A New Method for Assessment of Cultured Cardiac Myocyte Contractility Detects Immune Factor–Mediated Inhibition of β-Adrenergic Responses

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**Background.** Potentially reversible congestive heart failure accompanies disease states associated with an immune cell myocardial infiltrate such as cardiac allograft rejection and inflammatory myocarditis. We therefore examined the hypothesis that immune cells can produce noncytotoxic alterations in cardiac function.

**Methods and Results.** A novel system to evaluate cultured cardiac myocyte contractility was developed using neonatal rat cardiocytes grown on human amniotic membrane segments. Spontaneous synchronous cell beating produced macroscopic distortion of these membranes. Movement of free-floating membranes anchored within a perfusion chamber was visualized under low-power microscopy and measured from recordings of the rhythmic displacement of membrane-adherent markers. Additions of graded concentrations of isoproterenol to the perfusate produced up to threefold increases in the initial contractile phase velocity (contractile index), with an EC50 of 10^{-7} M. When the extracellular Ca^{2+} concentration was increased from 0.9 to 3.6 mM, 2.43-fold increases in this index occurred. Myocytes incubated for 72 hours in the presence of dilutions of medium conditioned by activated rat splenic macrophages and lymphocytes exhibited an isoproterenol contractile index inhibited by 62% compared with control cells. In contrast, responses of supernatant-exposed and control cells to increased extracellular Ca^{2+} concentrations were not significantly different. Parallel studies of increases in myocyte intracellular adenosine 3':5'-cyclic monophosphate concentrations in response to isoproterenol stimulation demonstrated correlated inhibition that was specific for exposure to medium conditioned by immune cells.

**Conclusions.** Thus, a new method of in vitro cardiac contractility assessment that has significant advantages over existing systems has been developed and characterized. This new method has enabled description of an inhibitor of cardiac contractile function produced by activated immune cells. (*Circulation* 1991;84:313–321)

Profound suppression of cardiac contractile function can accompany inflammatory myocarditis associated with idiopathic congestive cardiomyopathy1,2 and cardiac allograft rejection.3 This functional impairment occurs in a setting of infiltration of the myocardium by activated lymphocytes and macrophages.4,5 Restitution of or improvement in contractile performance occurs in a significant subset of patients, and this functional improvement correlates temporally with resolution of the mononuclear cell infiltrate.1,2,4,6 The histopathologic features of inflammatory cardiac disease are quite variable. To complicate matters, despite recent attempts to reach a consensus for the diagnosis and classification of myocarditis,7 inter-

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pertation of endomyocardial biopsy results is subject to rather dramatic interobserver variability. While myocyte necrosis and fibrosis are often seen on histopathologic analysis of myocardial biopsy specimens obtained from inflamed hearts, some investigators consider a focal interstitial cell infiltrate without myocyte degeneration or necrosis to be sufficient for diagnosis. Support for the latter perspective is derived from several studies in which fibrosis and myocytolysis were absent or minimal in the vast majority of cases of myocarditis and idiopathic dilated cardiomyopathy, despite concomitant severe cardiac dysfunction. The lack of fibrosis and myocytolysis and a reversible dysfunction in some patients with inflammatory heart disease led us to hypothesize that activated immune cells or their soluble products can alter cardiac function without cytotoxicity.

To examine this question, we developed a new system to evaluate cardiac myocyte contractility using cells established in monolayer culture on flexible biological membrane substrata. These preparations exhibited rhythmic motion resulting from the summed activity of spontaneously and synchronously beating cells that was readily observed visually. Videotapes of membrane-adherent markers under low-power inverted microscopy permitted precise tracking and measurement of myocyte-mediated membrane movements.

