Unique Topographical Distribution of M Cells Underlies Reentrant Mechanism of Torsade de Pointes in the Long-QT Syndrome

Fadi G. Akar, PhD; Gan-Xin Yan, MD, PhD; Charles Antzelevitch, PhD; David S. Rosenbaum, MD

Background—Specific ion channel mutations underlie the congenital long-QT syndrome (LQTS). However, the mechanisms by which dysfunction at the molecular level translates into functional electrical instability leading to torsade de pointes (TdP) in LQTS are poorly understood.

Methods and Results—The cellular basis of TdP was investigated using a novel approach of transmural optical imaging in the canine wedge preparation (n=11005). The spatial organization of repolarization and arrhythmogenesis were determined in a surrogate model of LQT2. Action potentials were recorded simultaneously from 128 sites spanning the transmural wall of the left ventricle. In LQT2, QT interval prolongation was paralleled by an abrupt rise in transmural dispersion of repolarization (DOR) from 2.7±0.9 ms/mm (controls) to 12.2±2.1 ms/mm (LQT2). Islands of midmyocardial (M) cells formed zones of increased refractoriness in LQT2, producing steep spatial gradients of repolarization that were directly responsible for conduction block and self-sustained intramural reentrant circuits underlying TdP.

Conclusions—These data provide direct evidence supporting the functional expression of M cells in intact myocardium and a central role for M cells in the development of reentrant TdP arrhythmias in LQTS. (Circulation. 2002;105:1247-1253.)

Key Words: torsades de pointes ■ action potentials ■ arrhythmia ■ ion channels ■ mapping

Torsade de pointes (TdP) is a life-threatening arrhythmia closely linked to abnormal cardiac repolarization.1-3 It is well recognized that specific mutations in ion channels controlling cellular repolarization underlie the various congenital forms of the long-QT syndrome (LQTS).4,5 Because exogenous factors such as antiarrhythmic drugs causing the acquired form of LQTS operate on the same ion channels implicated in congenital LQTS, both forms of the disease may share common electrophysiological mechanisms.6 However, the manner by which ionic alterations at the molecular level produce an electrophysiological substrate for TdP at the level of the whole heart remains unclear.

Previous clinical7 and experimental8-10 observations suggest 2 hypotheses regarding the electrophysiological basis of TdP. One theory states that TdP arises from triggered activity in competing ventricular foci. Evidence for the triggered activity hypothesis stems from experimental observations8-10 and computer models11 demonstrating an enhanced propensity of cardiac myocytes to generate early afterdepolarizations (EADs) in response to factors that prolong the action potential duration (APD). Because TdP observed in patients is associated with conditions favoring the development of EADs experimentally, TdP was attributed to EAD-induced triggered activity. This mechanism, however, was challenged because rapid rates accompanying the onset of TdP abruptly shorten repolarization, thereby eradicating the prerequisite condition for EAD-mediated TdP.

The second proposed mechanism is based on the association between dispersion of repolarization (DOR) and TdP, suggesting involvement of reentrant excitation. For example, patients with congenital LQTS manifest increased dispersion of QT interval. Moreover, recent observations from surrogate models of LQTS suggest a role for reentrant activity involving relatively large circuits around the cardiac chambers.9,10 However, focal (ie, nonreentrant) patterns of activation were also observed in these models, raising additional uncertainty regarding the underlying cellular mechanisms.

In this study, we focus on the potential role of transmural heterogeneities of cellular repolarization in the mechanism of TdP. Because of their relatively weak IKs current density, midmyocardial cells (M cells) are more sensitive to many APD-prolonging conditions than epicardial and endocardial cells.12 Therefore, M cells may play an important role in arrhythmias that are dependent on delayed cardiac repolar-
ization, such as LQTS. Despite inherent differences in the electrophysiological characteristics of different cell types, strong cell-to-cell coupling in the intact heart is expected to attenuate the functional expression of such heterogeneities.\textsuperscript{13} Hence, the extent to which M cells may functionally influence transmural DOR or arrhythmogenesis in intact myocardium remains unclear.\textsuperscript{14–16}

To establish definitively the functional topography of M cells and their role in promoting transmural DOR and arrhythmias in the presence of cell-to-cell electrotonic interactions, we developed a transmural optical mapping system capable of measuring electrical heterogeneities between hundreds of cells spanning the ventricular wall. This system was used to demonstrate a specific role of M cells in generating functional heterogeneities of repolarization that support intramural reentry in LQTS.

