## **EXPERIMENTAL STUDIES**

# Extension of Borderzone Myocardium in Postinfarction Dilated Cardiomyopathy

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OBJECTIVES	This study tests the hypothesis that hypocontractile, borderzone myocardium adjacent to an expanding infarct becomes progressively larger and more hypocontractile as remodeling continues.
BACKGROUND	Early infarct expansion following anteroapical myocardial infarction (MI) is associated with progressive ventricular dilation and heart failure. The contribution of perfused, hypocontrac- tile, borderzone myocardium to this process is unknown.
METHODS	Using a sheep model of anteroapical infarction, sonomicrometry array localization and serial microsphere injections were used to track changes in regional myocardial contractility, geometry, and perfusion. Eight sheep were studied before and after infarction and two, five, and eight weeks later. Thirty intertransducer chord lengths were analyzed to measure regional contractility and varial changes in regional geometry at and systel.
RESULTS	Beginning as a narrow band of fully perfused hypocontractile myocardium adjacent to the infarction, borderzone myocardium extends to involve additional contiguous myocardium that progressively loses contractile function as the heart remodels. Three distinct myocardial zones develop as a result of transmural MI: infarct, borderzone (perfused but hypocontractile), and remote (perfused and pormally functioning).
CONCLUSIONS	This study demonstrates that hypocontractile, fully perfused borderzone myocardium extends to involve contiguous normal myocardium during postinfarction remodeling. This borderzone myocardium is a unique type of perfused, hypocontractile myocardium, which is distinct from hibernating or stunned myocardium. Preventing extension of borderzone myocardium by medical or surgical means offers the prospect of preventing late-onset heart failure following transmural expanding MIs. (J Am Coll Cardiol 2002;40:1160–7) © 2002 by the American College of Cardiology Foundation

After acute myocardial infarction (MI), early infarct expansion predicts late generalized ventricular dilation (1,2), reduced longevity (3,4), and progressive loss of cardiac function. The mechanism of this process is not understood, but it is believed to involve a narrow hypocontractile perimeter of viable myocardium that surrounds an acute expanding infarct. In experimental animals this borderzone

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myocardium is fully perfused (5). Fully perfused, hypocontractile myocardium, termed "remodeled myocardium," has also been identified in patients with ischemic cardiomyopathy (6). The role of this remodeled myocardium in the borderzone of expanding infarctions during progression to heart failure is not defined. This study tests the hypothesis that infarct expansion causes recruitment of adjacent, normally contractile, fully perfused myocardium into an enlarging zone of fully perfused, hypocontractile myocardium in a self-perpetuating process that leads to heart failure.

## **METHODS**

**Surgical protocol.** Eight Dorsett hybrid sheep (Animal Biotech Industries, Doylestown, Pennsylvania) were induced with thiopental sodium (10 to 15 mg/kg intravenous [IV], intubated, anesthetized with isofluorane (1.5% to 2%), and ventilated with oxygen (Drager anesthesia monitor, North American Drager, Telford, Pennsylvania). All animals received glycopyrrolate (0.4 mg, IV) and cefazolin (1 g, IV). Animals were treated in compliance with National Institutes of Health Publication No. 85-23 as revised in 1985. The surface electrocardiogram (ECG) and arterial blood pressure were continuously monitored (Sonometrics, London, Ontario, Canada).

Using sterile surgical technique, a left anterolateral thoracotomy was performed. Polypropylene snares were placed around the left anterior descending and second diagonal coronary arteries (approximately 40% from the apex) (7). An

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Abbreviations and Acronyms									
ANOVA	= analysis of variance								
ECG	= electrocardiogram								
ED	= end diastole								
ES	= end systole								
$\%\Delta_{ m ES}$	= percent change in end systolic chord length								
$\%\Delta_{ m FS}$	= percent change in fractional shortening								
IV	= intravenous								
LV	= left ventricle								
LVP	= left ventricular pressure								
MDS	= multidimensional scaling								
MI	= myocardial infarction								
SAL	= sonomicrometry array localization								
SW	= stroke work								

ultrasonic flow probe (Transonic Systems, Ithaca, New York) was placed on the aortic root proximal to the origin of the innominate artery. Two epicardial pacemaker wires were sewn to the left atrium. Sonomicrometry transducers were placed, as described below. The animal was allowed to recover.