Methods

Myocyte Cell Culture

Neonatal rat cardiac myocytes were isolated and cultured using a modification of standard techniques. Briefly, hearts were removed aseptically from 1-day-old Sprague-Dawley rats and subjected to digestion with 200 units/ml collagenase (Wako, Dallas, Tex.) in diveral cation-free isotonic buffer (137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, and 5.5 mM dextrose) supplemented with 2% (by volume) fetal bovine serum (FBS) (HyClone Laboratories Inc., Logan, Utah). Intact viable myocytes were separated from nonmyocytes by centrifugation through a Percoll (Pharmacia Diagnostics Inc., Fairfield, N.J.) step gradient, effecting purification to greater than 95%. Myocytes were washed and suspended in culture medium consisting of Ham's F-12 nutrient solution supplemented with 10% (by volume) FBS, 10 mM N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 50 µg/ml streptomycin, and 50 units/ml penicillin. Cells were plated within 1-cm-diameter cloning rings on amniotic membranes in 35-mm petri dishes (contractility assay) or in 96-well Primaria (Becton Dickinson, Lincoln Park, N.J.) tissue culture plates (adenosine 3':5'-cyclic monophosphate [cAMP] assay) and incubated at 37°C in 5% CO₂ and humidified air. Attachment to substrate and formation of syncyta required 48 hours, after which experimental manipulations were initiated.

Contractility Assay

Fresh human placental amniotic membrane was separated from the chorion by blunt dissection and thoroughly rinsed of blood prior to incubation for 24 hours in 4 gm% deoxycholate at 4°C. Epithelial cells were removed from the underlying basement membrane by scraping with gauze pads, after which the membrane was washed and stored in Dulbecco's phosphate buffered saline (PBS) containing penicillin and streptomycin. Membrane pieces (2x2 cm) were placed stromal side down/basement membrane side up in 35-mm petri dishes. Isolated myocytes were then plated within 1-cm-diameter cloning rings placed on the membranes. Cloning rings were made by slicing 10×75-mm centrifuge tubes with a hot knife. A synchronously contracting monolayer that produced macroscopic displacement of the membrane was apparent after 48–72 hours. Following syncytium formation, there was a window of 4 days during which the simultaneous contractions of plated cells produced membrane displacements that could be easily and accurately measured under low-power microscopy. Decrements in myocyte-mediated membrane motion that produced this temporal window appeared to result from a gradual decrease in the compliance of the membrane accompanying the proliferation of the small population of nonmyocyte cells.

Following stated incubation conditions, small aliquots of a suspension containing 0.9-µm cationic fluorescent beads (Covalent Technology, Ann Arbor, Mich.) were added within the cloning rings and permitted to adhere to cells and interstices. For assays, individual membranes were transferred to a perfusion chamber within a circulating water bath mounted on a Zeiss inverted microscope stage (Thornwood, N.Y.). Membranes were tethered on six wire pins extending up from the bottom of the perfusion chamber arranged in a 2-cm-diameter circle. The chamber was perfused with modified Krebs buffer (128 mM NaCl, 4 mM KCl, 0.9 mM CaCl₂, 1.0 mM MgCl₂, 0.9 mM Na₂HPO₄, 22 mM HEPES, and 5.5 mM dextrose) supplemented with 1% (by volume) FBS, 1 mM ascorbate, and stated concentrations of isoproterenol or calcium ion (added as CaCl₂). Beads were then visualized under low-power microscopy and videotaped using a Hamamatsu fluorescence-enhancing video camera (Bridgewater, N.J.). Time-dependent linear displacement of single beads was measured directly from a video screen.

Immune Cell Cultures

Activated splenocyte culture supernatants were obtained from cultures of adult rat splenocytes suspended in medium containing 5 µg/ml concanavalin A. Medium was harvested after 24 hours, after which lectin was removed by batch adsorption with Sephadex G-25. Rat bidirectional primary mixed lymphocyte cultures were established using splenocytes harvested from Lewis strain and outbred Sprague-Dawley rats.
Cellular activation was confirmed by documenting accelerated proliferation in mixed lymphocyte cultures compared with syngeneic splenocyte cultures using [$^3$H]thymidine incorporation rates. Culture supernatants were harvested after 96 hours. All conditioned media were clarified by centrifugation at 2,000g for 10 minutes, sterile-filtered (0.2 μm), and stored in aliquots at -20°C. Immune cell cultures were established in medium identical to that used for myocyte cultures. Media contained less than 50 pg/ml lipopolysaccharide.