### Methods

#### Transmural Action Potential Mapping

We designed a system capable of recording action potentials with high spatial (1.0 to 1.5 mm), temporal (0.5 ms), and voltage (0.5 mV) resolutions from all myocardial layers spanning the transmural wall. This was achieved by applying a previously validated optical mapping technique\textsuperscript{17–19} to the transmural surface of the arterially perfused canine wedge preparation.\textsuperscript{20}

Details of the experimental procedure are provided elsewhere.\textsuperscript{21} Briefly, wedges (\( \times 3 \times 1.5 \times 1 \) cm) of myocardium surrounding secondary branches of the circumflex or the left anterior descending coronary arteries were dissected from the anterior, anterolateral, or posterior free walls of the left ventricle. Wedges were arterially perfused (at 40 to 50 mm Hg) with Tyrode solution, malperfused edges were removed, and leaking collaterals were ligated. Wedges were discarded when tissue perfusion was inadequate, as judged by the presence of pallor on perfusion. Homogeneity of arterial perfusion was confirmed in preliminary experiments by imaging the distribution of fluorescent-labeled microspheres injected into the coronary circulation after prolonged periods of perfusion.

Preparations were immersed in a temperature-controlled (35±1°C) perfusion chamber to prevent formation of intramyocardial temperature gradients. After staining with di-4-ANEPPS (15 \( \mu \text{mol/L} \)), wedges were stabilized against the imaging window using a piston (Figure 1). Excitation of the dye’s fluorescence was achieved with 540±10 nm light. Fluoresced light was long-wave pass filtered (610 nm) and focused onto a 12\( \times 12 \)-element photodiode array (MD144-ST, Centronics Ltd.). To record action potentials simultaneously from the entire transmural extent of myocardium, optical magnifications ranging between 1.0 and \( \times 1.5 \) were used. Wedges were stable for \( \geq 4 \) hours of perfusion, as judged by the stability of coronary resistance, APD, and QT interval. After each experiment, wedges were stained with 2,3,5-triphenyltetrazolium chloride to confirm absence of ischemic necrosis. Visually apparent contraction artifacts were present in \( \approx 10\% \) of recording sites and were discarded. APD of each cell type measured using transmural optical mapping was essentially identical to that using floating microelectrodes in earlier studies.\textsuperscript{16,20,22} and was highly correlated with local refractory period, additionally reaffirming the accuracy of our measurements of cellular repolarization.

#### Canine Wedge Model of LQT2

Transmural heterogeneities of repolarization were investigated in each preparation (\( n=14 \)) under the following 4 conditions: (1) stimulation at basic cycle length (BCL) of 500 ms (control); (2) stimulation at BCL of 2000 ms (bradycardia); (3) stimulation at BCL of 500 ms during perfusion with the \( I_{\text{Kr}} \) blocker d-sotalol (100 \( \mu \text{mol/L} \)); and (4) stimulation at BCL of 2000 ms during perfusion with d-sotalol (d-sotalol+bradycardia). Previously, \( I_{\text{Kr}} \) blockade during bradycardia was established as a surrogate model of LQT2.\textsuperscript{23} hence we refer to condition 4 as LQT2. Statistical comparisons between each condition were made using students paired \( t \) test with Bonferroni correction where appropriate.

#### Experimental Protocols

The topographical distribution of M cells was assessed by mapping optical action potentials simultaneously from 128 sites spanning the transmural extent of myocardium during steady-state endocardial pacing in each condition. To assess changes in susceptibility to and the mechanism of arrhythmias under each condition, programmed stimulation was performed in all preparations. After a 20-beat drive train, an epicardial premature stimulus (S2) was delivered at S1S2-coupling interval of 300 ms that was sequentially shortened by 10-ms decrements until refractoriness was reached or an arrhythmia was induced. Polymorphic VT was also induced in a subset of experiments (\( n=4 \)) by a short (300 ms)-long (5000 ms)-short (250 ms) pacing protocol.

### Results

#### Canine Wedge Model of LQT2

d-sotalol+bradycardia produced a model of LQT2 exhibiting many features of clinical LQTS. The QT interval prolonged significantly (by 133±26 ms, \( P<0.01 \)) compared with controls. Arrhythmias in this model were characterized by a polymorphic, undulating ECG morphology typical of TdP (Figure 2). Moreover, TdP induction was dependent on bradycardia (BCL 2000 ms), potassium channel blockade, and QT prolongation. TdP was never induced in the absence of d-sotalol and was induced in 10 of 14 (71\%) animals during bradycardia and d-sotalol perfusion (Figure 3). Also consistent with TdP is the fact that polymorphic VT was readily initiated by short-long-short coupling sequences (4 of 4 attempts) and typically terminated spontaneously after several seconds (Figure 2).

