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Development of cardiomyopathy and atrial tachyarrhythmias associated with activating autoantibodies to beta-adrenergic and muscarinic receptors

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Abstract

A 71-year-old male with well-controlled hypertension developed atrial tachyarrhythmias in 2002 and a restrictive cardiomyopathy in 2006 to 2007. Sera from 1992, 2001, and 2006 to 2008 demonstrated activating autoantibodies against β -adrenergic (AA β AR) and M2 muscarinic receptors (AAM2R). These sera have been characterized for bioactivity using in vitro assays of cardiac contractility and automaticity using a canine cardiac Purkinje fiber assay as well as protein kinase assay activation in H9c2 cells. These assays demonstrated concurrent positive β AR and inhibitory M2R effects that were blocked by nadolol and atropine, respectively. In a canine pulmonary vein atrial sleeve preparation, sera diluted 1:100 produced atrial hyperpolarization that was blocked by atropine. Atrial tachyarrhythmias developed in 2002 in the presence of a persistent bradycardia. Serial echocardiograms demonstrated progressive diastolic dysfunction in the absence of cardiac hypertrophy between 2006 and 2007. A dual-chamber pacemaker was installed with combined β AR (nadolol) and M2 β 3R (oxybutynin) blockade, resulting in marked suppression of atrial ectopy and improved diastolic function. The estimated pulmonary artery pressure decreased and exercise tolerance returned. Blood pressure has remained normal with β -blockade. AA β AR and AAM2R prospectively influenced atrial and ventricular function in this patient, and specific receptor blockade was associated with improved cardiac function.

Keywords

Premature atrial contractions; plasma aldosterone; converting-enzyme inhibitor; echocardiography

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Introduction

Activating autoantibodies to β -adrenergic receptors (AA β AR) have been observed as an associated etiologic and/or functional component of idiopathic dilated cardiomyopathy (DCM) since the early 1990s.^{1–4} More recently, activating autoantibodies to M2 muscarinic receptors (AAM2R) and AA β AR have been associated with atrial tachyarrhythmias.^{5,6} These studies have examined these relationships in a retrospective manner after the cardiomyopathy or tachyarrhythmias have developed. To date, there are no published prospective studies in humans examining whether the presence of one or more of these activating antibodies before development of the cardiac-related disorders is associated with the pathophysiologic outcome.

AA β AR production can be induced in experimental mouse⁷ or rat^{8,9} models and produces a functional cardiomyopathy that can be passively transferred to nonimmunized rodents⁸; thereby fulfilling Witebsky's postulates for an autoimmune disease. Lymphocytes from humans with DCM containing AA β AR also produce such a cardiomyopathy in a rodent recipient.¹⁰ There is therefore abundant evidence that these activating autoantibodies are associated with pathophysiologic states in humans and will produce such a state in animal models.

We have identified a 71-year-old male patient with a long history of well-controlled hypertension and a persistent bradycardia. There was no evidence for cardiac hypertrophy or atrial enlargement by echocardiography in 2001. One year later, he developed multiple runs of paroxysmal atrial tachycardia (PAT) and premature atrial contractions (PAC) superimposed on the bradycardia. In 2006, he demonstrated signs and symptoms of a progressive cardiomyopathy and worsening of the atrial tachyarrhythmias. A detailed evaluation with access to banked blood dating into the early 1990s has allowed the unusual opportunity to address the temporal relationship of the activating autoantibodies to the subsequent tachyarrhythmias and cardiomyopathy. Furthermore, directed interventions based on these pathophysiologic relationships have resulted in marked improvement in the tachyarrhythmias and cardiomyopathy.

