Longitudinal Analysis of Fibroblast Growth Factor Expression After Transplantation and Association With Severity of Cardiac Allograft Vasculopathy

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Background—Vascular smooth muscle cell growth factors are postulated to contribute to cardiac allograft vasculopathy (CAV). Few data quantitatively address the timing, location, or stimuli for growth factor expression and relationship to CAV.

Methods and Results—Acidic fibroblast growth factor (aFGF) mRNA expression was determined in serial endomyocardial biopsies during the first year after transplantation. Patients with high levels of aFGF mRNA and elevations after the early posttransplant period had significantly more severe CAV than patients with low aFGF and no late elevations.

Conclusions—Parenchymal aFGF expression varies between patients and in the same patient over time and correlates with development of CAV. (Circulation. 1999;100:2396-2399.)

Key Words: surgery ▪ transplantation ▪ growth substances ▪ circulation ▪ genes ▪ muscle, smooth

Cardiac allograft vasculopathy (CAV) describes proliferative neointimal lesions in coronary arteries of transplanted hearts and is part of the spectrum of pathology in chronic rejection. These lesions differ from atherosclerosis of native hearts in their prominence of concentric smooth muscle cell proliferation and extracellular matrix (reviewed in Reference 2). Immune responses to donor antigens are necessary to initiate the development of vascular lesions. However, vascular remodeling that occurs after transplantation most likely reflects the function of growth factors that act on vascular cells and extracellular matrix. Platelet-derived growth factor, acidic (aFGF) and basic fibroblast growth factors, vascular endothelial growth factor, and transforming growth factor-β1 are expressed in allografts, and each probably contributes shared and unique effects to vasculopathy.

FGF is expressed in many adult tissues in response to injury. Our studies have shown that myocytes and small myocardial vessels overexpress FGF and FGF receptors after transplantation. FGFs costimulate T-cell proliferation and cytokine production and thus may potentiate immune responses to donor antigens. FGF also mediates anti-MHC antibody–induced endothelial cell activation and proliferation.

Despite their presence in vascular lesions, our understanding of the contribution of growth factors to vascular disease has been limited by a number of constraints. Most studies in humans have focused on tissue at single time points, often late in the disease, and therefore could not observe potential variations over time that might be important in disease development. Histological assessment is limited in its ability to provide quantitative data that might identify patients at risk or physiological events that induce growth factor overexpression. The present study was performed prospectively to address these issues for aFGF by quantifying its expression in allografts over time and the development of CAV.

Methods

Study Population and Data Collection

Fifteen patients at Vanderbilt University Hospital were prospectively enrolled. The study was approved by the Vanderbilt Institutional Review Board, and all patients gave written informed consent. Patients received immunosuppression with prednisone, cyclosporine, and azathioprine or mycophenylate mofetil. They received standard posttransplantation care, including protocol biopsies to rule out rejection and annual coronary angiography. At each catheterization, 5 biopsies were obtained for histology, and 1 was obtained and frozen for RNA extraction. Biopsies were graded for acute cellular rejection according to criteria of the International Society for Heart and Lung Transplantation (ISHLT) independently of the studies on FGF. Intravascular ultrasound (IVUS) was available only for a subset of patients who enrolled; therefore, annual coronary angiography was used to assess CAV. Coronary angiograms from the second annual cardiac catheterization were reviewed independently by 2 cardiologists who were unaware of the results of studies on FGF. CAV was assessed according to the criteria of Gao et al.6

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including presence of focal stenoses, distal tapering or pruning, and loss of tertiary vessels, and were assigned a numerical rating for severity as absent (0), mild (1), moderate (2), or severe (3). Charts were reviewed for ISHLT rejection grade. Specific clinical variables were also assessed, including cold ischemia time, intraoperative and perioperative course (eg, assist devices, inotropes), hemodynamics, infections, blood pressure, donor/recipient cytomegalovirus (CMV) status, and subsequent CMV serology and culture or antigen assays, serum chemistries, cyclosporine level, and other medications.

**RT-PCR for aFGF and GAPDH Expression**

Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed as described. Briefly, RNA was extracted with RNAzolB (Tel-Test) and reverse transcribed with Superscript H (Gibco BRL). PCR was performed with primers for GAPDH and aFGF and included 32P-labeled dCTP to generate radioactively labeled PCR products for quantification. Standard curves within the linear range of amplification for GAPDH and aFGF were generated with known amounts of each cDNA and were run with each set of PCRs. The concentrations (in pmol) of FGF and GAPDH cDNA in each sample were calculated from these standard curves, and the resulting ratios ([pmol FGF/pmol GAPDH]×100) are presented as relative aFGF levels (Figure). To compare FGF transcription between patients, a mean aFGF level was calculated for each patient as the sum of the relative aFGF levels at each biopsy divided by the number of biopsies analyzed for that patient. The median for all patients was 0.4. Patients with levels above or below the median were classified as having high or low mean FGF levels, respectively.

**Data Analysis**

Differences in cold ischemia time, ISHLT rejection scores, and CAV severity between groups with high and low average FGF levels were analyzed by unpaired Student’s *t* test. A value of *P* < 0.05 was considered significant.

**Results**

**Temporal Pattern of FGF Expression After Transplantation**

Sufficient RNA for quantitative analysis was obtained from 149 endomyocardial biopsies from 15 patients during the first year after transplantation, or ~10 samples per patient. Three patterns of aFGF mRNA transcription were observed (Figure). Six patients showed no increase in aFGF levels throughout the first year (Figure, A). Three patients had early postoperative elevations that were evident at the first posttransplantation biopsy (usually posttransplantation day 7) and returned to low levels by 60 days after transplantation without further increases (Figure, B). Six patients had later elevations, either as multiple discrete increases (Figure, C) or as slowly rising but sustained increases lasting several weeks that subsequently declined (not shown). The results show marked differences between individuals in early postoperative expression of aFGF. Patients with postoperative elevations of FGF had somewhat longer cold ischemia times than those without (191±23 versus 164±44 minutes); however, this difference was not statistically significant (*P*=0.32), suggesting that other events and recipient characteristics contribute to FGF expression.