**Cardiac Nonmyocyte Cell Culture**

Acutely isolated neonatal cardiac cells were plated in 100-mm petri dishes at a density of $5 \times 10^5$ cells/cm². Dishes were washed exhaustively 30 minutes after plating, at which time selective adherence of nonmyocyte cells was achieved. Medium identical to that used for myocyte cultures was then added and harvested after 3 days. Supernatants were clarified by centrifugation at 2,000g for 10 minutes, sterile filtered, and stored in aliquots at -20°C.

**cAMP Assay**

Following the incubation of myocytes under stated control and experimental conditions for indicated periods, culture medium was aspirated from wells and replaced with assay buffer consisting of PBS, 10 mM HEPES, 5.5 mM dextrose, 1 mM ascorbate, and stated concentrations of isoproterenol. After incubation for 10 minutes at 37°C, samples were quenched and deproteinized with perchloric acid (final concentration 0.6 M) and cooled to 4°C. Aliquots of samples were neutralized with KHCO₃ and analyzed for cAMP content by radioimmunoassay. Duplicate assays of quadruplicate well myocyte cAMP concentrations were performed for each experimental condition. Total cellular protein in culture wells was determined by Bradford assay following solubilization with 0.1N NaOH.

**Statistics**

The significance of differences between control and immune cell culture supernatant-exposed cellular responses to agonist stimulation were determined by Student's *t* test for unpaired data. Data are reported as mean±SEM unless stated otherwise.

**Results**

**Purification of Cardiac Myocytes by Silica Sol Gradient Centrifugation**

Established methods to purify acutely isolated cardiac myocytes rely on differential kinetics of cell attachment to substrata. Contaminating fibroblasts and endothelial, mesothelial, and smooth muscle cells preferentially adhere to tissue culture substrata after plating such that the nonadherent myocytes remain in suspension and are proportionately enriched. Using this differential attachment technique, we were able to consistently obtain myocyte cell suspensions that included 10–20% nonmyocytes, often with a loss of 40% of the original myocyte population. Because of our particular interest in a potential functional myocyte response to immune factors, we sought to obtain a more homogeneous population of contracting myocytes.

Acutely isolated cardiac cells were subjected to density gradient centrifugation through Percoll silica sol. Preliminary experiments demonstrated that myocytes exhibited a buoyant density of approximately 1.06–1.08 g/ml in isotonic fluid (data not shown). Subsequently, aliquots of a suspension of cardiac cells were centrifuged over Percoll, with the density in different tubes ranging from 1.040 to 1.090 g/ml. As shown in Figure 1, cells migrating through Percoll solutions of greater than 1.070 g/ml density were uniformly 95–99% viable myocytes. Myocyte yield at the lowest density that effected this degree of purification was 45% of the initial cell number. These data compare favorably with those obtained by the selective adherence technique, with which only 80% purity could be achieved. For all studies presented here, contaminating blood cells were eliminated from the myocyte suspension by centrifugation over a Percoll step gradient (1.070 over 1.095 g/ml). Myocytes sedimented to the Percoll interface and segregated from both low-density nonmyocyte contaminants and high-density erythrocytes and leukocytes.

**Measurement of Myocyte-Mediated Amniotic Membrane Displacement**

In vitro assessment of cultured myocyte contractile function has been complicated by the necessity to
measure relatively small myocyte sarcolemma displacements as an index of cellular contraction.\textsuperscript{21–23} Because of myocyte attachment to rigid substrata in these studies, the degree of sarcolemma displacement is highly dependent on the membrane location being monitored.

