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Interleukin 1 and tumor necrosis factor inhibit cardiac myocyte β-adrenergic responsiveness

(myocarditis/cardiac transplantation/contractility/cAMP)

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ABSTRACT **Reversible congestive heart failure can accompany cardiac allograft rejection and inflammatory myocarditis, conditions associated with an immune cell infiltrate of the myocardium. To determine whether immune cell secretory products alter cardiac muscle metabolism without cytotoxicity, we cultured cardiac myocytes in the presence of culture supernatants from activated immune cells. We observed that these culture supernatants inhibit β-adrenergic agonist-mediated increases in cultured cardiac myocyte contractility and intracellular cAMP accumulation. The myocyte contractile response to increased extracellular Ca^{2+} concentration is unaltered by prior exposure to these culture supernatants, as is the increase in myocyte intracellular cAMP concentration in response to stimulation with forskolin, a direct adenyl cyclase activator. Inhibition occurs in the absence of alteration in β-adrenergic receptor density or ligand binding affinity. Suppressive activity is attributable to the macrophage-derived cytokines interleukin 1 and tumor necrosis factor. Thus, these observations describe a role for defined cytokines in regulating the hormonal responsiveness and function of contractile cells. The effects of interleukin 1 and tumor necrosis factor on intracellular cAMP accumulation may be a model for immune modulation of other cellular functions dependent upon cyclic nucleotide metabolism. The uncoupling of agonist-occupied receptors from adenyl cyclase suggests that β-receptor or guanine nucleotide binding protein function is altered by the direct or indirect action of cytokines on cardiac muscle cells.**

Profound suppression of cardiac contractile function can accompany inflammatory myocarditis associated with idiopathic dilated congestive cardiomyopathy (1) and cardiac allograft rejection (2), despite minimal necrosis of cardiac muscle (3). Contractile performance can be restored in a subset of patients after immunosuppressive therapy (4). The cellular basis of this reversible pathophysiology has not been established. These observations led us to hypothesize that activated immune cells or their soluble products can alter cardiac metabolism without cytotoxicity. Further, because the primary endogenous mediators of the inotropic state of the heart are catecholamines, we postulated that the reversible decrement in contractility in these clinical syndromes reflects, in part, impaired cardiac responsiveness to β-adrenergic stimulation. To examine these hypotheses, we established a model of the inflamed myocardium by using monolayers of spontaneously contracting neonatal rat cardiac myocytes cultured on a flexible biological substrate and cell-free culture supernatants from activated immune cells. We have observed marked inhibition of myocyte contractility after supernatant exposure and have established the mediators and fundamental elements of the mechanism of this phenomenon.

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MATERIALS AND METHODS

Myocyte Cell Culture. Neonatal rat cardiac myocytes were isolated and cultured using a modification of standard techniques (5). Briefly, hearts were removed aseptically from 1-day-old Sprague–Dawley rats and subjected to collagenase (200 units/ml) digestion in divalent cation-free isotonic buffer. Intact viable myocytes were separated from nonmyocytes by centrifugation through a Percoll 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% (vol/vol) fetal bovine serum, 10 mM Hepes, and antibiotics. Cells were plated in 96-well Primaria (cAMP assay) or vinyl (β-adrenergic receptor binding assay) tissue culture plates or on amniotic membranes in 60-mm Petri dishes (contractility assay). Cells were incubated at 37°C in an atmosphere of humidified 5% CO_2/95% air. Attachment to substrata and formation of a syncytium required 48 hr, after which experimental manipulations were initiated.