Methods

Patient Profile

The patient is a 71-year-old male with a history of well-controlled hypertension since age 35. Evaluation over the years has revealed suppressed plasma renin activity, but normal plasma aldosterone with no evidence for impaired suppression after converting-enzyme inhibitor administration. Electrocardiograms and echocardiography demonstrated no evidence for left ventricular hypertrophy or abnormal rhythms. In 1998, the patient had two episodes of abdominal and flank pain subsequently attributed to cholelithiasis. A severe bout of pancreatitis with development of a pseudocyst followed an endoscopic retrograde cholangiopancreatography; and recovery was followed by a successful cholecystectomy. Two years later, the patient developed Raynaud's syndrome and evaluation demonstrated a polyclonal Coombs positive autoimmune hemolytic anemia. No specific Rh antigens were identified and the anemia initially responded to 40 mg prednisone. This was tapered gradually to 10 mg/day when a flare was observed. Subsequently, a splenectomy has partially controlled the chronic anemia and the patient's serum hemoglobin has been maintained at 12 to 13 g/dL on 6.0 mg of prednisone/day.

The patient developed a sinus bradycardia around age 30 in proximity to a period of physical training and jogging. This bradycardia persisted despite cessation of the more intensive

training period. Five years ago, the patient developed frequent runs of PAT. Holter monitoring demonstrated these were generally unifocal and self-limited, with multiple PACs interspersed between the sinus bradycardia that averaged 48 to 52 beats/minute. The location of the atrial beats appeared to be in proximity to the sinoatrial node and near the ostia of the right pulmonary vein. An attempt to control these with a β -blocker was unsuccessful owing to development of marked sinus bradycardia with rates in the high 30s to low 40s. Flecainide 50 mg 3 times daily was partially successful in suppressing these PAT runs.

Echocardiography initially demonstrated no evidence for left ventricular hypertrophy and the patient has been maintained on a converting-enzyme inhibitor and low-dosage spironolactone and subsequently switched to eplerenone. Beginning in 2006, the gradual onset of decreased exercise tolerance was observed with increasing dyspnea on exertion when climbing two to three double sets of stairs at work. Estimated pulmonary artery (PA) pressures by echocardiography rose from 35 mm Hg in May 2005 to 60 mm Hg in January 2007 without significant left ventricular hypertrophy, right ventricular hypertrophy, or increased left atrial size. In mid-2007, the patient noted a significant increase in dyspnea on exertion while climbing even modest inclines or rapidly walking more than 100 yards. A repeat stress echocardiogram in May 2007 failed to demonstrate evidence for wall motion abnormalities or echocardiographic evidence for ischemia. The PA pressure, however, was estimated at 60 mm Hg and rose to 75 mm Hg with exercise. Spiral computed tomography failed to demonstrate evidence for thromboembolic disease, and cardiac size was confirmed as normal. Right heart catheterization was performed after implantation of a temporary pacemaker. The PA systolic pressure was 54/20 mm Hg (age adjusted, normal [nl] = 25 to 30) and pulmonary wedge pressure was 20 mm Hg (nl = 8 to 15). The PA pressure fell with progressively increasing rate of pacing, helping rule out primary pulmonary hypertension. The pulmonary vascular resistance was 3.7 Wood units (nl < 2.0). Left heart catheterization was performed and demonstrated an end-diastolic pressure of 32 mm Hg (age adjusted, nl = 8 to 12). An abnormal E/A ratio on echocardiography was consistent with diastolic dysfunction. A dual-chamber pacemaker was installed to protect against the profound bradycardia observed with β -blockade. Subsequent institution of combined blockade of β AR (nadolol 60 mg/day) and M2<3R (oxybutynin ER 5 mg twice daily) has resulted in a marked improvement in cardiac performance. The estimated PA pressure was 35 mm Hg by repeat echocardiogram after 5 months of combined blockade. A paced atrial minimum rate of 70 beats/minute combined with β and muscarinic blockade have markedly suppressed the atrial tachyarrhythmias.

This study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board and all subjects provided written informed consent.

Assays

Enzyme-Linked Immunosorbent Assay—The enzyme-linked immunosorbent assay (ELISA) was performed as described elsewhere.¹¹ Briefly, microtiter plates were coated with purified human cardiac myosin,¹² human β 1AR, or M2R expressed in Sf9 or CHO cell membranes (PerkinElmer, Waltham, WA) at 10 μ g/mL in coating buffer. Sera were diluted 1:100 and thereafter diluted two-fold. Goat anti-human immunoglobulin (Ig)G conjugated with alkaline phosphatase (Sigma, St. Louis, MO) and its substrate para-nitrophenyl-phosphate 104 (Sigma) were used to detect antibody binding. Optical density (OD) was measured at 405 nm in an ELISA plate reader (Dynex, Chantilly, VA). Titers were determined as the highest dilution with an OD value of 0.10 at 60 minutes as compared with positive and negative control sera. Controls included serum alone, secondary antibody conjugate alone, and 1% bovine serum albumin alone.

