderbilt University) was used as template for synthesis with oDT and MMLV reverse transcriptase (BRL). PCR amplification of cDNA was performed with the following primers: aFGF, ([5’]5’ATGGCTGAAGGAGAATCCATCACACAC; [3’]5’CAGAAGAAGACTGGCAGGGGGAG, Clontech); bFGF, ([5’]5’GGGAGCATCACCACGCCCGCC; [3’]5’CACATCAACACTGGTATTTCTTGTA, Stratagene); PDGF-A chain,[28] ([5’]5’TCTCCCGGCATCCACCGGAGCC; [3’]5’GGCTGCGGTCTCCTCCTCA); and PDGF-B chain,[28] ([5’]5’CCCCAGCCCCCACTTGGGCC; [3’]5’GGAATACGCAAGAAATACCA). Human GAPDH (Clontech) primers were used as a positive control: ([5’]5’TGAAGGTCGGAGTCAACGGATTTGGT; [3’]5’CTTGAGTCGGAGTCAACGGATTTGGT); and ([5’]5’GGAATACGCAAGAAATACCA). To ensure that negative PCRs on myocardial biopsies were not due to markedly different starting concentrations of mRNA, PCR analysis for constitutively expressed GAPDH mRNA was performed on serial twofold dilutions of cDNA for each sample. The last dilution giving a positive reaction for GAPDH was used to equalize the amount of cDNA used in each PCR. This analysis of GAPDH showed that the amount of mRNA present in cardiac allograft biopsies was generally similar to that in normal hearts, although a trend toward higher cDNA concentration was observed in samples derived from normal hearts (P<NS). PCR reactions were performed in 10 mmol/L Tris/HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.2 mmol/L dNTP, and 2 U Taq polymerase (Perkin Elmer Cetus). Primers were added at a final concentration of 0.5 μmol/L. Reactions were carried out in a DNA Thermal Cycler (Perkin Elmer Cetus) for 35 cycles, including denaturing at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes for each cycle. PCRs with no template (negative controls) and with a known positive control (usually kidney EC cDNA) were routinely performed in all experiments to ensure absence of contamination and to demonstrate that the primers, enzymes, and buffer functioned properly. PCR products were analyzed on 2% ethidium bromide–stained agarose gels.

**In Situ Hybridization and Immunohistochemistry**

Myocardial biopsies used for histology were embedded in O.C.T. compound (Miles) immediately after biopsy and stored at −70°C until cutting. Sections were cut (6 μm) and fixed in 3% paraformaldehyde–phosphate-buffered saline (PBS). In situ hybridization was performed with the In Situ Workstation (British Bio-techology Products) and solutions as recommended by the manufacturer. Hybridization with specific probes was carried out for 16 hours at 37°C. Probes were single-stranded DNA oligonucleotides (200 ng/mL) labeled by 3′ with digoxigenin-11-dUTP (Boehringer Mannheim).

The oligonucleotide probes used for in situ hybridization were antisense aFGF (5’-CAGAAGAGACTGGCAGGGGGAG-3’) and an antisense PDGF-A chain probe specific for exon 6 (British Bio-technology). An antisense probe for GAPDH (5’-CATGGCCATGAGTCCACCC-3’) was used for each sample as a positive control to ensure that mRNA in myocardial biopsies was intact. A sense probe for GAPDH (5’-TGAAGGTCGGAGTCAACGGATTGTG-3’) served as the negative control to demonstrate that hybridization was specific for the appropriate RNA and that hybridization to DNA was not being detected. After hybridization, slides were washed sequentially with 4×, 2×, and 0.2× SSC, all containing 30% formamide at 37°C and then with 0.1% Triton X-100/Tris-buffered saline (TBS). Alkaline phosphatase–conjugated Fab monoclonal antidigoxigenin was used for colorimetric detection of digoxigenin with nitro blue tetrazolium substrate (Boehringer Mannheim) and subsequently counterstained with hematoxylin-eosin.