Immune Cell Cultures. Rat bidirectional primary mixed lymphocyte cultures (MLCs) were established using splenocytes harvested from Lewis strain and outbred Sprague–Dawley adult rats. Cellular activation was confirmed by documentation of accelerated proliferation in MLCs compared to syngeneic splenocyte cultures by using [^{3}H]thymidine incorporation rates. Culture supernatants were harvested after 96 hr, clarified by centrifugation (2000 × *g*, 10 min), sterile-filtered, and stored in aliquots at −20°C. Activated splenocyte culture supernatants were obtained from 24-hr cultures of adult rat splenocytes suspended in medium containing Con A (5 μg/ml). Lectin was removed by batch adsorption to Sephadex G-25 prior to storage and use. Immune cell cultures were established in the same culture medium used for myocytes, except for the chromatographic fractionations when serum-free lectin-stimulated splenocyte culture supernatants were used. Myocyte culture medium served as control medium for experiments using MLC supernatants. Culture medium adsorbed with Sephadex G-25, in a manner identical to that used for lectin removal, served as control medium for experiments using activated splenocyte culture supernatants. All cell culture reagents used contained lipopolysaccharide at less than 10 pg/ml, as measured in the amebocyte lysate assay (Sigma). *Escherichia coli* lipopolysaccharide did not inhibit isoproterenol-stimulated cAMP increases in myocytes at concentrations up to 10 ng/ml.

Contractility Assays. Fresh human placental amniotic membrane was separated from chorion by blunt dissection and was thoroughly rinsed of blood prior to incubation for 24 hr in 4 g % deoxycholate at 4°C. Epithelial cells were then

Abbreviations: ICYP, [^{125}I]iodocyanopindolol; IL-1, interleukin 1; TNF, tumor necrosis factor; MLC, mixed lymphocyte culture.

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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 antibiotics (6). Membrane pieces (2 × 2 cm) were placed stromal-side down/basement membrane-side up in 35-mm Petri dishes. Freshly isolated myocytes were then plated in 1-cm-diameter cloning rings placed on the membranes. A synchronously contracting monolayer that produced macroscopic displacement of the membrane was apparent after 72 hr. A small aliquot (3 μl) of a suspension containing 0.9-μm cationic fluorescent beads (Covalent Technology) prepared as recommended by the manufacturer was added to cultures in the cloning rings and beads were permitted to adhere to cells and interstices. For assays, individual membranes were transferred to a perfusion chamber in a circulating waterbath mounted on a Zeiss inverted microscope stage. The chamber was perfused with modified Krebs buffer (128 mM NaCl/4 mM KCl/1.2 mM $CaCl_2$/1.0 mM $MgCl_2$/0.9 mM NaH_2PO_4/22 mM Hepes/5.5 mM dextrose) supplemented with 1% fetal bovine serum and 1 mM sodium ascorbate and various concentrations of calcium ion or isoproterenol. Beads were viewed by low-power microscopy and videotaped using a Hamamatsu fluorescence-enhancing video camera. Time-dependent linear displacement of single beads was measured directly from video screen. The average rate of displacement during the initial 33 msec of the contractile phase of five consecutive beats was determined before (v_o) and after (v_{ag}) agonist perfusion and expressed as a ratio (v_{ag}/v_o) to provide a relative contractility index.

cAMP Determinations. After incubation of myocytes under control and experimental conditions for the 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 sodium ascorbate, and various concentrations of isoproterenol or forskolin. After incubation for 10 min 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 $KHCO_3$ and analyzed for cAMP content by radioimmunoassay (7). Duplicate cAMP assays from quadruplicate myocyte wells were performed for each experimental condition. Total cellular protein in culture wells, after solubilization with 0.1 M NaOH, was determined by the Bradford assay (8).

β-Adrenergic Receptor Binding Assay. After incubation under control and experimental conditions for the indicated periods, intact myocyte β-adrenergic receptors were assayed by quantitating binding of the radioligand [^{125}I]iodocyanopindolol (ICYP). Culture medium was aspirated and replaced with assay buffer at 4°C consisting of PBS, 10 mM Hepes, 5.5 mM dextrose, 1 mM sodium ascorbate, and 0.1 g % bovine serum albumin with ICYP concentrations ranging from 2 to 250 pM in triplicate wells. After a 16-hr incubation at 4°C (determined in preliminary experiments to be required for equilibrium binding), aliquots of buffer were retrieved for determination of the concentration of free ICYP, and then wells were washed exhaustively with PBS. Radioactivity in individual vinyl culture wells was then measured to quantitate bound ICYP. Parallel wells received assay buffer containing 1 μM pindolol to determine nonspecific binding, which was less than 5% at K_D. Binding affinity and receptor density were determined by Scatchard transformation of binding data (9). For competition binding studies, 50 pM ICYP was included in assay buffer with isoproterenol from 10^{-10} to 10^{-4} M.