**Baseline data.** After 10 to 14 days, sheep were anesthetized with isofluorane, intubated, and placed supine. Surface ECG, arterial blood pressure, and pulmonary artery and capillary wedge pressures were continuously monitored. For all measurements the animal was disconnected from the ventilator and the heart atrially paced at 120 beats/min. Stroke work (SW) was calculated from simultaneous measurements of stroke volume and left ventricular pressure (LVP): SW (ergs) = LVP (mm Hg)  $\times$  1,330  $\times$  SV (ml), where SV is the stroke volume as determined by the aortic flow probe. Subdiaphragmatic echocardiographic images were obtained through a sterile, midline laparotomy, and they were used to calculate ventricular volumes, as described previously (8).

**Sonomicrometry array localization.** Sonomicrometry array localization (SAL) is an imaging technique that uses small piezoelectric transducers to permanently label specific locations of myocardium (9,10). In this study, 15 transducers (2 mm in diameter) were inserted into the myocardium of the left ventricular (LV) free wall to form a grid on the anterior LV. This array consisted of five transducers within the planned infarction, three transducers at the edge of the infarct, and nine transducers placed 2 to 5 cm cephalad to the infarct demarcation line. One reference transducer was sutured to the left fibrous trigone between the ascending aorta and left atrium.

Distance between all pairs of transducers (120 chord lengths) was measured once every 5 ms, in real time (Sonometrics). Using multidimensional scaling, the location of each transducer in a single, three-dimensional (3D) coordinate system was determined at end systole (ES) and end diastole (ED) (9,10).

**Infarction.** After obtaining baseline data an anteroapical infarction of 24% of the LV mass was created (8). Postin-farction measurements were made after the animal had stabilized. Sonometric, echocardiographic, and hemodynamic measurements were made before and after infarction and two, five, and eight weeks later in the eight animals. After the eighth-week study, animals were euthanized; hearts were excised; and the location of each transducer relative to the aneurysmal scar was recorded.

**Histology.** Sections of the myocardium from the infarct, borderzone, and remote myocardium were fixed in 5% buffered formalin, paraffin embedded, sectioned at 3 to 4  $\mu$ m, and stained with either hematoxylin and eosin or Masson's trichrome.

Assessment of myocardial perfusion in a separate group of animals. Seven additional Dorsett hybrid sheep from the same vendor underwent microsphere injections before and after infarction. These animals were instrumented identically to the first eight animals except that myocardial markers (2-mm stainless steel beads) were placed in the myocardium in lieu of sonomicrometry transducers and a permanent left atrial catheter was inserted. Fifteen million color-coded,  $15.5-\mu$ m-diameter NuFlow Fluorescent microspheres (IMT Laboratories, Irvine, California) were injected before infarction and two, five, and eight weeks after infarction to measure regional myocardial perfusion. A reference blood sample was also taken. These animals had the same echocardiographic and hemodynamic measurements as in the previous group.

Following the terminal eight-week study, animals were euthanized and hearts explanted. The steel beads were used to identify infarcted, borderzone, and remote myocardium for analysis. Myocardial samples and reference blood samples were analyzed using flow cytometry for microsphere content by IMT Laboratories. Regional perfusion was calculated using the following formula:  $Qm = (Cm \times Qr)/Cr$ , where  $Q_m =$  myocardial blood flow per gram (ml/min/g) of sample,  $C_m =$  microsphere count per gram of tissue in sample,  $Q_r =$  withdrawal rate of the reference