For immunohistochemistry staining, samples were initially processed as described above. After samples were cut and fixed, slides were treated with 3% hydrogen peroxide/methanol for 20 minutes and then washed with PBS. After 30 minutes of blocking with 5% bovine serum albumin (BSA), slides were incubated with anti-aFGF (15 μg/mL in 1% BSA/TBS) and anti-PDGF-AA (10 μg/mL in 1% BSA/TBS) for 2 hours at room temperature. The antibodies used for these studies were mouse monoclonal anti-aFGF (UBI) and rabbit polyclonal anti-PDGF-AA (UBI), which cross-reacts with PDGF-AB heterodimers. After incubation and washing with PBS, slides were then incubated with peroxidase-conjugated second antibody [goat anti-mouse IgG, 1:200 in 1% BSA/TBS (UBI) or goat anti-rabbit IgG, 1:500 dilution (Southern Biotechnoloy)] and subsequently stained with diaminobenzidine followed by counterstaining with hematoxylin.

**Results**

The myocardial biopsies were analyzed for aFGF, bFGF, and PDGF-A and -B gene expression by PCR. Sixty-one biopsies were from allografts, and 7 were from pretransplant normal donor hearts. Histology of allograft myocardial biopsies was studied in parallel with PCR. Of the 61 allograft biopsies, 43 were negative for rejection. Eight and 10 showed mild (grade I) and moderate (grades II and III) rejection, respectively. Included in this analysis were 43 biopsies taken from 13 patients before, during, and after rejection.

**aFGF and bFGF Gene Expression in Myocardial Biopsies**

Our previous experiments on 21 allograft myocardial biopsies demonstrated that 18 of 21 biopsies were positive for aFGF gene expression by PCR.[26] Our conclusions from those data were limited by the absence of information on aFGF expression in normal human hearts by use of the same techniques. We now have extended this analysis to normal donor hearts before transplantation and 40 additional allograft biopsies. aFGF gene expression was not detected by PCR in any of the 7 normal donor hearts before transplantation and 40 additional allograft biopsies.

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aFGF gene expression was not detected by PCR in any of the 7 normal donor hearts before transplantation and 40 additional allograft biopsies. (Fig 1, Pre-transplant). In contrast, aFGF mRNA was present in 36 of 40 allograft myocardial biopsies by PCR (Fig 1, Allograft; Table 1). Together with the previous results, aFGF gene expression was detected in 54 of 61 allograft myocardial biopsies (89%). Twenty-one of 23 biopsies obtained within 1 month of transplant and 33 of 38 obtained after 1 month were positive for aFGF gene expression (Table 1). Once present, aFGF gene expression persisted in the same individual and was not present.
correlated with histological rejection. As shown in Fig 1, multiple amplified aFGF DNA bands were found in allograft biopsies. We sequenced these products previously and found that they were amplified from mRNAs generated by alternative splicing within the coding sequence.26 The sequences included the usual aFGF coding sequence, a previously described alternatively spliced aFGF mRNA,30,31 and a novel variant.26

Six posttransplant biopsies were also available from 5 of the hearts that had been biopsied before transplantation (Fig 1, Post-transplant biopsies, lanes 11 through 15, are from the same hearts as the Pre-transplant normal donor heart biopsies, lanes 6 through 10). These biopsies were obtained at intervals of 1 week to 3 months after transplantation, and all were positive for aFGF gene expression.

PCR for bFGF expression was performed on biopsies from 26 allografts and 5 normal hearts. bFGF gene expression was not detected in either allografts or normal donor hearts but was present in control cDNA (data not shown).

**TABLE 1.** aFGF and Full-Length PDGF-A Chain Gene Expression in Myocardial Biopsies

<table>
<thead>
<tr>
<th>T</th>
<th>N</th>
<th>aFGF (n1)</th>
<th>F-PDGF-A (n2)</th>
</tr>
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<tbody>
<tr>
<td>&lt;1 mo</td>
<td>23</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>1-3 mo</td>
<td>13</td>
<td>12</td>
<td>11</td>
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<tr>
<td>3 mo-1 y</td>
<td>12</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>&gt;1 y</td>
<td>13</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Pre-Tx</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aFGF indicates acidic fibroblast growth factor; PDGF, platelet-derived growth factor; T, time after heart transplantation; N, number of biopsies examined; n1, number of biopsies that were positive for aFGF gene expression by polymerase chain reaction (PCR); F-PDGF, full-length PDGF; n2, number of biopsies that were positive for F-PDGF-A chain gene expression by PCR; and Tx, heart transplantation.