#### Topography of Cell Types Across the Transmural Wall

Figure 4 illustrates the heterogeneity of cell types across the ventricular wall as measured by transmural optical mapping.
Action potentials of epicardial and subepicardial cells (sites A and B) typically exhibited a distinct spike and dome morphology, whereas those of endocardial cells (sites D and E) lacked the dome and exhibited a negatively sloping phase-2. Moreover, M cells were characterized by a relatively longer APD at baseline (control) and a disproportionate prolongation of their action potential in response to LQT2 conditions (Figure 4, potential C).

The degree of epicardial, endocardial, and M-cell APD prolongation is summarized for all experiments in Figure 4B. M cells underwent the most pronounced prolongation of APD in response to either bradycardia or d-sotalol. Moreover, disproportionate prolongation of M-cell APD relative to other cell types was accentuated by the combination of bradycardia and d-sotalol. In controls, repolarization gradients were relatively small (Figure 6, control). The maximum spatial gradient of repolarization ($\nabla R_{\text{max}}$) measured as the maximum local gradient of repolarization times $24$ was $2.7 \pm 0.9$ ms/mm. In contrast, either bradycardia or d-sotalol enhanced transmural gradients of repolarization significantly (Figure 6, bradycardia and d-sotalol). Finally, during both bradycardia and perfusion with d-sotalol, transmural gradients of repolarization increased additionally ($\nabla R_{\text{max}}$ $12.2 \pm 2.1$ ms/mm) as zones of delayed repolarization extending from the midwall toward subepicardial or subendocardial borders emerged (Figure 6).

Figure 3 summarizes the QT interval, $\nabla R_{\text{max}}$, and TdP induction rate for each condition. C indicates control; B, bradycardia; S, d-sotalol; and S+B, d-sotalol+bradycardia. Each condition significantly ($P<0.01$) prolonged QT interval and $\nabla R_{\text{max}}$. TdP induction rate was markedly increased by the synergistic effect of S+B.

### Electrophysiological Substrate in LQT2

The electrophysiological substrate for TdP was investigated using contour maps of transmural depolarization and repolarization (Figure 6). Depolarization spread from the endocardial pacing site across the ventricular wall in $\approx 30$ ms. The direction, velocity, and pattern of propagation were similar in each experimental condition. In contrast, repolarization was highly sensitive to bradycardia and d-sotalol. In controls, repolarization gradients were relatively small (Figure 6, control). The maximum spatial gradient of repolarization ($\nabla R_{\text{max}}$) measured as the maximum local gradient of repolarization times $24$ was $2.7 \pm 0.9$ ms/mm. In contrast, either bradycardia or d-sotalol enhanced transmural gradients of repolarization significantly (Figure 6, bradycardia and d-sotalol). Finally, during both bradycardia and perfusion with d-sotalol, transmural gradients of repolarization increased additionally ($\nabla R_{\text{max}}$ $12.2 \pm 2.1$ ms/mm) as zones of delayed repolarization extending from the midwall toward subepicardial or subendocardial borders emerged (Figure 6).

Figure 3 summarizes the QT interval, $\nabla R_{\text{max}}$, and TdP induction rate for all preparations under each experimental condition. QT-interval prolongation in LQT2 was paralleled by a marked increase in transmural DOR, resulting from the formation of distinct M-cell zones. Furthermore, the sharp rise in transmural DOR was associated with enhanced susceptibility to TdP.

### Reentrant Mechanism of TdP

The mechanism underlying TdP in this model is shown in a representative example in Figure 7. After a single premature stimulus (S2), the impulse blocked in the region of most delayed repolarization (Figure 7A, cells c, d, m1, and m2). The S2 wavefront, however, successfully propagated in the orthodromic direction (Figure 7A, sites a’ through e’), circumventing the region of delayed repolarization (Figure 7A, hatched area). The zone of block of the premature beat (Figure 7A) coincided with the region of most delayed repolarization after the S1 beat (Figure 7R). When the former sites of block (sites c and d) regained...
excitability, the orthodromic impulse conducted from site e back to site a (Figure 7B), thereby completing the first beat of reentry. A broad area of functional conduction block was present during the initial beats of reentry; however, because of pronounced rate adaptation of M cells, these refractory islands rapidly collapsed and were replaced by functional lines of block on subsequent beats (Figures 7C through 7F). The polymorphic ECG characteristics of TdP were attributable to the fact that lines of block and trajectory of the reentrant circuit varied from beat to beat, initially within the mapped transmural surface and subsequently meandering into deeper myocardial depths. Similar reentrant mechanisms were observed in all experiments. Figure 8 shows epicardial depolarization contour maps recorded during the initiation of TdP. Planar propagation was observed on the epicardium, indicating