**Table 1.** Hemodynamic, Functional, and Echocardiographic Data for Ovine Model of LeftVentricular Aneurysm

	Baseline	30 Min	2 Weeks	5 Weeks	8 Weeks
LVEDP (mm Hg)	$1.7\pm1.0$	$8.2 \pm 3.5^{*}$	$6.4 \pm 5.7^{*}$	$6.0 \pm 1.9^{*}$	$5.7 \pm 2.8^{*}$
ES volume (cc)	$28 \pm 7$	$40 \pm 10$	$68 \pm 18^{*}$	$66 \pm 11^{*}$	$76 \pm 19^{*}$
Ejection fraction (%)	$47 \pm 9$	$41 \pm 7$	$26 \pm 9^{*}$	$27 \pm 9^{*}$	$23 \pm 6^{*}$
Stroke work (ergs $\times$ 10 <sup>3</sup> )	$253\pm78$	$214\pm80$	$178 \pm 73^{*}$	$170 \pm 52^{*}$	$139 \pm 54^{*}$

\*p < 0.05 compared to baseline.

ES = end systolic; LVEDP = left ventricular end diastolic pressure.



Figure 1. Regional myocardial blood flow (ml/g/min) at baseline and at two, five, and eight weeks' postinfarction, as determined by serial microsphere injections.



**Figure 2.** Regional changes in fractional shortening during the cardiac cycle for three different myocardial segments (1 = infarct, 2 = borderzone, 3 = remote) before and at each time point after infarction. Note progressive expansion of all segments during remodeling and the progressive loss of contractility in segment 2 (borderzone) as remodeling progresses. AO = aorta; LAD = left anterior descending.



**Figure 3.** Graphs depicting the relationship between the geometrical change (expansion) and functional change (contractile function) for each of 30 intertransducer segments in the left ventricular myocardium. **Red symbols** represent segments located within the anatomical infarction; **green symbols** represent segments located outside the infarction in normally perfused myocardium. No graph is shown for the preinfarction time point because changes (from baseline) in chord length and contractile function are plotted; before infarction all chords lie at the origin. The horizontal line y = 0 represents a segment of myocardium that has exactly the same fractional shortening as it had at the baseline study; segments that fall between that line and y = -100% are hypocontractile relative to baseline; segments falling below y = -100% demonstrate systolic dyskinesis. In the corresponding left ventricular diagrams, **dashed lines** indicate segments that fall below the line y = 0. Therefore, **dashed green lines** represent hypocontractile but perfused myocardium (borderzone myocardium) and **dashed red lines** represent infarcted myocardium. Note the progressive numerical increase and location of the hypocontractile, dashed segments. The data presented represent a composite constructed from averages of all eight animals. ES = end systole.



**Figure 4.** At postinfarction and eight weeks, chord lengths were clustered by z-score using a k-clustering algorithm (with respect to percent change in contractility and percent change in chord length, perfusion status was not considered in the analysis). The plot above is similar to Figure 3, depicting each cluster using a different color. In this figure, each chord in the ventricular array is depicted using the color corresponding to the cluster to which it belongs. **Dashed lines** in the lower portion of the figure denote cord lengths with reduced contractility below baseline. **Solid lines** denote chord lengths with contractility at or above the baseline value. Note that the blue cluster includes all of the borderzone myocardium defined in Figure 3 as well as several infarcted segments. (The significance of this finding is presented in detail in the Discussion section.) The data presented represent a composite constructed from averages of all eight animals. ES = end systole.

blood sample (ml/min), and  $C_r$  = microsphere count in the reference blood sample.

**Data analysis.** End diastole was identified as the peak of the QRS complex. End systole was identified at the minimum (most negative) dP/dt.

Using 3D transducer coordinates from SAL, 30 selected intratransducer distances (chord lengths) at ES and ED were calculated for each serial time point. Changes in serial ES chord lengths were represented by calculating a percent change from the baseline value ( $\%\Delta_{ES}$ ).

Likewise, in each animal, for each chord length at each experimental time point, fractional shortening was calculated by subtracting ES length from ED length, then dividing by ED length; a percent change from the preinfarction value ( $\&\Delta_{FS}$ ) was then calculated. Finally, the mean

 $\%\Delta_{\rm FS}$  and  $\%\Delta_{\rm ES}$  for each chord length was calculated for all animals at each time point.