Contractility Bioassay—Contractility assays were performed using isolated canine Purkinje fibers as described elsewhere.¹¹ Briefly, free running Purkinje fibers from canine ventricles were transferred to a $36 \pm 0.1^\circ\text{C}$ perfusion chamber mounted on the stage of an inverted microscope (Olympus; Olympus America Inc., Melville, NY). The fibers were perfused with normal Tyrode's solution at 36°C and paced via extracellular platinum electrodes at a constant rate of 120 beats/minute. Contractions were recorded digitally using a video edge detector (Model VED-205; Crescent Electronics, Sandy, UT) at 120 Hz. After achieving stable contractile responses, IgG equivalent to 1:100 serum dilutions were examined. IgG was purified from the patient or a healthy control's serum using the NAb Protein A/G Spin Kit (Pierce Biotechnology, Rockford, IL). Contractions continuously were recorded before, during and following the washout. After the washout, IgG plus atropine (100 nmol/L) was assayed to determine βAR activation attributable to the $\text{AA}\beta\text{AR}$ component of the IgG. IgG also was tested in the presence of dual βAR and M2R blockade using nadolol (100 nmol/L) and atropine (100 nmol/L). The βAR nonselective agonist isoproterenol (10 nmol/L) served as a positive control. Contractility indices were recorded and analyzed offline using pClamp 9.2 (Axon Instruments, Foster City, CA) and Sigmastat statistical software. Contractility indices were calculated as the mean of at least 15 consecutive contraction cycles after a stable baseline or agonist response was elicited.

Automaticity Bioassays—These were performed in free-running Purkinje fiber preparations attached to a canine endocardial section in a perfusion chamber.¹¹ Intracellular potentials were recorded from the central Purkinje strand using a glass microelectrode filled with KCl (3.0 mol/L). A bipolar electrogram was recorded from the distal free-running Purkinje strand, proximal to the endocardial insertion. Rates were determined after reaching equilibrium, generally within 1 to 2 minutes of exposure to the test substance. The average recorded rate was computed from 1-minute intervals. Rates were allowed to stabilize and the test serum was then infused as described previously. Serum infusion was followed by a 10- to 15-minute wash to allow a new baseline to be established. When the effect of blockade of βAR , M2R, or their combination was tested, they were first added to the perfusate to allow the new baseline to be established. Then serum was added into the buffer/blocker containing perfusate to determine if the previously documented activity was altered. Three separate measurements were made for each observation period and the values represent the mean for each period.

Protein Kinase Assay—Assays to determine protein kinase A (PKA) activity were performed as described¹¹ using serum or IgG from the patient. Briefly, 1×10^7 rat cardiac H9c2 cells (American Type Culture Collection, Manassas, VA) were plated in T75 culture flasks overnight. Sera (1:100 dilution) or IgG equivalent to 1:100 serum dilution with and without atropine (100 nmol/L) were incubated with cells in a final volume of 15 mL of medium for 1 hour before reactions were stopped by ice-cold phosphate-buffered saline. Cells were mechanically dislodged from flasks, centrifuged, and solubilized in 0.3 mL of protein extraction buffer before homogenization. Protein kinase activity of H9c2 cells was measured using a SignaTECT PKA assay system (Promega, Madison, WI). For inhibition of antibody-induced PKA activity, the β -blocker nadolol (100 nmol/L) was added. The βAR agonist isoproterenol (100 nmol/L) was used as a positive control. Calculation of the units of PKA per microgram of protein was determined and computed in the formula used in the assay kit purchased from Promega. The specific activity of the enzyme was determined in pmol/L per minute per microgram for each sample and the results were presented as percentages above the basal level of PKA activity.