Figure 4. Panel A illustrates optical action potentials recorded across the transmural surface in control and LQT2 (d-sotalol + bradycardia). ∆APD indicates APD prolongation–caused bradycardia in each transmural layer of myocardium. Note that the midmyocardial cell (C) exhibits disproportionate APD prolongation relative to cells in layers A, B, D, or E. Panel B summarizes for all experiments disproportionate APD prolongation in M cells in response to bradycardial and d-sotalol. Epicardial (EPI) and endocardial (ENDO) recordings were obtained from cells within 1.5 mm of the epicardial and endocardial surfaces, respectively. M cells were defined as non-EPI and non-ENDO cells with the longest (10th percentile) APDs.

Figure 5. Topography of M cells across the transmural surface in LQT2 from 4 representative experiments. Midmyocardial cells exhibited the longest APDs (red) and extended toward endocardial or epicardial surfaces. Typically, epicardial cells exhibited the shortest APDs (blue). Optical action potentials shown for selected transmural sites (A through D) demonstrate that M cells can express markedly different APDs from neighboring cells even in multicellular tissues under conditions of normal cell-to-cell coupling, and that M cells are not necessarily distributed uniformly across each transmural layer. EPI indicates epicardial surface; ENDO, endocardial surface.
the absence of epicardial reentrant circuits and supporting the hypothesis that transmural heterogeneities underlie transmural block and reentry in TdP.

**Discussion**

LQTS is characterized by QT prolongation often resulting in sudden death attributable to TdP. To date, several distinct mutations have been identified in patients with LQTS. These mutations involve ion channel proteins that control repolarization. Both triggered activity and reentry have been implicated in the mechanism of TdP. However, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.

We hypothesized that transmural electrical heterogeneities may underlie a reentrant mechanism for TdP. Although inherent differences in the electrophysiological and pharmacological properties of cell types across the ventricular wall have been implicated in the mechanism of TdP, however, because of limitations of conventional electrophysiological recording techniques, an integrated understanding of the mechanistic relationship between genetically determined alterations of cellular repolarization, QT interval prolongation, and the underlying mechanism of TdP has remained elusive.
are well recognized, electrotonic flow of current between cells is expected to reduce the functional expression of electrical heterogeneities across the heart. Hence, the extent to which M cells may functionally influence transmural DOR and arrhythmogenesis remained controversial.14–16

In this study, the functional topography of M cells and their role in TdP were investigated using transmural optical mapping in the canine wedge preparation. This technique allowed a detailed and simultaneous measurement of cellular repolarization of each cell type across the transmural wall of an intact preparation where the influence of cell-to-cell coupling was present, thereby eliminating the need for defining, a priori, what characteristics or locations constitute an M cell. This approach provided a novel and quantitative assessment of transmural DOR under various electrophysiological conditions, including a surrogate model of LQT2. We provide direct evidence that the topographical distribution of M cells causes unidirectional block and reentrant excitation underlying TdP in this model.

**Functional Transmural Heterogeneities of Repolarization**

Using transmural optical mapping, fundamental differences in action potentials recorded from different layers of myocardium were evident. In controls, a 30- to 40-ms difference in APD was measured between populations of cells displaying the longest and shortest action potentials, as described previously.16,25 As expected, these differences were smaller than those reported from isolated cells because of the homogenizing effect of cell-to-cell coupling present in the wedge and the intact heart. Importantly, the transmural gradients of repolarization present in controls (<3 ms/mm) were not of sufficient magnitude to cause TdP.

Unlike depolarization, which remained relatively constant (Figure 6), repolarization was highly sensitive to each experimental condition. Marked gradients of repolarization were formed in the presence of bradycardia, d-sotalol, and especially when both were present. (Figures 3 and 6). Bands of M cells exhibiting prolonged repolarization appeared in mid-myocardial layers and extended to either deep subepicardium to subendocardium. Previously, it was not possible to directly map the precise topographical distribution of M cells. Our ability to do so has revealed that M cells can vary considerably in their position within the wall. As a result of their complex distribution and their exquisite sensitivity to rate and $I_Kr$ blockade, M cells formed spatial gradients of repolarization ($\approx 12$ ms/mm) in LQT2 of sufficient magnitude to cause functional block and reentry in response to a premature stimulus (Figure 7).