Sarcolemma membranes were prepared from myocytes plated in 60-mm plates. After thorough washing with PBS, cells were scraped with a rubber scraper and cellular material was collected in a small volume of buffer consisting of 20 mM potassium phosphate, 1 mM EDTA, and 5 mM $MgCl_2$ (pH 7.2). Cells were subjected to four freeze–thaw cycles and further homogenized with 20 strokes in a Dounce homogenizer. Material was then centrifuged for 10 min at 800 × g, and the supernatant fraction containing sarcolemmae was pelleted by centrifugation for 60 min at 100,000 × g. Competition binding studies were conducted with 5 μg of protein per tube in 200 μl of assay buffer with 50 pM ICYP and isoproterenol from 10^{-10} to 10^{-4} M. Membranes were incubated for 90 min at 37°C, after which binding reactions were terminated by filtration through Whatman GF/C filters and rapidly washed twice with 5 ml of PBS.

Chromatography. Serum-free culture supernatant from lectin-activated splenocytes (50 ml) was dialyzed against 20 mM Mes (pH 6.0; 1 liter of dialysate and three exchanges) and loaded onto a Mono S (Pharmacia) cation-exchange column equilibrated with buffer. Fractions (1 ml) eluting from the column with a NaCl gradient were collected and dialyzed against PBS. Collected fractions were reconstituted with fetal bovine serum to 10% and diluted 1:3 with myocyte culture medium prior to use in myocyte culture experiments.

Cytokine Bioassays. Crude culture supernatants and reconstituted chromatographic fractions were assayed in parallel for interleukin 1 (IL-1) using the D10.G4.1 T-cell proliferation assay (10). Serial dilutions of samples were incubated in 96-well plates with 2 × 10^4 D10 cells per well and Con A (2.5 μg/ml) for 72 hr. Wells were pulse-labeled with 0.4 μCi (1 Ci = 37 GBq) of [^{3}H]thymidine after 48 hr of culture, such that [^{3}H]thymidine incorporation into cellular DNA during the final 24 hr of culture was determined and compared to that of cultures containing IL-1 standards. Tumor necrosis factor (TNF) activity was assayed by L929 fibrosarcoma cytotoxicity (11). L929 cells were established at a density of 2 × 10^4 cells per well in 96-well culture plates and dilutions of samples and recombinant TNF standards were added to wells. After a 72-hr incubation, cytotoxicity was scored on a semi-quantitative scale (0, 1+, . . . , 4+) and samples were compared to standards.

RESULTS

Immune Cell Culture Supernatants Inhibit Cardiac Myocyte Contractility. Myocytes were established in monolayer culture on acellular basement membrane segments prepared from human amnion. Spontaneous synchronous myocyte contractions produced visible rhythmic distortion of the membranes. The time-dependent displacement of membrane-adherent fluorescent microspheres during contractile activity was viewed and recorded by using low-power fluorescent microscopy. Increases in velocity of contraction and maximal displacement were exhibited by control cells in response to the β-adrenergic agonist isoproterenol (Fig. 1 *Upper*). Under otherwise identical conditions, myocyte preparations incubated in medium conditioned by activated immune cells demonstrated markedly inhibited contractility augmentation in response to isoproterenol (Fig. 1 *Lower*). This impairment was reproducibly present and was quantitated in subsequent experiments. An index of agonist-mediated contractile response was defined as the ratio of initial contractile phase velocity after agonist stimulation to basal contractile phase velocity. Myocytes incubated under control conditions exhibited an isoproterenol-dependent increase in this index, from 1.0 in the absence of agonist (by definition) to 3.0 ± 0.28 (mean ± SEM) in response to 1 μM isoproterenol. The isoproterenol responsiveness of myocytes incubated with a 1:4 dilution of activated immune cell culture supernatant was 66% inhibited, with contractility augmentation to an index of only 1.69 ± 0.10 (Fig. 2). Importantly, increases in contractility mediated by a non-ligand-specific mechanism (increased extracellular Ca^{2+} concentration) were preserved in supernatant-exposed cells (Fig. 2). Thus,

