Skeletal Myoblast Transplantation in Ischemic Heart Failure
Long-Term Follow-Up of the First Phase I Cohort of Patients

Albert A. Hagège, MD; Jean-Pierre Marolleau, PhD; Jean-Thomas Vilquin, PhD; Armelle Alhérithière, MD; Séverine Peyrard; Denis Duboc, MD; Eric Abergel, MD, PhD; Emmanuel Messas, MD, MSc; Elie Mousseaux, MD; Ketty Schwartz, PhD; Michel Desnos, MD; Philippe Menasché, MD, PhD

Background—Skeletal myoblast (SM) transplantation (Tx) in a post-myocardial infarction (MI) scar experimentally improves left ventricular (LV) ejection fraction (EF). Short-term follow-up (FU) studies have suggested that a similar benefit could clinically occur despite an increased risk of LV arrhythmias.

Methods and Results—We report the long-term FU of the first worldwide cohort of grafted patients (n = 9, 61.8±11.6 years, previous MI, EF ≤35%) operated on (autologous SM Tx and bypass surgery) in 2000 to 2001 and evaluated before Tx, at 1 month (M1) and at a median FU of 52 (18 to 58) months after Tx (37 patient-years). NYHA class improved from 2.5±0.5 to 1.8±0.4 at M1 (P = 0.004 versus baseline) and 1.7±0.5 at FU (P ≈ not significant versus M1; P = 0.0007 versus baseline). EF increased from 24.3±4% to 31±4.1% at M1 (+28%, P = 0.001 versus baseline) and remained stable thereafter (28.7±8.1%, +18% versus baseline). There were 5 hospitalizations for heart failure in 3 patients at 28.6±9.9 months, allowing implant in 2 patients with a resynchronization pacemaker. An automatic cardiac defibrillator (ACD) was implanted in 5 patients for nonsustained (n = 1) or sustained (n = 4) ventricular tachycardia at 12.2±18.6 (1 to 45) months. Despite a beta-blocker/amiodarone combination therapy, there were 14 appropriate shocks for 3 arrhythmic storms in 3 patients at 6, 7, and 18 months after ACD implantation.

Conclusions—In this cohort of severe heart failure patients both clinical status and EF stably improve over time with a strikingly low incidence of hospitalizations for heart failure (0.13/patient-years) and the arrhythmic risk can be controlled by medical therapy and/or on-request ACD implantation. (Circulation. 2006;114[suppl I]:I-108–I-113.)

Key words: follow-up studies ■ heart failure ■ myocardial infarction ■ skeletal myoblasts ■ transplantation

Cellular transplantation is receiving a growing interest as a means of partially compensating for the loss of cardiomyocytes after myocardial infarction (MI). Among the candidate cells, skeletal myoblasts, which are responsible for skeletal muscle formation and repair, have been widely investigated because they feature clinically attractive characteristics (ease of procurement, autologous origin, marked growth potential, myogenic commitment, resistance to ischemia). Myoblast transplantation in a post-MI scar experimentally improves left ventricular (LV) ejection fraction (EF) and short-term follow-up. Phase I surgical studies of patients with ischemic cardiomyopathy and concomitant indication to coronary artery bypass graft (CABG) surgery have suggested that they could incur a similar benefit despite an increased risk of ventricular arrhythmias. However, if short-term results of the procedure are encouraging, long-term follow-up (FU) of a homogeneous cohort of patients is lacking. We report here the long-term FU of the first worldwide cohort of grafted patients operated on in a single center in 2000 to 2001.

Materials and Methods

Study Group

Eligibility for the procedure was based on: (1) systolic LV dysfunction reflected by an echocardiographic LVEF ≤35%; (2) history of MI with a residual akinetic and nonviable scar, as demonstrated by the lack of response to low-dose dobutamine echocardiography and of metabolic activity on fluorine-18 fluorodeoxyglucose positron emission tomography; and (3) indication for concomitant CABG in remote areas (ie, different from the transplanted area). Patients with significant mitral regurgitation (MR) (>2+ on a scale of 4+) by extension of the color jet within the atrium) or poor echogenicity were excluded. The protocol was approved by the French Regulatory Health Authorities and an independent Ethics Committee. Patients were included after having given their informed written consent.


Presented at the American Heart Association Scientific Sessions, Dallas, Tex, November 13–16, 2005.