The distribution of M cells varied considerably between experiments. Previously, M cells were encountered predominantly in subendocardial layers of the anterior left ventricle and subepicardial layers of the posterior left ventricle. In the present study, wedges were isolated from the anterior, anterolateral, and posterior surfaces, which may account, in part, for the variability in location and distribution of M cells. Importantly, these data may explain the discrepancy in earlier studies regarding the functional expression of M cells in intact myocardium. Anyukhovsky et al14 reported that when studied in isolation, M cells were identified in all myocardial layers except for the most superficial endocardium and epicardium but were not functionally present in vivo. We demonstrate that M cells exist in myocardial clusters that vary in spatial extent and location across the heart. Because these clusters are not present uniformly at a given depth of myocardium, and because they may extend to the epicardial and endocardial surfaces, it is not surprising that M cells were not consistently identified in earlier studies where action potentials were not monitored simultaneously across the transmural wall.12,14 Our data suggest that relatively high (<5 mm) mapping resolution is required for their precise localization. Importantly, because of electrotonic interactions between cells, the M-cell zone is necessarily blurred in space, possibly explaining why subepicardial cells could exhibit surprisingly long APDs when adjacent to M cells (Figure 7). The mechanism underlying this island-like distribution of M cells reported here clearly requires additional investigation.

**Reentry Underlies Mechanism of TdP**

Arrhythmias induced in this model of LQT2 exhibited many characteristics seen in clinical TdP (Figure 2). Ventricular
tachycardia characteristically exhibited a polymorphic undulating ECG morphology, was only initiated in the setting of a prolonged QT-interval, typically self-terminated, and was readily induced by long-short coupling sequences. Clinical TdP can also persist for long time periods and degenerate into VF, possibly because of superimposed influences of myocardial ischemia caused by tachycardia and hypotension. Alternatively, the relatively confined muscle mass of the wedge preparation may result in premature termination of TdP from annihilation of wavefronts along tissue boundaries. In these experiments, TdP did not initiate spontaneously; however, spontaneous TdP was reported previously in this model at slightly warmer temperatures. By perfusing at 35°C, we intentionally suppressed spontaneous activity to measure the electrophysiological substrate and propagation patterns underlying TdP in a controlled fashion. One cannot rule out from our data the potential role of triggered activity in the initiating beats of TdP, although our data clearly implicate reentry as the mechanism for sustenance of this arrhythmia. We found that the M-cell zone produced discrete refractory borders (Figure 5), which were directly responsible for conduction block and reentry that underlie TdP (Figure 7). Conduction slowing was not a requirement for reentry, because the path length dictated by the M-cell zone was sufficiently long to allow partial recovery of excitability at former sites of block (Figure 7). In other experiments (not shown in Figure 7), an analogous pattern of reentry was observed with the arc of block forming between the M-cell zone and the endocardial border. Because of exquisite sensitivity of M cells to rate, the broad zones of block delineated by M cells collapsed into functional lines of block that shifted dynamically on successive beats. Despite relative normalization of the M-cell APD on subsequent beats, reentry persisted as the leading edge of the wavefront propagated into the recovering tail of the circuit. Such dynamic M-cell APD adaptation undoubtedly accounted for the rapidly changing trajectory of the reentrant circuit producing the characteristic polymorphic ECG morphology of TdP. The presence of uniform propagation on the epicardium (Figure 8) may explain the appearance of a monomorphic waveform configuration in certain ECG leads but not others. Taken together, these findings suggest the existence of a single rotor during TdP that initially forms in the transmural wall and subsequently meanders into deeper layers of myocardium.

Acknowledgments
Supported by National Institutes of Health grants HL54807 (to Dr Rosenbaum) and HL47678 (to Dr Antzelevitch) and grants from the American Heart Association (to Drs Yan and Antzelevitch).

References
Unique Topographical Distribution of M Cells Underlies Reentrant Mechanism of Torsade de Pointes in the Long-QT Syndrome

Fadi G. Akar, Gan-Xin Yan, Charles Antzelevitch and David S. Rosenbaum

*Circulation*. 2002;105:1247-1253; originally published online February 18, 2002;
doi: 10.1161/hc1002.105231

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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/105/10/1247

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/