To circumvent these methodological difficulties, we used human placental membrane as a flexible biological substrate on which to culture myocytes. The synchronous activity of spontaneously contracting cells caused the circular area on which cells were plated to contract regularly. For measurement, membranes were transferred to a perfusion chamber for recording of motion under low-power microscopy. Linear displacement of areas on the membrane was visualized readily by tethersing several points on the periphery of the otherwise free-floating membrane. The time-dependent displacement of one point on a representative preparation, measured from a videotape, is depicted in Figure 2, top. Of particular note, maximal membrane displacement is nearly 200 \( \mu \text{m} \), equivalent to approximately four cell lengths. In addition, beat-to-beat temporal variability and displacement variability are minimal. Maximal displacements varied by less than 5% under a given perfusion condition.

Despite the well-behaved motion of single points on a given membrane, three factors contributed to variability in myocyte-mediated membrane motion between different membranes and between different points on the same membrane. First, the placental membrane segments varied in thickness and compliance, and compliance decreased with time after myocyte syncytium formation. Second, peripheral tethering necessarily altered the imposed load, and this could not be experimentally fixed. Third, the central portion of the circular area on which cells were plated experienced forces generated by contracting cells on all sides, while forces imposed on the peripheral regions were more unidirectional. Thus, we concluded that determinations of contractile force could not be compared between different membrane segments, even of the same preparation. However, for a single point on a given membrane preparation, a relative change in contractile state in response to a stimulus could be measured as long as the load and mass of the system were not altered.

**Myocyte Inotropic Response to \( \beta \)-Adrenergic Stimulation**

To examine the effect of \( \beta \)-adrenergic agonist stimulation on myocyte contractile responses using this system, myocyte-mediated membrane motion was recorded before and after addition of isoproterenol to the perfusate. The motion of a single point on each membrane was recorded before and after agonist exposure without intervening manipulation of the membrane to ensure that load was not altered. As shown in Figure 2, bottom, isoproterenol produced increases in beating rate and maximal displacement.

To quantify changes in contractile velocity, the average rate of displacement during the initial two frames of the contractile phase of five consecutive beats was determined before \( (v_0) \) and after \( (v_{ag}) \) agonist perfusion and expressed as a ratio \( (v_{ag}/v_0) \) to provide an index of contractile response to the agonist. This two-frame time interval was selected to maximize the detection of changes in maximal contractile velocity and to reduce error. Comparison of displacement measurements from longer intervals underestimated true changes in contractile velocity because of inflection in the displacement versus time curves. Subjectivity in assignment of the frame in each cycle during which contractile motion could first be detected and variation in the actual duration of motion in the first video frame, since motion could be initiated at any time during that frame, constituted the major sources of error. Determination of displacement over a two-frame interval and averaging the readings of five consecutive beats reduced the contribution of these error terms.
Figure 3. Isoproterenol-mediated increases in cultured cardiac myocyte contractility. Time-dependent cardiac myocyte-mediated displacement of amniotic membrane segments was determined under basal conditions and after 5 minutes of perfusion with isoproterenol. Measurements of five consecutive beats were averaged under each condition. Ratio of displacement during first two video frames of contraction in each cycle after isoproterenol perfusion to displacement before isoproterenol perfusion was calculated for each preparation to provide index of contractile response to agonist (contractile index). Four different membrane preparations perfused with single isoproterenol concentration were used to generate data for each point, depicted as mean±SEM.

Importantly, the basal contractile state did not change during the time required for measurements. Thus, the index did not differ significantly from unity when measurements were obtained from preparations perfused without agonist over a 10-minute period. This confirmed the legitimacy of the contractile index and ensured that responses to agonist exposure would not be blunted by decrements in basal contractile function during the assay period. The index increased in a monotonic fashion to 3.0 with increasing isoproterenol concentrations, with an EC50 of approximately 10^{-7} M (Figure 3). Somewhat blunted responses were demonstrated when a single membrane preparation was exposed to graded increases in isoproterenol concentration (which required up to 45 minutes) compared with responses of individual myocyte preparations challenged a single time with one isoproterenol concentration. In the former case, contributions from desensitization and from decrements in basal contractile state could not be segregated. The latter protocol was judged to be more accurate and was used in subsequent experiments.