Correspondence to Albert A. Hagège, Cardiology Department, Hôpital Européen Georges Pompidou, 20 Rue Leblanc, 75015 Paris, France. E-mail albert.hagege@egp.ap-hop-paris.fr
© 2006 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.105.000521

I-108
TABLE 1. Study Group

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Infarction Territory</th>
<th>Cell N (×10⁶)</th>
<th>CD56+ (%)</th>
<th>FU (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>Posterior</td>
<td>820</td>
<td>67</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>66</td>
<td>Anterior</td>
<td>870</td>
<td>91</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>Posterior</td>
<td>620</td>
<td>97</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>Posterolateral</td>
<td>950</td>
<td>95</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>Posterior</td>
<td>880</td>
<td>96</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>Anterior</td>
<td>980</td>
<td>92</td>
<td>52</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>Anterior</td>
<td>1,100</td>
<td>85</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>Anterior</td>
<td>500</td>
<td>75</td>
<td>51</td>
</tr>
<tr>
<td>9</td>
<td>67</td>
<td>Anterior</td>
<td>840</td>
<td>81</td>
<td>48</td>
</tr>
<tr>
<td>Mean</td>
<td>61.8</td>
<td>840</td>
<td>871</td>
<td>86.6</td>
<td>49.4</td>
</tr>
<tr>
<td>SD</td>
<td>11.6</td>
<td>182</td>
<td>62</td>
<td>10.4</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Age indicates age at transplantation; Cell N, number of injected cells; CD56+, % of CD56+ injected cells; FU, follow-up.

Staging of the Procedure
The procedure was described previously.6 From a 10- to 15-grm biopsy of the vastus lateralis muscle, a 2- to 3-week cell culture was started using Good Manufacturing Practices grade products according to the French Regulatory Agency requirements. The cell-containing suspension was transferred into sterile syringes and, on completion of CABG anastomoses in nontransplanted areas, 4 to 8 mL were injected in 27 to 57 sites within and around the scar. The areas of injection were drawn on a map based on the echocardiographic LV segmentation and subsequently used to target echocardiographic analysis of functional changes ascribed to cell transplantation.

Predischage Evaluation
It has previously been reported in detail.1 Briefly, there were no early postoperative complications related to muscular biopsy or cell transplantation, particularly no bleeding, infection, or clinically manifest embolic episodes. Patient 5 died within the first 24 hours after transplantation from a mesenteric infarction, leaving 9 patients available for further evaluation.

Long-Term FU
Long-term FU included repeat (4 per year) clinical (New York Heart Association [NYHA] functional class, hospitalizations for congestive heart failure [CHF] or death), rhythmologic (24-hour Holter electrocardiogram monitoring and/or implantable automatic cardiac defibrillator [ACD] interrogations), and echocardiographic examinations.

Echocardiographic studies were conducted according to a standard protocol. Transthoracic 2-dimensional examinations used fundamental and second-harmonic imaging. Global LVEF (%) was calculated from measurements of LV end-diastolic (LVEDV) and LV end-systolic (LVESV) volumes using the bpline Simpson rule. Regional contractile function was assessed semi-quantitatively after division of the LV into 16 segments, as recommended by the American Society of Echocardiography.10 Care was taken to optimize endocardial visualization on 3 parasternal LV short-axis views (at the base, mid-ventricular, and apex levels) and apical 2-, 3-, and 4-chamber views. The baseline assessment was performed within 1 month before surgery, off episodes of cardiac failure, at rest. Thickening of each segment within the grafted area was visually graded (as dyskinetic, akinetic, severely hypokinetic, moderately hypokinetic, or normal) and serial changes were evaluated semi-quantitatively and classified as unchanged, improved (≥1 grade) or worsened (<1 grade). These grafted areas were localized by reference to the anatomic diagram established intraoperatively and depicting the sites of injections. Image analysis was performed by a single senior investigator, blinded to the timing of echocardiographic data, number and location of CABG, and sites of injections. Excellent reproducibility of segmental thickening evaluation was previously reported in the same cohort of patients.6

Statistics
All results are reported as the mean±SD. Safety and efficacy were evaluated between the first and second months after procedure (M1) and at the end of FU (October 1, 2005) and compared with baseline data (collected within 1 month before the procedure) using a paired nonparametric Wilcoxon test with P<0.05 as the limit of significance.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results
Study Group
Nine male patients formed the study group (Table 1). Age of MI ranged from 3 months to 19 years. Its location was anterior (5), posterior (3), or postero-lateral (1). Four patients were in NYHA functional class II and 5 in class III. Patients received 2 (n=8) to 3 (n=1) bypass grafts. Patient 1 died from a stroke at 17.5 months FU and pathologic examination of the explanted heart showed in-scar clusters of myotubes.11 Thus, 9 patients were evaluated before transplantation (baseline) and at M1, and 8 were available for long-term FU. All received optimal medical therapy before surgery, including diuretics, angiotensin-converting enzyme (ACE) inhibitors, and beta-blockers at maximal tolerated doses and these treatments were maintained at follow-up.