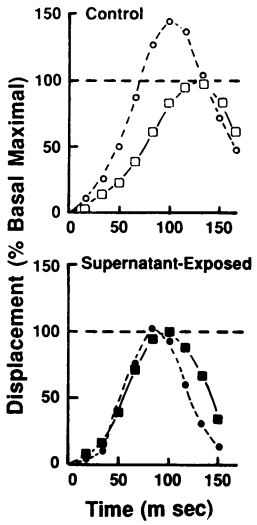

FIG. 1. Supernatant-induced inhibition of myocyte contractility. Spontaneously contracting cardiac cells established on acellular human amniotic membrane segments were incubated for 3 days with a 1:4 dilution of control medium or activated splenocyte culture supernatant. Time-dependent rhythmic displacement of membrane-adherent fluorescent beads on anchored free-floating membranes was recorded during buffer perfusion before (squares) and 5 min after (circles) addition of 1 μM isoproterenol. Displacement measurements of five consecutive contractions were averaged for each membrane and condition and data were then normalized to maximal displacement exhibited prior to agonist perfusion. Representative movement tracings are depicted for control (*Upper*) and supernatant-exposed (*Lower*) preparations.

immune-cell-conditioned culture medium contained activity that inhibited the contractile response of myocytes to β-adrenergic stimulation but did not alter the capacity of the myocyte to augment contractility.

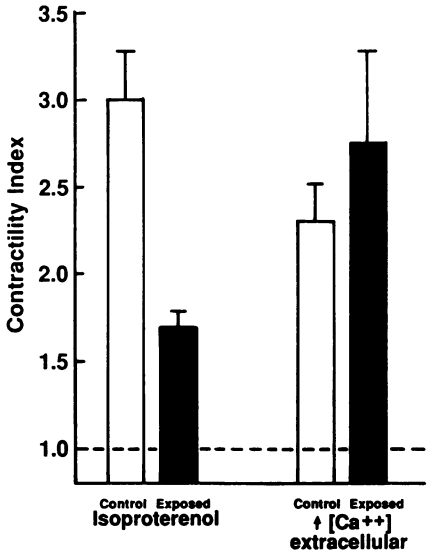

FIG. 2. Contractile augmentation of myocytes in response to isoproterenol and increased Ca^{2+}. Cultured myocytes were preincubated with a 1:4 dilution of activated splenocyte culture supernatant or control medium for 3 days prior to contractility assays. The average rate of contractile phase velocity during the initial 33 msec of five consecutive beats was determined before and after agonist perfusion and expressed as a ratio to provide an index of contractile response. Responses of control (open bars) and supernatant-exposed (solid bars) cells to 1 μM isoproterenol and to 3.6 mM Ca^{2+} were determined for multiple membrane preparations (n = 5) for each incubation condition. Values are expressed as mean ± SEM.

Immune Cell Culture Supernatants Inhibit β-Adrenergic Agonist-Stimulated Intracellular cAMP Increases in Cardiac Myocytes. Because β-adrenergic agonists effect enhanced myocyte contractility by increases in intracellular cAMP (12), we next examined the cAMP concentration in myocytes preincubated with activated immune cell culture supernatant or with control medium. The basal myocyte cAMP level was unaffected by exposure to supernatant (17.1 ± 0.4 vs. 17.2 ± 4.1 pmol/mg of protein, control vs. supernatant-exposed, respectively). Myocytes incubated in control medium demonstrated intracellular cAMP increases to a maximum of 150 pmol/mg of protein with isoproterenol stimulation. This maximal cAMP increase occurred at a stimulating isoproterenol concentration of 1 μM, and the EC_{50} for the effect was 30 nM. In contrast, myocytes incubated in the presence of splenocyte culture supernatant exhibited a maximal cAMP concentration of only 75 pmol/mg of protein with agonist stimulation up to an isoproterenol concentration of 0.1 mM (Fig. 3). Maximal suppression (65%) of isoproterenol-stimulated myocyte cAMP increases occurred in cells preincubated with a 1:4 dilution of supernatant, and the IC_{50} for the effect was a 1:20 dilution of supernatant (13). The inhibitory effect was first manifest after 24–48 hr of supernatant exposure. Maximal suppression was typically observed after 72 hr and persisted for up to 1 week. Reversal of the suppression was complete by 72 hr after removal of conditioned medium. Thus, time-dependent and reversible supernatant-mediated inhibition of myocyte intracellular cAMP increases in response to β-adrenergic stimulation correlated with inhibition of contractility augmentation.