Inhibition of β-Adrenergic Responsiveness by Activated Splenic Cell Culture Supernatants

Myocyte preparations were cultured in the presence of dilutions of medium conditioned by mitogen-activated splenocytes or control medium for 3 days prior to the assessment of contractile responses. No gross differences in basal contractions were seen between control and supernatant-exposed preparations. In contrast, responses of splenocyte supernatant-exposed myocytes to isoproterenol stimulation were markedly impaired. At a stimulating isoproterenol concentration of 1 μM, supernatant-exposed preparations exhibited a 62% inhibited contractile index of 1.58±0.06 compared with a control response of 2.54±0.16 (p<0.0005) (Figure 4). To examine whether these cells were capable of an augmented contractile state, the response to increased extracellular Ca^{2+} concentrations was determined for both control and supernatant-exposed myocytes. Index changes in response to 3.6 mM Ca^{2+} were not significantly different (2.43±0.16 versus 2.33±0.29, control versus supernatant-exposed, p>0.25). Thus, the impaired response induced by immune cell–conditioned medium appeared at least partially selective for β-adrenergic stimulation.

Myocyte chronotropic responses to adrenergic stimulation were also impaired in cells preincubated with immune cell supernatants. Control cells exhibited a 2.07-fold increase in beating rate when stimulated with 1 μM isoproterenol, from 57±7 to 118±21 (mean±SD) beats/min. Under otherwise identical conditions, myocytes preincubated with a 1:4 dilution of lectin-activated splenocyte supernatant exhibited a 1.52-fold increase, from 60±6 to 91±22 beats/min. The basal beating rates of control and supernatant-exposed cells did not differ significantly (p>0.25). Thus, immune cell culture supernatants inhibited the myocyte chronotropic response to isoproterenol by 52%, significant at the 0.005 level.

Inhibition of contractile responsiveness was apparent after 3 and 4 days of supernatant exposure. Because of the 4-day window between syncytium formation at day 3 and limiting decrements in myocyte-mediated membrane motion at day 7, it was not...
possible to measure the effect of longer periods of supernatant exposure. Similarly, these same constraints precluded analysis of reversibility of this physiological effect.

**Correlative Inhibition of β-Adrenergic Agonist-Mediated Myocyte cAMP Concentration Increases by Supernatants**

Because β-adrenergic agonists produce enhanced contractile function via stimulation of adenylyl cyclase and consequent increases in intracellular cAMP concentrations, correlative metabolic effects of immune cell factors on this system in the myocyte were investigated. Medium conditioned by lectin-activated rat splenic cells inhibited myocyte cAMP concentration increases in response to β-adrenergic stimulation. Isoproterenol increased the control cell intracellular cAMP concentration from a basal level of 10 to 145 pmol/mg protein. Supernatant exposure for 72 hours produced inhibition of cAMP concentration increases by up to 60%, with an EC50 of 10–20%. Detailed analysis of the time-dependence of this cAMP-suppressive activity revealed that the effect was first manifest after 24–48 hours, persisted for up to 7 days, and was reversible within 3 days upon discontinuation of supernatant exposure.

To eliminate attribution of this immune cell culture supernatant effect to medium exhaustion or nutrient depletion, medium harvested from cultures of cardiac nonmyocytes (fibroblasts and mesothelial, endothelial, and smooth muscle cells) was tested for myocyte cAMP-suppressive activity. As shown in Table 1, myocytes that were susceptible to splenocyte supernatant–induced inhibition of agonist-mediated cAMP concentration increases were not affected by fibroblast-conditioned media. Myocytes incubated in 50% (by volume) fibroblast-conditioned medium had a 134 ± 19 pmol/mg intracellular cAMP concentration following stimulation with isoproterenol, which was not significantly different from the control cell cAMP concentration (p = 0.11). Studies using supernatants from other cell culture sources indicated that myocyte cAMP-suppressive bioactivity was produced exclusively by activated macrophages and/or lymphocytes.

This bioactivity was immunologically nonrestricted since conditioned media from Lewis, Buffalo, Brown-Norway, and outbred Sprague-Dawley adult rats (including mothers of the animals killed for myocyte preparations) all contained virtually equivalent bioactivities.