**TABLE 2.** Correlation of aFGF and Full-Length PDGF-A Chain Gene Expression in Allograft Myocardial Biopsies

<table>
<thead>
<tr>
<th>aFGF(+)</th>
<th>aFGF(+)</th>
<th>aFGF(-)</th>
<th>aFGF(-)</th>
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<tbody>
<tr>
<td>PDGF-A(+)</td>
<td>PDGF-A(-)</td>
<td>PDGF-A(+)</td>
<td>PDGF-A(-)</td>
</tr>
<tr>
<td>Number</td>
<td>39</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

aFGF indicates acidic fibroblast growth factor; PDGF, platelet-derived growth factor; (+), negative for gene expression; and (+), positive for gene expression.

PDGF-A and -B Chain Gene Expression in Myocardial Biopsies

Myocardial biopsies from allografts and normal donor hearts were analyzed by PCR for expression of PDGF-A and -B chain transcripts. PDGF-A chain primers corresponding to sequences in exons 4 and 7 were chosen so that alternative usage of exon 6 could be determined. Alternative splicing of exon 6 has been shown previously to result in long-form (full-length) and short-form PDGF-A chain messages that encode molecules with different carboxy terminal sequences.32,33 RNA from cultured kidney ECs, which constitutively express PDGF-A and -B chains, was used as a positive control. A representative agarose gel is shown in Fig 1. Amplification of kidney EC cDNA with PDGF-A chain primers revealed two products with sizes corresponding to those expected for both alternatively spliced A chain transcripts. The full-length form (F-PDGF-A) including exon 6 was 250 bp, and the short form (S-PDGF-A) excluding exon 6 was 181 bp. The short form was the predominant transcript. PCR for PDGF-B chain on kidney ECs showed a single band corresponding to the predicted size of 867 bp (data not shown).

Full-length PDGF-A chain transcripts were detected in 44 of 61 allograft biopsies (Fig 1 and Table 1). Its expression was not correlated with histological rejection. Compared with aFGF, F-PDGF-A chain gene expression was delayed after transplantation. Thirteen of 23 biopsies (versus 21 of 23 for aFGF, P=.001) obtained within 1 month and 31 of 38 biopsies (versus 33 of 38 for aFGF, P=.75) obtained after 1 month posttransplantation were positive for F-PDGF-A chain gene expression (Table 1). F-PDGF-A chain message was not detected in any of the 7 normal donor hearts (Fig 1). Of 6 biopsies obtained from these hearts after transplant, 5 were negative at 1 to 3 weeks after transplantation, and 1, at 3 months, was positive for F-PDGF-A chain gene expression. Short-form PDGF-A chain messages were detected in all allografts and normal donor hearts (Fig 1). As shown in Table 2, 39 of the 61 allograft myocardial biopsies were positive for both aFGF and F-PDGF-A chain gene expression, 15 were positive for aFGF but negative for F-PDGF-A chain, 5 were negative for aFGF but positive for F-PDGF-A chain, and 2 were negative for expression of both genes. PCR for PDGF-B chain was performed on 26 allograft and 5 normal donor heart biopsies. PDGF-B chain message was not found in either allografts or normal donor hearts (data not shown).
Expression of aFGF and PDGF mRNA in Cardiac Allografts by In Situ Hybridization and Immunohistochemistry

The results described above demonstrated enhanced expression of aFGF and full-length PDGF-A chain genes in cardiac allografts by use of PCR. However, these experiments cannot identify the cells producing aFGF and PDGF. Therefore, we used in situ hybridization and immunohistochemistry to identify the cellular sources of aFGF and PDGF and to confirm the differences in allografts and normal hearts. The results of the in situ hybridization are shown in Fig 2. Negative and positive controls for hybridization with both allograft (upper panels) and normal heart (lower panels) biopsies show that the hybridization was specific for RNA (Fig 2A and 2E) and that the RNA in the biopsies was
intact (Fig 2B and 2F). Positive control hybridization for constitutively expressed GAPDH mRNA was similar in the allograft and normal heart biopsies (Fig 2B and 2F). In contrast, intense, diffuse hybridization was seen for aFGF mRNA in the allograft, with little or no signal evident in the normal heart (Fig 2C and 2G). Myocytes were clearly the predominant cells producing aFGF message, but vascular structures also demonstrated positive hybridization with aFGF. These results are consistent with the PCR data showing that aFGF mRNA is virtually undetectable in normal hearts and is induced after transplantation.