At each time point these two quantities, representing mean percent change in regional myocardial expansion ( $\%\Delta_{\rm ES}$ ) and mean percent change in regional function ( $\%\Delta_{\rm FS}$ ), were plotted on orthogonal axes. These graphs were inspected to identify chord lengths lying in regions of myocardium with distinctive properties. We note that the preinfarct plot was not presented because it consists of 30 data points all lying at (0,0).

To justify separation of the LV free wall into regions of myocardium with distinct properties, cluster analysis was performed using both hierarchic and partitioning methods in SPSS (SPSS, Inc., Chicago, Illinois). Nondimensional z-scores were constructed from  $\&\Delta_{ES}$  and  $\&\Delta_{FS}$  at postin-



Figure 5. Percent expansion for myocardial chords within each cluster plotted over time. Clusters are identified by color as in Figure 4. Note that the rate of expansion is similar in all three myocardial regions after the immediate postinfarction period.

farction, two weeks, five weeks, and eight weeks. An eight-dimensional cluster analysis was then performed. The number of clusters was taken to be greater than or equal to two, and for each case the resulting clusters were subsequently compared to one another using analysis of variance (ANOVA).

For all other measurements, values are reported as means and standard deviations. Differences between baseline measurements and measurements at subsequent times were compared using one-way ANOVA with the Bonferroni correction for repeated measures. If the time effect was significant (p < 0.05), pairs were compared using the *t* statistic.

## RESULTS

Hemodynamic, echocardiographic, and perfusion data. Selected data appear in Table 1; complete hemodynamic and quantitative echocardiographic results for this sheep model have been reported (7). All sheep developed LV aneurysms and clinical signs of heart failure between the fifth and eighth week. Mean myocardial blood flow at each time point for each region is presented in Figure 1. Blood flow to uninfarcted myocardium did not significantly differ between regions or times.

**Sonomicrometry data.** Figure 2 depicts regional changes in fractional shortening during one cardiac cycle for infarct, borderzone, and remote myocardium at each postinfarction time point. The increasing distance of each contractile trace

from the preinfarction trace indicates progressive expansion of the three selected myocardial segments. After infarction, we noted the immediate loss of contractility in the infarct, the progressive loss in borderzone myocardium, and absence of loss in remote myocardium.

Figure 3 presents changes in expansion, contractility, and perfusion for each chord length during the study. Figure 3 illustrates the progressive loss of fractional shortening of normally perfused myocardial segments and progressive expansion of all segments.

Figure 4 depicts results of the cluster analysis with respect to percent change in contractility and percent change in chord length performed using three groups. When cluster analysis was performed assuming three clusters, the groups were found to be significantly different by ANOVA (p < 0.01). If two or more than three groups were assumed, the divisions became statistically arbitrary. Unlike the analysis depicted in Figure 3, perfusion status was not included in the clustering analysis. The significance of this distinction will be presented in the Discussion section.

Expansion was greatest in the infarct immediately after infarction. After two weeks, the rate of expansion did not differ among the three different myocardial clusters (Fig. 5). **Histology.** Analysis of the histologic sections from the region of the infarct demonstrated fibrosis consistent with healed infarction. Specimens from the borderzone showed patchy interstitial and replacement fibrosis. Myocyte vacuolization (myofibrillarlytic cells) and mild hypertrophy were

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**Figure 6.** A low magnification panoramic histologic view of the ovine model of anteroapical infarction (**A**, trichrome,  $1\times$ ) is magnified to individually represent myocardial infarction region (**B**, trichrome,  $20\times$ ), borderzone (**C**, trichrome,  $20\times$ ), and remote myocardium (**D**, trichrome,  $20\times$ ). The region of fibrosis stains blue and the viable myocytes stain red. The region of healed infarct (**B**) shows fibrosis; the borderzone (**C**) shows patchy interstitial and replacement fibrosis with myofibrillarlysis. The remote (**D**) area shows focal interstitial fibrosis.

also evident in the borderzone sections. Sections of the remote myocardium demonstrated mild patchy interstitial fibrosis (Fig. 6).