Clinical data were reviewed to determine whether FGF levels at each biopsy could be related to events potentially associated with allograft injury, such as ISHLT rejection score, hypertension, infection, etc. Among these, a single episode of primary CMV infection occurred in 1 patient (patient 4, Table) and was associated with a rise in FGF (indicated by * in Figure, B). This CMV-seronegative recipient received a transplant from a CMV-seropositive donor. After an initial early postoperative increase, FGF transcripts declined to virtually undetectable levels and then began a second rise, which was followed within a week by clinical CMV syndrome and seroconversion, indicating primary infection. In primary CMV infection after transplantation, the heart itself is the source of CMV and the initial site of viral replication. Thus, the recurrent increase in FGF in this graft
Occurrence of Vascular Disease After Transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>FGF/GAPDH, Mean</th>
<th>Late FGF Rise</th>
<th>CAV Score</th>
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<tbody>
<tr>
<td>1</td>
<td>0.04±0.04</td>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.04±0.04</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.13±0.14</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.45±0.41</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1.31±0.87</td>
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<td>0</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
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<tr>
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<td>2</td>
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<tr>
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<td>3</td>
</tr>
<tr>
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<td>2.03±1.4</td>
<td>Yes</td>
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</tr>
<tr>
<td>13</td>
<td>0.44±0.4</td>
<td>Yes</td>
<td>3†</td>
</tr>
<tr>
<td>14</td>
<td>1.08±1.2</td>
<td>Yes</td>
<td>1‡</td>
</tr>
</tbody>
</table>

*Patient was unable to undergo second annual angiography because of renal insufficiency secondary to cyclosporine nephrotoxicity.
†Patient died 27 months after transplantation, with severe CAV confirmed on autopsy.
‡Patient died 21 months after transplantation and had no second angiography. Mild disease was detected at 1 year after transplantation.

After the initial postoperative rise probably reflects injury induced by local viral replication before symptomatic viral dissemination. No other cases of active CMV infection were identified.

FGF Transcript Levels Are Associated With Severity of CAV

To compare FGF expression with development of CAV, patients were classified as having high or low FGF expression on the basis of whether their mean FGF/GAPDH ratio was above or below the median value (0.4) for all 15 patients. Two years after transplantation, coronary angiography was used to assess the development of vascular disease (Table). One patient (patient 15) died 21 months after transplantation of complications resulting from grade 4 acute cellular rejection. Vascular disease assessment in this patient was based on studies at 1 year after transplantation. Disease severity was assigned a numerical score as described in the Methods section. Patients with high FGF levels had significantly increased severity of CAV (P=0.03). Consistent with this result, all of the patients with severe or moderate CAV were included among the group with late elevations of FGF, whose average FGF levels were generally higher (Table). One patient in the high-FGF group (patient 13) was unable to undergo angiography because of renal insufficiency. Endomyocardial biopsy was negative for acute cellular rejection, and echocardiographic evaluation and right heart catheterization demonstrated poor graft function and decreased cardiac output compatible with CAV. In contrast, patients without late rises, who generally had low levels of FGF, had either no or mild vascular disease at 2 years after transplantation. No other clinical variables, including rejection score, showed a statistically significant association with FGF levels.

Discussion

This is the first study, to the best of our knowledge, that investigates changes in growth factor expression in human cardiac allografts longitudinally over time. The results demonstrate that aFGF varies both between individuals and in the same individual at different times. High FGF expression was significantly associated with increased severity of CAV. That FGF production in the heart is induced after surgery is perhaps not surprising in view of the injurious events associated with ischemia, reperfusion, and surgery. However, a substantial number of patients had no postoperative increase in FGF transcription. Whether this reflects a more benign perioperative course or implies genetic heterogeneity in response to injury is unknown. We suspect that perioperative events play a large role, as exemplified by patient 5, whose high mean FGF level reflects marked elevation in the postoperative period only, and this patient had a stormy perioperative course. In either case, the results establish that growth factor overproduction occurs early in some patients, followed by return to low levels, with resolution of acute injury.

Subsequent elevations were significantly associated with increased severity of CAV. Because patients with late elevations generally had higher mean FGF levels, we suspect that it is the total quantity and cumulative exposure rather than the temporal pattern of expression that is the major contributor to increased risk of CAV. The use of angiography, rather than the more sensitive IVUS, undoubtedly limited our detection to more severe CAV. Because IVUS studies indicate the most rapid progression of intimal hyperplasia during the first year, the results point to a relationship between FGF and this period of rapid progression. Although we focus on FGF, we believe that similar results would be observed for other growth factors, because many of the same inflammatory stimuli induce their expression, and recent data show that platelet-derived growth factor-β and FGF receptors activate a largely overlapping set of genes.

Finally, the finding that FGF elevation occurred at the time of CMV reactivation in the donor heart is novel and intriguing. CMV infection is associated with development of CAV. Initiation of viral replication requires transcription from the CMV immediate early gene promoter, and we have found that FGF enhances transcription from this promoter in vitro (G.G.M., unpublished data). Therefore, FGF expression in the graft may contribute to CMV reactivation from latency, and conversely, induction of FGF by CMV replication within the graft may be one of the mechanisms leading to increased vascular disease with CMV infection.

Acknowledgments

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References


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