Preliminary characterization studies revealed that the activity was proportionately increased by the concentration of supernatants by pressurized filtration (Amicon YM-10 membrane [Beverly, Mass.], 10-kDa cutoff). As shown in Figure 5, serum-free medium conditioned by concanavalin A–activated rat splenic cells had myocyte cAMP-suppressive activity. After fivefold concentration, this same medium was significantly more potent, producing 31% inhibition at a concentration of 6% by volume compared with 14% inhibition produced by the 10% by volume unconcentrated supernatant. Thus, activity could be attributed to a substance with a molecular mass greater than 10 kDa.

![Figure 5](https://example.com/figure5.png)

**FIGURE 5.** Immune factor inhibition of increases in cardiac myocyte adenosine 3′:5′-cyclic monophosphate (cAMP) concentrations in response to isoproterenol stimulation (Iso-Stim.). Cardiac myocytes established in monolayer culture in 96-well plates were incubated with control medium or stated dilutions of unconcentrated (*) or fivefold concentrated (10-kDa-cutoff membrane) (△) concanavalin A–activated rat splenocyte culture supernatant for 72 hours. Myocyte intracellular cAMP concentration following stimulation with 10−7 M isoproterenol was determined by radioimmunoassay for quadruplicate wells for each condition and each was then normalized to well protein concentrations. Data were transformed to reflect inhibition of myocyte cAMP concentration increases compared with cells incubated in control medium.
Discussion

Determination of the contractile state of muscle is complicated by difficulties in controlling preload and afterload, particularly in vivo. This is of particular concern in the assessment of cardiac inotropic response to a mediator that has secondary or other primary effects on vascular tone and therefore on load. Efforts to circumvent this have included the in vitro direct measurement of tension development in isolated atrial and papillary muscle strips, where load can be experimentally manipulated and fixed. While these systems have enabled accurate assessment of physiological responses, the limited time of viability of these tissues precludes the assessment of physiological effects that are manifest only after extended periods of exposure. In addition, delivery of mediators requires passive diffusion through numerous nonperfused cell layers.

Physiological responses of isolated cultured cardiac cells have also been examined. These systems permit monitoring of physiological characteristics over extended periods and have the advantage of direct delivery of mediators to cells, which exist in monolayers in culture. However, complex equipment capable of detecting minute displacements is required. In addition, the movement of a single cell sarcolemma has been used as an index of cellular contractile function. Because cells are grown on fixed, nondeformable substrata, the extent of sarcolemmal displacement is highly dependent on the sarcolemma location being monitored with respect to adherent cell borders and adjacent cell attachment sites.

The present method represents an improvement in existing techniques for the in vitro assessment of cardiac myocyte inotropic responses. Use of a flexible biological substratum amplifies the physiological response by reflecting the cumulative contractile activity of numerous beating cells. Thus, a point on the membrane substratum moves several times the linear dimension of a single cell, or across approximately one half of a low-power microscopic field. The system shares the advantages of an ability to directly deliver mediators to cells and carries the potential for fixing load. Unlike established systems in which contractile responses of cultured cells are determined by measurements of maximal sarcolemmal displacement, this method permits the determination of contractile phase velocity.

In inflammatory myocardial diseases, a major subpopulation experiences reversible and often profound congestive heart failure. In early and mild or moderate disease, histopathologic analysis of myocardium reveals little muscle necrosis or scarring. Functional reversibility and minimal cell death implicate a noncytotoxic process. A sparse distribution of mononuclear leukocytes, which are not present in normal myocardium, exists in inflammatory disease. These cells or their soluble products are likely candidates as mediators of noncytotoxic cardiac dysfunc-

tion. Humoral and cytotoxic cellular immune responses in myocarditis and allograft rejection have been demonstrated in humans and animal models, but there exists no unifying scheme to explain immune-mediated myocardial injury in the absence of myocyte necrosis.