In situ hybridization was also performed with a probe specific for exon 6 of PDGF-A chain that hybridizes to mRNA only for full-length PDGF-A chain. These results also showed that expression of this gene was increased in cardiac allografts compared with pretransplant normal hearts (Fig 2D and 2F), and mRNA was present mainly in myocytes and to a small degree in vascular cells. Little or no full-length PDGF-A chain mRNA was identified in normal hearts. Although present in the allograft biopsies examined, the intensity of PDGF-A chain hybridization was clearly less pronounced than that for aFGF.

Immunohistochemistry with antibodies for aFGF and PDGF (Fig 3) gave results similar to those found with in situ hybridization. Dense staining with anti-aFGF was observed in cardiac allograft biopsies (Fig 3B), predom-
inantly in myocytes and vascular walls. Faint staining was seen in normal hearts (Fig 3E). Antibody to PDGF-AA also demonstrated that expression of PDGF proteins was increased in allografts compared with normal hearts (Fig 3C and 3F) and was localized in vascular structures and cardiac myocytes. Again, faint staining was present in the normal heart (Fig 3F). This antibody is directed against PDGF-AA homodimer; it does not differentiate the long form from the short form and may also react with PDGF-AB heterodimers.

**Discussion**

The long-term success of heart transplantation remains limited at present by the frequent development of CAV. Because of the diffuse vascular intimal hyperplasia observed in this disease, we have begun to investigate the expression of growth factors for vascular SMCs and ECs that could evoke these proliferative lesions. Our present studies examined the effects of heart transplantation on cardiac expression of two potent SMC growth factors, aFGF and PDGF. The PCR, in situ hybridization, and immunohistochemistry experiments demonstrate that aFGF and PDGF mRNA and protein are markedly increased in human hearts after transplantation.

aFGF is a potent growth factor for vascular SMCs and ECs. Thus, the major changes in aFGF gene expression and protein production that occur in the heart after transplantation may be expected to have significant effects on these cells. Previous studies by other laboratories have examined the cardiac effects of exogenous and endogenous aFGF in experimental models. The effects of exogenous aFGF applied to the epicardium were investigated by Banai et al.\(^4^4\) using a canine model of ischemia induced by an amiodar constrictor. In these experiments, aFGF caused rapid vascular intimal hyperplasia in areas of ischemic injury, whereas vessels in adjacent normal myocardium were unaffected. The effects of endogenously produced aFGF were examined in elegant experiments reported by Nabel and her colleagues.\(^3^5\) These investigators introduced a recombinant, secreted form of aFGF into normal porcine iliofemoral arteries by direct gene transfer. Endogenously produced aFGF induced extensive, obliterative intimal hyperplasia within 21 days, and these lesions were similar to those observed in CAV. These physiologically distinct experiments show clearly that aFGF can produce vascular intimal hyperplasia in vivo. Our in situ hybridization and immunohistochemistry experiments demonstrate that cardiac myocytes are the predominant source of aFGF in transplanted hearts. Together with the data from other groups, these experiments provide strong support for the hypothesis that aFGF contributes to the diffuse lesions of intramyocardial vessels in CAV.

The presence of small amounts of aFGF protein, despite absence of aFGF mRNA by PCR in biopsies from normal hearts, is consistent with previous studies from other laboratories. Schmidt et al.\(^3^6\) using PCR and ethidium bromide–stained gels, identified aFGF mRNA in only one of three preparations of normal porcine myocardium starting from 0.5 g tissue, several hundred-fold more tissue than present in our biopsy specimens. In the absence of an experimental ischemic stimulus, which is an inducer of aFGF expression, Sharma et al.\(^3^7\) were unable to demonstrate aFGF mRNA in porcine myocardium. Similarly for bFGF, protein expression was observed in the absence of detectable mRNA in both myocytes and heart tissue.\(^3^8,3^9\) In contrast to mRNA, aFGF protein has been identified in normal hearts at low concentrations (150 ng/g tissue),\(^4^0\) and our immunohistochemistry data are concordant with these observations.

Our studies did not detect mRNA for bFGF, another member of the heparin-binding growth factor family. However, Gordon and colleagues\(^4^0\) found bFGF protein by immunohistochemistry in coronary arteries obtained from transplanted hearts. Staining was prominent in the segments from the heart 10 days after transplant but was focal and less pronounced in long-term transplants. The discrepancy between these two studies may be due to the difference between mRNA and protein levels for bFGF discussed above.