Intracellular cAMP increases in myocytes after direct adenyl cyclase stimulation with forskolin were not altered by prior supernatant exposure. Thus, control cells had an intracellular cAMP concentration of 416 ± 14 pmol/mg of protein when stimulated with 30 μM forskolin, whereas myocytes incubated with a 1:4 dilution of splenocyte supernatant had a cAMP concentration of 392 ± 33 pmol/mg. Parallel studies showed no changes in cellular protein synthetic rate, ATP concentration, and microscopic appearance after supernatant exposure (13), eliminating nonspecific toxicity as a cause of supernatant-induced changes in contractility and cyclic nucleotide metabolism.

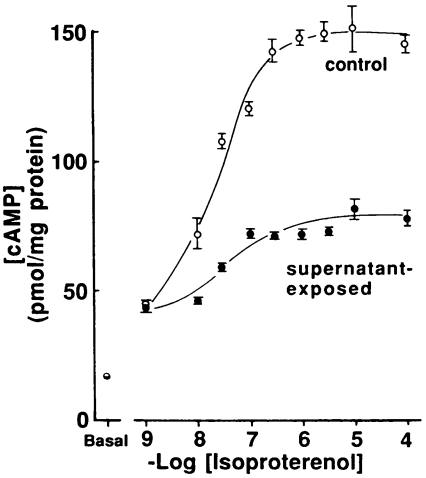

FIG. 3. Inhibition of myocyte isoproterenol-stimulated intracellular cAMP increases. Myocytes plated in 96-well tissue culture wells were incubated for 3 days in a 1:4 dilution of medium conditioned by activated splenocytes or control medium. Monolayers were washed once and incubated in buffer containing (−)-isoproterenol as indicated for 10 min, after which reactions were quenched with perchloric acid and intracellular cAMP content was determined by radioimmunoassay. Values are normalized to total cellular protein and are expressed as mean ± SEM.

β-Adrenergic Receptor Expression and Ligand Affinities Are Not Altered in Supernatant-Exposed Myocytes. To assess possible supernatant-mediated alteration of myocyte β-adrenergic receptor density or affinity, equilibrium binding studies were conducted using the β-adrenergic antagonist radioligand ICYP. Saturation binding of receptors was demonstrated in control and immune cell supernatant-exposed myocytes, and no significant differences in either receptor density or antagonist affinity were found (K_D = 6.3 vs. 7.3 pM; B_{max} = 275 vs. 282 fmol/mg of protein; control vs. supernatant-exposed, respectively). Competitive displacement of ICYP binding by the agonist isoproterenol was identical in control and supernatant-exposed cells, with respect to affinity (IC_{50} = 300 nM) and to maximal radioligand displacement (data not shown). Competition binding studies using washed sarcolemma membranes harvested from control and supernatant-exposed cells in the absence of guanine nucleotides or nonhydrolyzable analogues also showed comparable displacement of ICYP by isoproterenol. These data suggested that the suppression of cAMP accumulation in myocytes exposed to activated immune cell supernatants was secondary to impaired coupling of agonist-occupied receptors to cAMP production.