Atrial Electrical Recording—An atrial electrogram was recorded using a canine pulmonary vein atrial sleeve preparation.¹³ The preparation was cut lengthwise, opened, and

pinned for superfusion of the endocardial surface (20 mL/minute). Up to three bipolar electrograms (0.10 mm diameter Teflon-coated silver wires, 1 mm apart) and an intracellular glass microelectrode (3 mol/L KCl, 10 to 30 M Ω) were recorded on a Gould Windograf recorder. The preparation was paced at 2 to 3 $\times$ diastolic threshold using 2-millisecond duration stimuli.

Results

ELISA and Activity Assays

The antibody titers for the β 1AR, M2R and cardiac myosin (CM) are shown in Figure 1. Each value for a given autoantibody was measured in the same assay, with the earliest serum available from 1992. Previous studies of control subjects demonstrated a normal range of antibody titers of 0 to 1600 for β 1AR and 0 to 800 for M2R and CM.¹¹ An increase (two-fold or greater) in the antibody titers for the β 1AR and M2R was already present in 1992. These titers were four-fold higher for anti- β 1AR and anti-M2R in 2001, and then decreased somewhat through the present. Thus anti- β AR and anti-M2R antibodies have been present at varying, but measurable levels throughout the last 16 years. The anticardiac myosin levels were within normal range throughout this period.

In our laboratory, IgG purified from 2001 sera (insufficient 1992 sera existed for this assay) demonstrated an increase of 111% of baseline in the contractility of the superfused Purkinje fiber ($P < .005$ vs. control IgG) (Figure 2). This contractility was significantly increased to 121% of baseline when atropine was superfused along with the IgG to block any M2R activity ($P < .05$ vs. IgG alone). The nonselective β -blocker nadolol decreased the IgG-mediated effect to 95% of baseline ($P < .005$ vs. IgG alone). Combined blockade with atropine and nadolol eliminated any change in contractility. The negative inhibitory M2R effect was therefore uncovered when atropine was added to the superfusate. IgG purified from control sera had no significant effect either in the absence or presence of atropine, nadolol, and their combination. The β AR agonist isoproterenol was also tested for comparison of its inotropic effect.

Automaticity effects from the serum of 2001 were studied (Figure 3). Serum alone produced a small but not significant decrease in the intrinsic automaticity of the Purkinje fiber. The addition of atropine, however, caused a 61% increase in the rate ($P < .001$ vs. sera alone) and this was completely blocked by addition of the non-selective β -blocker nadolol. Sera from a normal control subject did not show any significant chronotropic effect with or without atropine.

Sera from 1992, 2001, and 2007 were studied for their ability to stimulate PKA activation in cultured H9c2 cardiac cells (Figure 4A). In sera from 1992, PKA activity increased by 179% ($P < .001$ vs. control) and 200% ($P < .05$ vs. sera alone) of basal level in the absence and presence of atropine, respectively. This effect was partially blocked by concurrent treatment with nadolol ($P < .005$ vs. sera alone). Sera from 2001 demonstrated quantitatively greater activity than in 1992, but are proportionately similar for both AA β AR and AAM2R. This activity in 2007 both in the absence and presence of atropine was essentially identical to that observed in 2001 sera. The values for 2008 (Figure 4B) were studied after the subject was placed on combined β AR and M2/3R blockade therapy. IgG was purified from the serum to examine the effect of eliminating the impact of concurrent therapeutic β AR and M2R blockade on PKA activation. Both serum and purified IgG produced an increase in PKA activation of 160% to 180% of basal activity. The sera (containing similarly diluted β AR and M2R blockers) had no significant increase in PKA activation with atropine ($P > .05$ vs. sera alone), and β -blockade failed to produce any significant additional drop in activity. By contrast, the IgG preparation which did not contain the blocking agents had a dramatic

increase in β AR-mediated PKA activation in the presence of atropine ($P < .001$ vs. IgG alone), which returned to the initial IgG alone value with addition of the β -blocker. It is of interest that addition of the β -blocker to the in vitro assay failed to suppress the activity completely to basal level.