FU
Mean FU was 49.4 months (median, 52 [18–58]), corresponding to 37 patient-years.

Clinical Data
NYHA class improved from 2.5±0.5 at baseline to 1.8±0.4 at M1 (P=0.004 versus baseline) and 1.7±0.5 at end of FU (P=1.0 versus M1; P=0.0007 versus baseline) (Table 2). There were 5 hospitalizations for CHF in 3 patients at 28.6±9.9 (range, 13 to 71) months, corresponding to 0.13/patient-years. Because of persistent symptoms, 2 patients (7 and 9) were implanted with a ventricular resynchronization system.
pacemaker, whereas a third one (2) received ACD with a resynchronization function on the referring physician’s request.

**Echocardiography**

There was no statistical difference between systolic blood pressures measured before each ultrasonic examinations and MR remained minimal or mild. LVEDV remained unchanged over time (201±56 mL at baseline, 209±61 mL at M1, and 215±72 mL at FU, \( P > 0.4 \) for all comparisons). At these time points, LVEF increased from 24.3±4% (18 to 31) to 31±4.1% (25 to 35) at M1 (+28%, \( P = 0.001 \) versus baseline) and remained stable thereafter (28.7±8.1% [17 to 40] at FU [±18% relative to baseline, \( P = 0.64 \) versus M1, \( P = 0.24 \) versus baseline]) (Figure 1). A high interindividual variability was observed as 3 patients continuously improved over time, whereas 3 showed a late deterioration (below baseline values) after an initial increase. According to the surgical scheme of the grafted segments, a total of 24 in-scar akinetic nonviable segments were treated (Table 2). At 1 month, 14 of them (58.3%) demonstrated a new-onset echocardiographic systolic shortening (\( P = 0.015 \)), whereas at end of FU only 8 (33.3%) remained improved (\( P = 0.03 \) versus baseline, \( P = 0.15 \) versus M1) (Figure 2). The corresponding number of improved patients were 7 of 9 at M1 and 6 of 8 (75%) at end of FU.

**Arrhythmias**

An ACD was implanted in 5 patients at 12.2±18.6 (1 to 45) months of FU (Table 3). Indications were asymptomatic nonsustained ventricular tachycardias (SVT) at 45 months after procedure in patient 2 (with no firing during the next year of FU) or SVT within the first month after the procedure in 4 patients (4, 6, 7, and 8). Three of them had evidence for ventricular hyperexcitability (Lown grade 2 to 3) on preoperative Holter recordings. Except for 1 severe syncopal form (patient 4), which necessitated invasive electrophysiologic stimulation for recovery of sinus rhythm, all other episodes were clinically well-tolerated and successfully managed by \( \beta \)-blockers and amiodarone.

---

**Table 2. Functional Evaluation**

<table>
<thead>
<tr>
<th>Patient</th>
<th>NYHA Class</th>
<th>LVEF (%) PRE-M1-FU</th>
<th>Akinetic Grafted Segments PRE-M1-FU</th>
<th>HF Admissions (delay, months)</th>
<th>Resynchronization (delay, months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>III-II-II</td>
<td>20–30-35</td>
<td>4-1-1</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>III-II-II</td>
<td>25–35-22</td>
<td>2-0-2</td>
<td>0</td>
<td>Yes (45)</td>
</tr>
<tr>
<td>3</td>
<td>II-II-I</td>
<td>31–35-40</td>
<td>3-1-2</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>II-II-II</td>
<td>22–35-40</td>
<td>2-0-1</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>III-II-II</td>
<td>26–30-29</td>
<td>2-2-2</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>III-II-II</td>
<td>28–30-23</td>
<td>2-1-1</td>
<td>1 (13)</td>
<td>Yes (27)</td>
</tr>
<tr>
<td>8</td>
<td>II-II-II</td>
<td>25–34-17</td>
<td>3-1-2</td>
<td>2 (33, 35)</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>II-II-II</td>
<td>18–25-25</td>
<td>2-2-1</td>
<td>2 (25, 38)</td>
<td>Yes (38)</td>
</tr>
<tr>
<td>10</td>
<td>III-I-I</td>
<td>24–25-27</td>
<td>4-2-4</td>
<td>0</td>
<td>No</td>
</tr>
</tbody>
</table>