Identification of cAMP-Suppressive Activity as IL-1 and TNF. Activity was reproducibly present in multiple supernatant preparations from mitogen-activated splenocyte cultures and MLCs. Conditioned medium from fibroblast cultures did not exhibit myocyte cAMP-suppressive activity (13). Activity was precipitable in saturated $(NH_4)_2SO_4$, was not dialyzable, and was eluted at an apparent molecular mass of 15–40 kDa by molecular sieve chromatography (data not shown). It did not bind to an anion-exchange resin (Mono Q, Pharmacia) at pH 8.5 but was resolved into two distinct peaks by Mono S cation-exchange chromatography at pH 6.0 (Fig. 4 *Upper*). Myocytes cultured in the presence of either active fraction exhibited a 50% reduction in isoproterenol-stimulated intracellular cAMP concentration compared to control cells incubated with buffer or inactive column fractions. Concurrent bioassays of several immune cytokines demonstrated comigration of one cation-exchange chromatographic peak of myocyte cAMP-suppressive activity with D10.G4.1 T-cell activation and of the other peak with a L929 fibrosarcoma cytotoxicity (Fig. 4 *Lower*). These data suggested identity with IL-1β and TNF, respectively. This was subsequently confirmed using recombinant preparations of human cytokines (Table 1). At physiologic concentrations, recombinant IL-1β (Genzyme) was a potent inhibitor of isoproterenol-stimulated myocyte cAMP concentration increases, with maximal inhibition of 50% and an IC_{50} of 5 pM (8 units/ml). Similarly, recombinant TNF-α (Genzyme) inhibited cAMP increases by 40% with an IC_{50} of 50 units/ml. The potency of myocyte cAMP-suppressive activity paralleled IL-1 activity in crude immune cell culture supernatants, such that K_i supernatant concentrations contained IL-1 activity at 5–10 units/ml.

The recombinant cytokines produced an inhibition of myocyte contractile responses to isoproterenol. Cell preparations preincubated with 10 pM IL-1β demonstrated an increase in the contractility index to only 1.75 ± 0.12 in response to 1 μM isoproterenol, representing a 62% inhibited response compared to myocytes incubated under control conditions.

DISCUSSION

To date, most clinical and experimental investigations of immunologically mediated myocardial injury have focused on cytotoxic and humoral immune responses, which have not consistently correlated with active disease (14, 15). We report here observations of a physiologically relevant modulation of cardiac function and metabolism by a noncytotoxic cellular immune response. In myocarditis and cardiac allograft rejection, the cellular composition of the myocardium is altered by infiltration of activated macrophages and T lymphocytes (16). This occurs in a context of potentially reversible congestive heart failure. We have shown that macrophage-derived IL-1 and TNF are potent inhibitors of cardiac contractile responsiveness to β-adrenergic stimulation. Since the primary endogenous mediators of the inotropic state of the heart are catecholamines, particularly in cardiac failure and the denervated allograft (17, 18), this immune cytokine-mediated effect may contribute to reversible impairment of cardiac function *in vivo*. Although cytokine-induced inhibition of cAMP concentration increases in myocytes is a