IgG derived from the 2006 serum was independently assayed in the laboratory of Dr. A. Baba (Kitasato Institute Hospital, Tokyo, Japan). These values confirmed the presence of M2R second extracellular loop peptide-reactive autoantibodies with a positive titer of 1:160 (normal $<1:40$). There was no negative chronotropic effect of this preparation in an embryonic chick bioassay.⁵ The IgG, however, significantly decreased the ejection fraction of the chick embryo from a control value of $84 \pm 2\%$ to $58 \pm 5\%$ (negative inotropic effect).

Electrophysiologic Studies

Multiple electrocardiograms (EKG) from the 1980s to 2001 demonstrated an underlying regular rhythm with a relative bradycardia becoming more noticeable in the last 7 years. Runs of PAT superimposed on this background of a bradycardia became evident in 2002. These were characterized by recurrent runs lasting from just a few beats to 5 to 30 seconds and generally were self-terminating. When noted, a valsalva maneuver would terminate any longer runs. Holter monitoring in February 2007 demonstrated 16,703 atrial beats, 1,896 runs, and 5,912 beats in runs, all appearing to arise from a single focus or region adjacent to the SA node and accentuated during sleep.

A recent interrogation of the pacemaker (February 25, 2008 to April 29, 2008) disclosed only 27 runs of atrial tachycardia, each less than 1 minute, during therapy with nadolol and oxybutynin. Echocardiography in May 2008 showed the estimated right ventricular systolic pressure had decreased to 35 mm Hg.

Serum from 2001 caused hyperpolarization and shortening of action potential duration in a canine pulmonary vein atrial sleeve preparation, which was blocked by the muscarinic blocker atropine (Figure 5). This supports the presence of cholinergic agonistic autoantibodies in the patient serum. The same serum also induced an early afterdepolarization during rapid pacing. No early afterdepolarization was present with treatment of control serum.

Discussion

This patient has provided historical information relating the presence of activating autoantibodies directed toward the β AR and M2R to the presence of a recently recognized form of mild hypertension.¹¹ We do not have sera antedating the onset of hypertension in this subject, but his biochemical correlates, clinical course, and response to therapy are consistent with the presence of continuous activation of the β AR by the autoantibodies that are directed to these receptors. Importantly, the presence of banked serum has allowed us to correlate these activating autoantibodies to the onset of atrial tachyarrhythmias and subsequently diastolic dysfunction and cardiomyopathy. The time interval of 10 and 15 years to these cardiac sequelae is compatible with studies in other autoimmune diseases such as lupus erythematosus wherein a time interval of 10 to 12 years was observed between development of autoantibodies and observable disease.¹⁴ Although shorter intervals from development of antibodies to onset of disease have been observed in rodent studies,⁷⁻¹⁰ the relationships proportional to animal lifespan are comparable. In addition, there will be an expected variability in activity of various autoantibodies. The important issue is the presence of active autoantibodies clearly antedated development of the cardiac dysfunction and supports a causative role rather than an epiphenomenon. There was no associated increase in autoantibodies against human cardiac myosin that might be associated with earlier cardiac

damage and development of molecular mimicry to myosin autoantibodies. Because earlier sera were not available and the activating autoantibodies were already present, this possibility cannot be excluded.¹⁵

There was a temporal relationship between development of an intense inflammatory abdominal reaction related to the endoscopic retrograde cholangiopancreatography–induced pancreatitis and higher autoantibody titers and activity observed in 2001. The subsequent onset of atrial tachyarrhythmias likely reflects the expected effects of both M2R and β AR activation on atrial action potentials and development of atrial cell ectopy.¹⁶ M2R activation causes hyperpolarization of the resting membrane potential and a slowed atrial sinus node intrinsic pacemaker. Atrial cell M2R activation also leads to a shortened action potential. This combined with β AR activation produces conditions predisposing to early afterdepolarization and ectopy in this patient. Besides tachycardia and ectopic beats, increased AA β AR and AAM2R have been associated with the development of atrial fibrillation. Although this patient had not progressed to this point, it appeared likely that this eventuality could not be ruled out.