HF admissions indicates number of hospitalizations for CHF during FU, with the delay in months between surgery and each admission. Resynchronization indicates implantation of a pacemaker for ventricular synchronization, with the delay in months between transplantation and device implantation. PRE indicates before the procedure; M1, 1 month; FU, end of follow-up. Akinetic grafted segments indicates number of akinetic segments that have been grafted as shown on the diagram established intraoperatively.
Despite this combination therapy, late SVTs were observed in patients 4 (at 5 months, motivating ACD implantation, and at 7 months, with no further SVT recorded over the next 49 months), 6 (at 8 months, leading to ACD implantation, with no SVT recorded over the next 45 months), and 7 and 8 who had implantation before discharge (at 18 and 6 months of FU, with no recurrence during the next 34 and 46 months, respectively). Three of these late arrhythmias featured an arrhythmic storm (patients 4, 7, and 8), necessitating a total of 14 ACD firings. In 2 of these patients, there were 6 nonappropriate shocks for atrial arrhythmias or sinus tachycardia.

**Figure 2.** Recovery of segmental thickening of a grafted area (arrows). Upper panel, Short-axis endsystolic echocardiographic LV view. Lower panel, Short-axis end systolic MRI LV view. Post indicates at follow-up.

**Discussion**

More than 15 years of experimental data have suggested a beneficial effect of skeletal myoblast transplantation within injured hearts.1–3 Since the first operation performed in June 2000,12 3 surgical studies of small cohorts of patients have combined autologous myoblast transplantation and CABG in post-MI patients with low LVEF.7–9 These phase I studies were not designed or powered to demonstrate the efficacy of the technique, but rather its feasibility and safety. However, the current study differs noticeably from previous publications. Although most of the previous studies were multicentric, with patients occasionally undergoing additional procedures
The current study includes 3 data points functional evaluations, adding more longitudinal information than previous publications, particularly concerning the contrast between improved EF at short-term, as previously shown (+6.8% to +18%), and the decreased beneficial effect at long-term, suggesting a potential loss of efficacy over time. However, a marked interindividual variability, because one-third of the patients exhibited an initial increase followed by a decrease below the pre-transplant values. While improved EF might be consecutive only to associated CABG surgery, it is the only study in which the grafter scar was not bypassed. This is particularly relevant to an accurate interpretation of thickening recovery in the grafted areas and allows a more reliable "proof of concept" demonstration, because contractility of nonviable and nonrevascularized transmural infarcted areas is uncommon after revascularization of remote myocardial territories. Although more than half of the grafted segments demonstrated systolic thickening recovery at short-term, this percentage slightly decreased to 33% at long-term. This is consistent with a high rate of cell death, which is incompletely compensated for by in vivo proliferation within the scar. Cell manipulations to increase graft proliferation, survival, and electrical coupling with the host tissue look to further enhance safety and efficacy of myoblast engraftment. Nevertheless, the majority of the patients still exhibited at least 1 improved segment at end of FU. That was consistent with serial magnetic resonance imaging (MRI) studies performed in 2 non-ACD patients (Figure 2) and position emission tomography data showing recovery of metabolic activity within the grafted scar. Such an improvement has been also reported in animals using tissue Doppler imaging or MRI techniques and in humans, despite the fact that the grafted myotubes remain isolated from the host tissue and do not express connexin-43, thereby precluding the possibility of transmural delivery of paracrine signaling.

Finally, functional class stably improved over time after the procedure, along with a strikingly low incidence of hospitalizations for congestive heart failure (CHF) (0.13 patient-years) as compared with similar CHF populations. The anti-remodeling effect of the procedure, suggested by animal experiments, was confirmed by the absence of (like aneurysmectomy), the current study includes a very homogeneous cohort of patients from a single center. Second, the number of injected and viable cells is markedly higher than in the previously published articles (mean 953 versus <300 million cells), as is the purity of the injected solution, which may be clinically relevant in view of the suggested link between the number of injected cells and the efficacy and arrhythmogenicity of the procedure. Third, the FU is the longest ever reported (up to 5 years, versus 3, 12, and 24 months), allowing to confirm the absence of tumorigenicity of the procedure and to systematically check for long-term arrhythmogenicity. The short-term occurrence of SVT has been highlighted since the first clinical study but, so far, the long-term risk of the procedure has remained unknown.