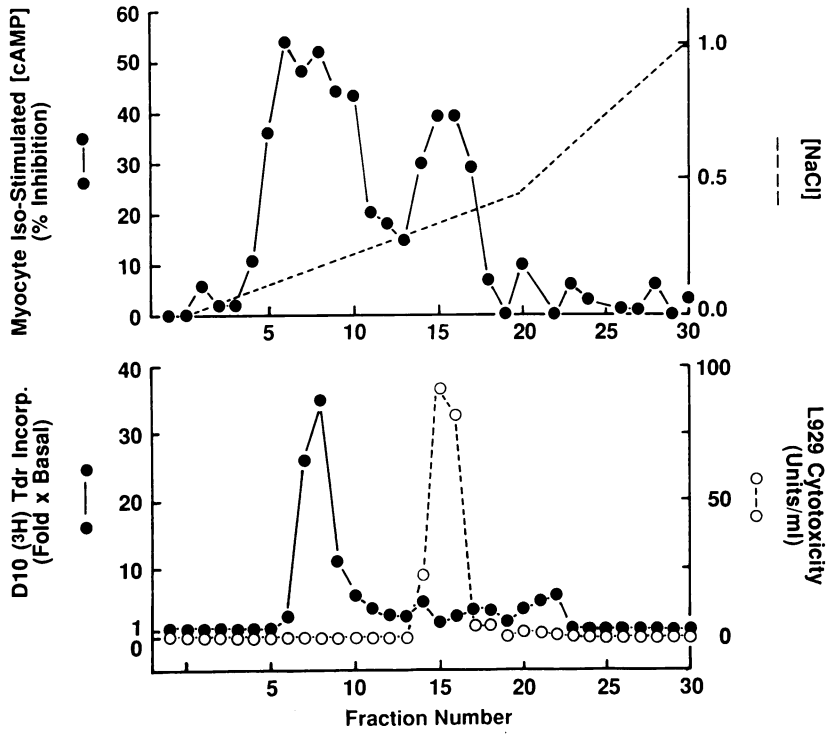

FIG. 4. Identification of cAMP-suppressive activity as IL-1 and TNF. Splenocyte culture supernatant prepared in serum-free medium was clarified by centrifugation and dialyzed vs. 20 mM Mes (pH 6.0) at 4°C. A 50-ml sample was loaded onto a Mono S (Pharmacia) FPLC column equilibrated with buffer, and 1-ml fractions, eluted with a NaCl gradient, were collected, dialyzed against PBS, sterile-filtered, and diluted 1:3 in myocyte culture medium. (*Upper*) Isoproterenol-stimulated myocyte cAMP was determined after incubation of cells in reconstituted column fractions for 3 days. Values are expressed as percent inhibition of cAMP increases (●) compared to control cells incubated in PBS diluted in medium. (*Lower*) Reconstituted chromatographic fractions were assayed in parallel for ability to stimulate D10.G4.1 T-lymphocyte proliferation by measuring [^{3}H]thymidine incorporation (●) and for L929 fibrosarcoma cytotoxicity (○), bioassays for IL-1 and TNF, respectively.

Table 1. Inhibition of isoproterenol-stimulated intracellular cAMP increases in cardiac myocytes by splenic-cell conditioned medium and recombinant macrophage cytokines

Medium supplement	Myocyte cAMP	
	pmol/mg	% inhibition
None	131 ± 5	0
Splenic cell culture supernatant		
2% (vol/vol)	105 ± 3	20
20% (vol/vol)	50 ± 3	62
None	127 ± 5	0
rIL-1β		
5 pM (8 units/ml)	74 ± 3	42
20 pM (34 units/ml)	66 ± 4	48
rTNF-α		
20 units/ml	106 ± 6	17
100 units/ml	78 ± 4	39

Cardiac myocytes plated in 96-well culture plates were incubated in medium supplemented with various concentrations of immune cell culture supernatant or recombinant (r) cytokines for 72 hr. Myocyte intracellular cAMP concentration after stimulation with 0.1 μM isoproterenol is shown. Values are expressed as mean ± SEM. Data are from two experiments.

correlative finding, these observations are seen in a context of an established role for cAMP in mediating contractile responses to adrenergic stimulation (12).

The observed impairment of contractile response to β-adrenergic stimulation is not secondary to alteration in the capacity of the myocyte to augment contractility, since responses to increased extracellular calcium ion concentration were normal. There were no changes in total cellular β-adrenergic receptor density or affinity for ligands. Identical maximal isoproterenol binding argues against β-adrenergic receptor internalization as an explanation for this effect, since this is a hydrophilic ligand that does not diffuse into cells (19). Further, equipotent isoproterenol binding implies intact stimulatory guanine nucleotide regulatory protein β-receptor coupling, since high-affinity agonist binding requires this interaction. The preserved response to direct adenyl cyclase activation with forskolin suggests that the effect is not due to acceleration of cAMP phosphodiesterase activity, loss of membrane adenyl cyclase, or nonspecific toxicity. Thus, the inhibition of cAMP accumulation in myocytes exposed to immune supernatants or constituent cytokines is secondary to impaired coupling of receptors to cAMP production. A membrane composition change that affects β-receptor/guanine nucleotide regulatory protein/adenyl cyclase coupling, impairment of GTP/GDP exchange by guanine nucleotide binding protein, or alterations in the stoichiometry or structure of guanine nucleotide binding proteins or subunits in the sarcolemma exist as possible explanations for this modulation of myocyte metabolism. These observations are distinct from those (20) of a non-specific relaxation response of vascular smooth muscle after acute exposure to IL-1. We have not observed acute responses of cardiac myocytes to either IL-1 or TNF; longer exposure selectively modulates ligand-specific stimulation of contractility and intracellular cAMP increases.