There have been no prospective trials reported regarding the development of a cardiomyopathy associated with pre-existing activating autoantibodies. This patient previously demonstrated no significant effects of his well-controlled hypertension by EKG or echocardiography. He had not been able to tolerate previous β -blockade because his pulse would drop to the 38 to 44 range with associated symptoms. The insidious onset of a restrictive cardiomyopathy was noted by gradual loss of exercise tolerance while climbing sets of stairs, an exaggerated response to high altitude and later when even walking for one to three blocks. A stress echocardiogram failed to document evidence of ischemia but did demonstrate a significant E/A reversal and evidence for an increased estimated pulmonary artery pressure. The presence of an increased left ventricular end-diastolic pressure *in the absence* of significant left ventricular hypertrophy was confirmed by right and left heart catheterization. Primary pulmonary hypertension was excluded by the reduction in PA pressures during incremental pacing of the heart.

It is recognized that long-term stimulation by β AR agonists will alter ventricular performance. Increased β AR activity through constitutive genomic activation will produce a cardiomyopathy in experimental animals.^{17,18} Less well known is the markedly negative effect of M2R activation on ventricular contractility. This appears to primarily act via Gi activation through the M2R receptor.^{19,20} The impact of the resultant decrease in adenylate cyclase activity on ventricular energy requirements for diastolic relaxation would also be expected to decrease ventricular compliance. Thus, the marked diastolic dysfunction may be newly recognized sequelae of intense β AR and M2R activation by these autoantibodies.

Placement of a cardiac pacemaker in this subject and atrial pacing at 70 beats/minute has allowed use of the nonselective β -blocker nadolol in dosages that have suppressed atrial ectopy. Concurrent M2R blockade was established using oxybutynin, which, although directed primarily toward M3R, also contains inherent M2R blocking activity. With this “tailored” medical therapy, follow-up echocardiography showed reduction of right ventricular systolic pressure and there were no changes in cardiac morphology or function. It is of interest that PKA activation in serum taken while on combined blockade continued to demonstrate an increase over basal level, but addition of atropine or additional nadolol produced no significant change in PKA activity. When IgG was purified to eliminate additional serum effects, there was no change in PKA activity compared with serum, but there was a significant rise in β AR-induced PKA activation. These data suggest that serum dilution was proportionately altered by the antagonists taken by the patient. These data support the concept that “tailored” therapy based on the presence of multiple activating

autoantibodies may alter the associated cardiomyopathy. The failure to suppress PKA activation further with β -blockade is compatible with a recent report that activating autoantibodies directed against the second extracellular loop are incompletely (~60%) blocked by β -blockers.²¹ Although there is no proof that the existent blockade of the activating autoantibodies was the primary reason for the subsequent improved cardiomyopathy, there is significant evidence this was successful for management of the atrial tachyarrhythmias.

Conclusion

In summary, we have provided evidence, albeit with an individual case report, that the presence of activating autoantibodies directed toward β AR and M2R prospectively influence both atrial and ventricular function, and that therapeutic measures directed against their presence are associated with improved cardiac function. These data support the development of prospective trials of sufficient size and duration to confirm these issues.