In the current study, patients with SVT at long-term FU always exhibited SVT during the first postoperative month. Except for patient 4, arrhythmias were controlled by drug regimen and/or on-request ACD implantation. Presence of short-term non-SVT was not predictive of future events. Late arrhythmic storms have been observed until 18 months after grafting and were correctly managed by multiple ACD firings. Even if a direct link between the procedure and arrhythmias remains speculative, because of the underlying arrhythmogenic substrate inherent to CHF, absence of electrical coupling, and a resulting inhomogeneous electrical propagation might account for reentry circuits. Of note, no SVT was observed among those patients with continuously improved EF, whereas 3 of 4 patients who exhibited SVT showed a marked decrease in EF from short-term to long-term, raising the possibility that those arrhythmic events were not procedure-related but reflected CHF evolution, graft closure, or progression of atherosclerosis in native vessels (a control coronary angiogram was only performed after the first SVT and was not repeated thereafter). Because an equivalent proportion (31%) of patients issued from a similar (SCD-HeFT) population (age, 60 years; NYHA II/III; EF ≤35%; FU, 45.5 months) and systematically implanted with an ACD received shocks from their device, it is clear that only the ongoing randomized placebo-controlled MAGIC trial, which entails implantation of a defibrillator in all patients, will allow to support or refute a causal relationship between myoblast implantation and ventricular arrhythmias.

**TABLE 3. Arrhythmias and ACD**

<table>
<thead>
<tr>
<th>Patient</th>
<th>ACD Delay</th>
<th>Indication (delay)</th>
<th>ACD Interrogation</th>
<th>Shocks*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>M45</td>
<td>NSVT (M45)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>M5</td>
<td>Syncopal SVT (M5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SVT + NSVT (M5)</td>
<td>SVT (M7)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>6</td>
<td>M9</td>
<td>SVT (D7, M8)</td>
<td>NSVT (M44)</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>D28</td>
<td>NSVT + SVT (D10–13)</td>
<td>SVT (M18)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NSVT (M26)</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>D23</td>
<td>NSVT + SVT (D12)</td>
<td>SVT (M6)</td>
<td>9 (5)</td>
</tr>
</tbody>
</table>

*Nonappropriate shocks are in parentheses.

ACD indicates automatic cardiac defibrillator, with the delay in days (D) or months (M) between surgery and ACD implantation; indication (delay), delay between surgery and occurrence of arrhythmias; NSVT and SVT, nonsustained and sustained ventricular tachycardias, respectively.
Further LV dilatation over time, possibly caused by the scaffolding properties of the graft.

Limitations
The improvement in LV function was not the primary end point of this study, which was designed to rather address the long-term safety of the procedure, particularly with regard to tumor development and arrhythmias that might have been related to the high number of injected cells. Echocardiography was the only method used to track the changes in systolic function after surgery; MRI, assumed to be more accurate, was excluded because of ACD implantation in several patients. However, the consistency of ultrasonic measurements of EF and segmental scoring for 2 examinations performed by the same observer reached 99% in the previously published phase I study. Second, the associated CABG surgery could clearly confound interpretation of functional outcomes, although this study, in contrast to others, entitled exclusive revascularization of the noncell-transplanted segments. Finally, it is clear that improved LV function does not necessarily equate myocardial regeneration and as such the present results still fail to provide a mechanistic insight of myoblast transplantation.

In conclusion, the present long-term study confirms that autologous skeletal myoblast transplantation is a feasible and relatively straightforward procedure. In a very homogeneous cohort of severe CHF patients, both functional class and ventricular function stably improved over time, whereas the incidence of hospitalizations for CHF remained strikingly low. In these patients receiving the highest number of cells ever injected, severe arrhythmias may occur at long-term, but they can be controlled by a prophylactic drug regimen and/or on-request ACD implantation. Larger randomized, placebo-controlled, double-blind studies, such as the MAGIC trial that we are currently conducting, should soon generate meaningful safety and efficacy data allowing to accurately assess the risk-to-benefit ratio of the procedure.

Sources of Funding
This study was supported by a French Public Institution, the Département de la Recherche Clinique et du Développement Ile de France, Assistance Publique - Hôpitaux de Paris, Paris, France.

Disclosures
This study was promoted by the Département de la Recherche Clinique et du Développement Ile de France, Assistance Publique Hôpitaux de Paris, Paris, France (http://www.drrc.aphp.fr/), with the promotion number P 990503. Since completion of this phase I study, Genezyme has provided research and consulting support, but there is no conflict relevant to the present work, which was exclusively academia-driven and funded by a public grant.

References
Skeletal Myoblast Transplantation in Ischemic Heart Failure: Long-Term Follow-Up of the First Phase I Cohort of Patients

Albert A. Hagège, Jean-Pierre Marolleau, Jean-Thomas Vilquin, Armelle Alhéritière, Séverine Peyrard, Denis Duboc, Eric Abergel, Emmanuel Messas, Élie Mousseaux, Ketty Schwartz, Michel Desnos and Philippe Menasché

Circulation. 2006;114:I-108-I-113
doi: 10.1161/CIRCULATIONAHA.105.000521

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/1_suppl/I-108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/