In the absence of available neutralizing antibody to rat IL-1, the possibility of additional cAMP-suppressing factors in the complex culture supernatants cannot be excluded. Nonetheless, quantitative comparison of the effects of recombinant IL-1 and TNF and supernatants containing defined cytokine activities indicates that >90% of this bioactivity is attributable to these two factors. Although IL-1 and TNF may be exerting this effect directly, the time course for expression of cAMP suppression is equally consistent with cytokine-induced secretion of secondary effector factors.

In summary, we have demonstrated that the macrophage-derived cytokines IL-1 and TNF induce potent inhibition of cardiac myocyte contractile responses to β-adrenergic stimulation. Inhibition of augmented contractile force correlates with impaired synthesis of cAMP. This impaired responsiveness occurs without evidence for nonspecific cellular toxicity—preserved capacity to augment contractility, unchanged β-adrenergic receptor density and affinity for ligands, and normal responses to direct adenyl cyclase stimulation. These findings suggest that a mechanism of reversible impairment of cardiac function in inflammatory heart disease may be the down-regulation of adrenergic responsiveness induced by IL-1 and TNF elaborated *in situ* in the myocardium. In a wider context, these observations implicate cytokines as potential modulators of non-immune cell hormonal responsiveness and guanine nucleotide regulatory proteins as a target cell site of cytokine action.

1. Kawai, C., Matsumori, A. & Fujiwara, H. (1987) *Annu. Rev. Med.* **38**, 221–239.
2. Murdock, D. K., Collins, E. G., Lawless, C. E., Molnar, Z., Scanlon, P. J. & Pifarre, R. (1978) *Heart Lung* **16**, 237–245.
3. Tazelaar, H. D. & Billingham, M. E. (1986) *Am. J. Surg. Pathol.* **10**, 405–412.
4. Fenoglio, J. J., Ursell, P. C., Kellogg, C. F., Drusin, R. E. & Weiss, M. B. (1983) *N. Engl. J. Med.* **308**, 12–18.
5. Harary, I. & Farley, B. (1963) *Exp. Cell Res.* **29**, 451–454.
6. Liotta, L. A., Lee, C. W. & Morakis, D. J. (1980) *Cancer Lett.* **11**, 141–152.
7. Tamayo, J., Bellorin-Font, E., Sicard, G., Anderson, C. & Martin, K. J. (1982) *Endocrinology* **111**, 1311–1317.
8. Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254.
9. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–665.
10. Kaye, J., Porcelli, S., Tite, J., Jones, B. & Janeway, C. A. (1983) *J. Exp. Med.* **158**, 836–856.
11. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. S., Palladino, M. A. & Shepard, H. M. (1985) *Science* **230**, 943–945.
12. Evans, D. B. (1986) *J. Cardiovasc. Pharmacol.* **8**, Suppl. 9, S22–S28.
13. Gulick, T., Chung, M. K., Pieper, S. J., Schreiner, G. F. & Lange, L. G. (1988) *Biochem. Biophys. Res. Commun.* **150**, 1–9.
14. Wynne, J. & Braunwald, E. (1988) in *Heart Disease*, ed. Braunwald, E. (Saunders, Philadelphia), 3rd Ed., pp. 1414–1415.
15. Cramer, D. V. (1987) *CRC Crit. Rev. Immunol.* **7**, 1–30.
16. Gokel, J. M., Reichart, B. & Struck, E. (1985) *Pathol. Res. Pract.* **178**, 354–364.
17. Borow, K. M., Neumann, A., Arensman, F. W. & Yacoub, M. H. (1985) *Circulation* **71**, 866–872.
18. Pope, S. E., Stinson, E. B., Daughters, G. T., Schroeder, J. S., Ingels, N. B. & Alderman, E. L. (1980) *Am. J. Cardiol.* **46**, 213–218.
19. Staehelin, M. & Hertel, C. (1983) *J. Recept. Res.* **3**, 35–43.
20. Beasley, D., Cohen, R. A. & Levinsky, N. G. (1989) *J. Clin. Invest.* **83**, 331–335.