### DISCUSSION

The experimental methods used in this study allow nearperfect registration of expansion (stretch), contractility, and perfusion of myocardial segments during remodeling after acute MI. These methods control for ischemic contractile dysfunction due to stunned or hibernating myocardium. The results clearly demonstrate that fully perfused, hypocontractile, borderzone myocardium *expands* (stretches) during the remodeling process and *extends* by recruiting previously normally contracting perfused myocardium into an enlarging hypocontractile zone.

Borderzone myocardium is determined only by perfusion status (perfused) and contractility (reduced as compared to preinfarction). Immediately after infarction the extent of the borderzone is small, and function is only moderately impaired. However, over two weeks the area of dysfunctional myocardium enlarges (due to expansion and extension) and the degree of functional impairment becomes severe.

Figure 3 demonstrates changes in regional expansion and contractility as remodeling progresses. Infarcted myocardium is depicted in red and normally perfused myocardium in green. Three distinct myocardial regions are evident: infarcted myocardium (unperfused, hypocontractile), borderzone myocardium (perfused and hypocontractile), and remote myocardium (perfused with preserved contractility). The cluster analysis presented in Figure 4 also delineates three discrete groups. The group depicted in blue in Figure 4 does not correspond exactly with the borderzone myocardium defined by Figure 3 (i.e., green squares that fall below y = 0). Cluster analysis considers only regional expansion and contractility without regard for perfusion status. The cluster analysis suggests that, as remodeling occurs, borderzone myocardium develops deformation and contractility profiles identical to some regions of infarcted myocardium.

This profound degree of contractile impairment in segments of borderzone myocardium seen in this study demonstrates how such regions could have been confused for infarct extension or expansion in previous experimental and clinical studies.

The cause of the progressive reduction in contractility of uninfarcted, borderzone myocardium is not established; however, increased stress and the resulting strain in this region of myocardium is likely important. Early infarct thinning and expansion causes an immediate increase in the radius of curvature of adjacent borderzone myocardium. The increased radius of curvature raises wall stress in borderzone myocardium. As regional stress and strain both increase and persist (11), borderzone myocardium may be fundamentally altered on a biochemical and cellular level.

The presence of vacuolated myocytes in normally perfused borderzone myocardium supports this hypothesis and suggests a mechanism for the contractile dysfunction that develops in this model. We have shown (J. Narula, personal communication, March 2001) that the majority of vacuolated myocytes, also termed *myofibrillarlytic* or *myocytolytic cells*, are apoptotic and are associated with an upregulation of caspase-3. This reasoning raises the intriguing possibility that early infarct expansion initiates as a progressive myopathic process in normally perfused myocardium that ultimately produces global ventricular dysfunction by nonischemic myocyte loss secondary to stretch-induced myocyte apoptosis.

Progressive enlargement of uninfarcted borderzone myocardium (by extension and expansion) following an expanding transmural myocardial infarction has been identified clinically. Narula et al. (6), using a novel four-stage singlephoton emission computed tomographic imaging protocol, demonstrated that over 50% of the severely dysfunctional myocardium in patients with ischemic, dilated cardiomyopathy had normal blood flow. These data indirectly suggest that the extension of borderzone myocardium described in this study contributes significantly to the development of postinfarction cardiomyopathy in patients. Narula et al. (6) have proposed that this hypocontractile but normally perfused myocardium be called "remodeled myocardium." This might be a better term than "borderzone myocardium" given its apparent wide distribution during fully developed ischemic cardiomyopathy in patients.

The present study demonstrates that an expanding regional infarction can initiate a myopathic process that spreads beyond the immediate peri-infarct region and may involve the entire ventricle. This process provides an explanation for progressive dysfunction and ventricular dilation that occur in some patients after a single myocardial infarction. Once advanced postinfarction, dilated cardiomyopathy occurs, medical treatment options and surgical procedures, except for cardiac transplantation, are ineffective and temporary (12–14). Preemptive medical, catheterbased, or surgical strategies designed to prevent acute infarct expansion and extension of borderzone, remodeled myocardium may offer a new paradigm for preventing progression of postinfarction LV remodeling to postinfarction dilated cardiomyopathy.

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