These studies demonstrate that a soluble product of activated immune cells is capable of interfering with cardiac myocyte β-adrenergic responsiveness. Exposure of cells to immune cell soluble factors significantly decreased contractile velocity and chronotropic responses to isoproterenol stimulation. As a caveat, it is necessary to note that, since supernatant exposure resulted in an inhibited chronotropic response to stimulation, it is possible that the observed inhibition of augmentation of contractile phase velocity was due exclusively to a frequency–force relation and not to a true alteration in contractility. Definitive evidence would require measurements of cells paced at a rate exceeding the maximal agonist-stimulated rate. Nevertheless, supernatant exposure clearly interfered with myocyte responsiveness to adrenergic stimulation.

Sterin-Borda et al reported that lectin-activated human lymphocytes produced positive chronotropic and inotropic effects on isolated rat atria. That study differed significantly from our work in several crucial aspects, in addition to the obvious methodological differences. In that study, immune cells were coincubated directly with atrial strips, the time course of the effect was acute (10–50 minutes), the effect was seen on otherwise unstimulated muscle and β-adrenergic antagonists did not influence the effect, and the stimulation of inotropy and chronotropy was sensitive to inhibitors of lipoxygenase and appeared specific for a subpopulation of T cells. Given these numerous differences, those results are not in conflict with ours.

The inhibition of physiological responses was associated with a corresponding decrease in adrenergically stimulated intracellular cAMP concentration increases. Importantly, the basal contractile function and cAMP concentration of immune-conditioned myocytes did not differ from that of controls. This is consistent with previous observations that these cells do not differ from normal with respect to general cellular metabolism. Temporal limitations of the system used to measure cultured cell contractile responses precluded assessment of reversibility of the immune factor–induced inhibitory effect. However, parallel studies have demonstrated reversal of myocyte cAMP suppression upon discontinuation of supernatant exposure.

Since catecholamines are the principal physiological mediators of augmented cardiac contractility, these observations may have important implications for understanding the congestive heart failure of inflammatory heart disease. There are several potential mechanisms by which immune factors might produce blunted adrenergic responses without affecting basal metabolism and function. We have shown that β-receptor density and affinity for ligands...
are not altered in myocytes exposed to immune cell–conditioned media. However, other possible explanations include immune factor modulation of receptor internalization, interference with the formation of functionally active high-affinity receptor–G protein complexes, alterations in membrane G protein density or stoichiometry, changes in adenyl cyclase activity, or acceleration of cAMP phosphodiesterase activity. The mechanism of immune cytokine inhibition of β-adrenergic agonist stimulation of myocyte intracellular cAMP concentration increases is the subject of another report.

Lectin-activated splenocytes release numerous soluble cytokines with myriad effects. Studies designed to purify and characterize the responsible factor(s) have demonstrated that interleukin-1 and tumor necrosis factor-α each have activity in suppressing isoproterenol-stimulated increases in intracellular cAMP concentration. However, there is bioactivity in complex immune cell culture supernatants that cannot be attributed to these two defined cytokinins, and experiments are underway to further segregate and identify all of the active constituents of the complex conditioned media. Once complete, this will permit the exploration of interactions between effectors vis-à-vis myocyte metabolism, including efforts to determine whether each is a primary or secondary mediator. Detailed examination of myocyte contractile responses following exposure to each factor will then be undertaken.

In summary, a novel system to evaluate cultured contractile cell function was developed and characterized. Positive contractile velocity and chronotropic responses of cardiac myocytes to β-adrenergic agonist stimulation were markedly impaired in cells preincubated in medium conditioned by activated immune cells. Parallel studies demonstrated correlative reversible supernatant-induced inhibition of isoproterenol-stimulated intracellular cAMP concentration increases in a setting of normal general cellular metabolism. This inhibitor of cardiac contractile function may have relevance in disease states characterized by impaired contractility, alterations in β-adrenergic receptor expression and function, and the presence of leukocytes within the myocardium.

References

29. Marban E, Kusuoka H, Yue DT, Weisfeldt ML, Wier WG: Maximal Ca2+-activated force elicited by tetanization of ferret papillary muscle and whole heart: Mechanism and character-

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