PDGF is also a potent mitogen for SMCs.\(^3^4,3^5\) Two distinct PDGF genes, A and B, encode homologous polypeptides that combine into three dimers: AA, AB, and BB.\(^4^1\) Fibroblasts\(^4^2\) and SMCs\(^3^3\) express only PDGF-A chain, whereas ECs\(^4^4,4^5\) and monocytes\(^2^9\) express both A and B chain. aFGF,\(^4^3,4^6\) TGF-\(\beta^3^3,4^7\) IL-6, and TNF-\(\alpha^4^6\) stimulate PDGF-A chain expression in ECs and SMCs. Alternative splicing of exon 6 in PDGF-A chain RNA generates two isoforms of A chain. They differ by the presence or absence of a hydrophilic carboxyl terminus consisting of 18 amino acids.\(^3^2,3^3\) In vitro studies demonstrate that normal resting human umbilical vein ECs\(^3^2,4^8\) and resting monocytes\(^2^9\) predominantly express short-form A chain, whereas tumor cells,\(^2^8,4^8\) activated monocytes,\(^2^9\) and activated ECs\(^3^2\) express both forms. The functional significance of exon 6 is not entirely clear. Collins et al.\(^3^2\) demonstrated that full-length PDGF-A chain increased efficiency of assembly and secretion of the active A-chain homodimer. Recent studies suggest that full-length, extracellular PDGF-A chain may be compartmentalized by binding to matrix rather than diffusible.\(^4^9\) In the present study, full-length PDGF-A chain transcripts were found in the majority of allograft biopsies, regardless of rejection, but not in normal hearts before transplantation. Increased expression of PDGF-A chain has been described in native atherosclerotic lesions.\(^1^0\) Recent studies by Nabel et al.\(^5^0\) have shown that PDGF-B chain expression in porcine vessels induced by direct gene transfer results in severe intimal hyperplasia similar to that produced by aFGF in a similar experimental model.\(^3^5\) Thus, both aFGF and PDGF are capable of inducing vascular intimal hyperplasia in large and small vessels.

aFGF and PDGF are distributed diffusely throughout transplanted myocardium and therefore may account for CAV in intramyocardial vessels; however, the disease is clearly not limited to these vessels. Epicardial vessels and even donor aortas, far removed from myocardial tissue, also develop the lesions of CAV, which often include mononuclear cell infiltrates as well as intimal hyperplasia.\(^3^4\) Banai et al.\(^4^4\) induced CAV-like lesions by applying aFGF to the epicardium via sponges, suggesting that diffusible aFGF can cause disease, thereby raising the possibility that aFGF produced in the myocardium could reach epicardial vessels. Alternatively, vascular SMCs or infiltrating T cells may be the
source of growth factors in larger vessels. Our previous studies have shown that some human T cells produce aFGF.26 Such T cells may be included in lymphocytic infiltrates present in larger vessels,51 initiating or augmenting local production of aFGF and consequent smooth muscle hyperplasia.

Our data do not address two important questions. First, is augmented expression of these growth factors specific to transplantation or a nonspecific response to injury? A clear answer awaits examination of biopsies from other human heart diseases, but data on experimental animal ischemia show that expression of aFGF is increased in vessel walls in ischemic myocardium.37 Thus, at least two forms of injury, immunologic and ischemic, can result in reexpression of a gene that is generally quiescent in the heart after fetal development. This suggests that although aFGF may be induced by multiple kinds of injury, the nature of the inciting stimulus, its location, and its duration may all influence the phenotype that results from aFGF production.

The second question that remains to be addressed is whether other factors or events influence the development of CAV. The finding that more than 80% of allografts express aFGF by 1 year after transplant is inconsistent with the clinical incidence of CAV, even though the latter may substantially underestimate the true frequency of disease. This discrepancy suggests that expression of aFGF may be necessary but not sufficient.
for progression of intimal hyperplasia. Recent experiments in our laboratory indicate that the effects of aFGF may be dependent not only on expression of the growth factor itself but also on the number and type of aFGF receptors expressed. These studies show that reaction episodes modify the normal pattern of FGF receptor isofrom expression and induce the production of receptor isofroms with increased capacity for ligand-induced signal transduction.

In summary, these experiments show that transplantation induces marked increases in expression of two potent growth factors for vascular SMCs, aFGF, and PDGF-A chain. Both are expressed diffusely in myocytes and vascular cells, and an isofrom of PDGF-A chain not found in normal hearts is expressed in cardiac allografts. We propose that these growth factors play important roles in the pathogenesis of accelerated vascular disease in transplanted hearts.

Acknowledgments

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References


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