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References

1. Limas CJ, Goldenberg IF, Limas C. Autoantibodies against beta-adrenoceptors in human idiopathic dilated cardiomyopathy. *Circ Res*. 1989; 64:97–103. [PubMed: 2535798]
2. Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation*. 1994; 89:2760–2767. [PubMed: 8205690]
3. Rosenbaum MB, Chiale PA, Schejtman D, Levin M, Elizari MV. Antibodies to beta-adrenergic receptors disclosing agonist-like properties in idiopathic dilated cardiomyopathy and Chagas' heart disease. *J Cardiovasc Electrophysiol*. 1994; 5:367–375. [PubMed: 8019712]
4. Luft FC, Dechend R, Dragun D, Müller D, Wallukat G. Agonistic antibodies directed at cell surface receptors and cardiovascular disease. *J Am Soc Hypertens*. 2008; 2:8–14. [PubMed: 20409879]
5. Baba A, Yoshikawa T, Fukuda Y, Sugiyama T, Shimada M, Akaishi M, et al. Autoantibodies against M2-muscarinic acetylcholine receptors: new upstream targets in atrial fibrillation in patients with dilated cardiomyopathy. *Eur Heart J*. 2004; 25:1108–1115. [PubMed: 15231368]
6. Chiale PA, Garro HA, Schmidberg J, Sanchez RA, Acunzo RS, Lago M, et al. Inappropriate sinus tachycardia may be related to an immunologic disorder involving cardiac beta-adrenergic receptors. *Heart Rhythm*. 2006; 3:1182–1186. [PubMed: 17018348]
7. Buvall L, Tang MS, Isic A, Andersson B, Fu M. Antibodies against the beta1-adrenergic receptor induce progressive development of cardiomyopathy. *J Mol Cell Cardiol*. 2007; 42:1001–1007. [PubMed: 17362986]
8. Jahns R, Boivin V, Hein L, Triebel S, Angermann CE, Ertl G, et al. Direct evidence for a beta 1-adrenergic receptor- directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy. *J Clin Invest*. 2004; 113:1419–1429. [PubMed: 15146239]
9. Buvall L, Bollano E, Chen J, Shultze W, Fu M. Phenotype of early cardiomyopathic changes induced by active immunization of rats with a synthetic peptide corresponding to the second extracellular loop of the human beta-adrenergic receptor. *Clin Exp Immunol*. 2006; 143:209–215. [PubMed: 16412044]
10. Omerovic E, Bollano E, Andersson B, Kujacic V, Schulze W, Hjalmarson A, et al. Induction of cardiomyopathy in severe combined immunodeficiency mice by transfer of lymphocytes from patients with idiopathic dilated cardiomyopathy. *Autoimmunity*. 2000; 32:271–280. [PubMed: 11191286]

11. Kem DC, Yu X, Patterson E, Huang S, Stavrakis S, Szabo B, et al. Autoimmune hypertensive syndrome. *Hypertension*. 2007; 50:829–834. [PubMed: 17923587]
12. Galvin JE, Hemric ME, Ward K, Cunningham MW. Cytotoxic mAb from rheumatic carditis recognizes heart valves and laminin. *J Clin Invest*. 2000; 106:217–224. [PubMed: 10903337]
13. Patterson E, Jackman WM, Beckman KJ, Lazzara R, Lockwood D, Scherlag BJ, et al. Spontaneous pulmonary vein firing in man: relationship to tachycardia-pause early after depolarizations and triggered arrhythmia in canine pulmonary veins in vitro. *J Cardiovasc Electrophysiol*. 2007; 18:1067–1075. [PubMed: 17655663]
14. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med*. 2003; 349:1526–1533. [PubMed: 14561795]
15. Li Y, Heuser JS, Cunningham LC, Kosanke SD, Cunningham MW. Mimicry and antibody-mediated cell signaling in autoimmune myocarditis. *J Immunol*. 2006; 177:8234–8240. [PubMed: 17114501]
16. Tai CT, Chiou CW, Chen SA. Interaction between the autonomic nervous system and atrial tachyarrhythmias. *J Cardiovasc Electrophysiol*. 2002; 13:83–87. [PubMed: 11843490]
17. Kudej RK, Iwase M, Uechi M, Vatner DE, Oka N, Ishikawa Y, et al. Effects of chronic beta-adrenergic receptor stimulation in mice. *J Mol Cell Cardiol*. 1997; 29:2735–2746. [PubMed: 9344768]
18. Hata JA, Williams ML, Koch WJ. Genetic manipulation of myocardial beta-adrenergic receptor activation and desensitization. *J Mol Cell Cardiol*. 2004; 37:11–21. [PubMed: 15242731]
19. Brodde OE, Bruck H, Leineweber K, Seyfarth T. Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic Res Cardiol*. 2001; 96:528–538. [PubMed: 11770070]
20. Brodde OE, Michel MC. Adrenergic and muscarinic receptors in the human heart. *Pharmacol Rev*. 1999; 51:651–690. [PubMed: 10581327]
21. Nikolaev VO, Boivin V, Stork S, Angermann CE, Ertl G, Lohse MJ, et al. A novel fluorescence method for the rapid detection of functional beta1-adrenergic receptor autoantibodies in heart failure. *J Am Coll Cardiol*. 2007; 50:423–431. [PubMed: 17662395]

Figure 1.

The ELISA titers for antibodies against β1AR, M2R, and CM in patient sera between 1992 and 2007. β1AR, β1-adrenergic receptor; CM, cardiac myosin; ELISA, enzyme-linked immunosorbent assay; M2R, M2 muscarinic receptor.

Figure 2.

The effect of purified IgG from 2001 sera on canine Purkinje fiber contractility. Data are expressed as percent of basal buffer control (dashed line) (mean $\pm$ SD). IgG alone induced a positive inotropic response of isolated Purkinje fibers (* P < .005 vs. control IgG). IgG in the presence of ATR increased the contractile response (** P < .05 vs. IgG alone). The nonselective β -blocker NAD decreased the IgG-mediated contractile response (* P < .005 vs. IgG alone). Combined blockade with atropine and nadolol eliminated any change in contractility. The normal control IgG did not demonstrate any significant contractile response, either alone or in the presence of atropine, nadolol and their combination. The positive control, ISO (10 nmol/L), significantly increased Purkinje fiber contractility (* P < .001 vs. control IgG). ATR, atropine; IgG, immunoglobulin; ISO, isoproterenol; NAD, nadolol; SD, standard deviation.

Figure 3.

The effect of the 2001 sera on Purkinje fiber automaticity. Each value represents the mean of 20 consecutive contractions and was measured after the preparation had stabilized. The data are expressed as the percentage of basal levels of the buffer control (dashed line; mean $\pm$ SD). This effect was similar to the contractility data. The presence of ATR significantly increased the rate (* P < .001 vs. sera alone), and this accelerated response again was completely eliminated by previous treatment with the nonselective β -blocker NAD. The positive control, ISO (10 nmol/L) significantly increased Purkinje fiber automaticity (** P < .01 vs. control sera). ATR, atropine; ISO, isoproterenol; NAD, nadolol; SD, standard deviation.

Figure 4.

The effect of patient sera on PKA activation. Values are expressed as percent of basal levels of PKA in medium control (dashed line) in the H9c2 cardiac cells (mean $\pm$ SD, $n = 3$). (A) Comparison between sera from 1992, 2001, and 2007. The serum-induced PKA activation was increased in the presence of muscarinic blocker ATR (** $P < .05$ vs. sera alone). The PKA activity was returned toward baseline when the β -blocker NAD was added (* $P < .005$ vs. sera alone), indicating the concurrent presence of activating antibodies to β AR and M2R in the patient sera. Isoproterenol (ISO, 100 nmol/L) was used as a positive control to activate PKA (* $P < .005$ vs. control sera). (B) Comparison between the sera from 2008, taken after β AR and M2<3R blockade had been established, and IgG purified from the same sera,

which did not contain the blocking agents. The serum and purified IgG both demonstrated residual β AR activity approximately 160% to 180% of basal levels. The serum containing the blocking agents failed to increase activity when atropine was added, suggesting that inherent β AR blockade was present. In the presence of atropine, the IgG preparation from the same serum had a robust increase in β AR activity (* $P < .005$ vs. IgG alone), strongly suggesting that the diluted sera still contained sufficient β -blocker to block the expected increase in β AR activity. The addition of nadolol to the in vitro assay suppressed this observed increase of β AR activity but only back to the level observed with serum or IgG alone. ATR, atropine; IgG, immunoglobulin; NAD, nadolol; PKA, protein kinase A; SD, standard deviation.

Figure 5.

The effect of patient sera on atrial action potentials and production of EAD. Serum from the patient caused hyperpolarization and shortening of action potential duration, which was blocked by muscarinic blocker atropine, indicating the presence of cholinergic agonistic autoantibodies in the patient serum. The same serum also induced an EAD during rapid pacing. No EAD was present with treatment of control serum. EAD, early after depolarization.