Identification of α– and β–Cardiac Myosin Heavy Chain Isoforms as Major Autoantigens in Dilated Cardiomyopathy

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Background. Immunization with cardiac myosin induces experimental autoimmune heart disease in genetically predisposed mice. These mice produce heart-specific autoantibodies, some of which are directed against the cardiac myosin isoform.

Methods and Results. We have reported the presence of circulating heart-specific autoantibodies in 26% of patients with idiopathic dilated cardiomyopathy (DCM) using indirect immunofluorescence. To identify the autoantigen(s) recognized by heart-specific autoantibodies in human disease, we tested, by Western blotting, sera from 26 DCM patients, 14 of whom were cardiac antibody-positive and 12 antibody-negative, as well as sera from 12 patients with cardiac failure from ischemic or valvular heart disease and from 13 normal subjects who were cardiac antibody-negative. Crude myofibrillar proteins and myosin preparations extracted from human atrial or ventricular specimens were used as antigens. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed. The proteins were electrophoretically transferred to nitrocellulose sheets. The paper strips were incubated in sera from patients or controls at 1:100 dilution; the reaction was revealed with a peroxidase-labeled second antibody against human immunoglobulin. Twelve of the 14 DCM sera (86%) containing heart-specific antibodies reacted with both the α– (atrial specific) and β– (ventricular and slow skeletal) myosin heavy chain isoforms; none of the 13 normal sera ( p=0.0001) and one of the 24 heart failure–negative control sera (4%, p=0.0001) contained antibodies against myosin heavy chain.

Conclusions. These findings indicate that α– and β–cardiac myosin heavy chain isoforms as in the murine model of autoimmune heart disease are major autoantigens in patients with idiopathic DCM. (Circulation 1992;85:1734–1742)

Key Words • cardiomyopathies • autoantigens • myosin

Immunization of genetically predisposed mice with cardiac myosin causes cardiac enlargement, the production of heart-specific antibodies, and a heart condition that resembles human dilated cardiomyopathy (DCM).1 Circulating autoantibodies to different cardiac antigens, such as the β–adrenergic receptor,2–4 the M7 mitochondrial antigen,4 and the adenine nucleotide translocator,5 have been reported in patients with DCM. Conclusive evidence for cardiac myosin being a relevant autoantigen in human DCM is lacking.

We have reported the presence of circulating heart-specific autoantibodies in 26% of patients with DCM by indirect immunofluorescence.6 Recently, Neumann et al7 reported a similar prevalence (20%) of such antibodies in DCM sera. The authors used Western blotting on human heart extract and were unable to identify specific autoantigens, but purified myocardial antigenic preparations were not tested. The aims of this study were to identify by immunoblotting contractile and noncontractile proteins as potential autoantigens recognized by the heart-specific antibodies found in DCM sera and to directly test the hypothesis that cardiac myosin is one of these autoantigens. In addition to whole human heart homogenates, crude myofibrillar extracts and purified myosin preparations were used as antigens; monoclonal antibodies to contractile and cytoskeletal components were also applied to confirm the identity of the bands recognized by the autoantibodies present in the patients’ sera.

Methods

Patients

The study included 26 patients with DCM, 12 control patients with heart failure from ischemic or valvular disease, and 13 normal individuals.

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Dilated cardiomyopathy. We have previously identified by indirect immunofluorescence “organ-specific” cardiac antibodies in sera from 17 of 65 patients with DCM. Skeletal muscle “cross-reactive” antibodies were detected in an additional seven patients. Organ-specific cardiac antibodies gave a diffuse cytoplasmic staining of atrial and, to a lesser extent, of ventricular myocytes; cross-reactive antibodies stained both heart and skeletal muscle sections with a striational pattern. 6 Eleven of the 17 DCM patients with organ-specific cardiac antibodies and three of the seven patients with cross-reactive antibodies were selected for this study on the basis of serum availability and previously detected high antibody titer. Twelve DCM patients who were cardiac antibody-negative were included as controls. The 26 DCM patients were 24–70 years of age (mean, 47±10 years); 20 were men and six women. The diagnosis of DCM was based on the World Health Organization criteria 8 with the demonstration of the demonstration of a dilated and poorly contracting left and/or right ventricle in the absence of a known cause. The diagnosis was made within 2 years of the study in all patients. Patients were excluded if there was evidence of 1) coronary heart disease at selective coronary arteriography; 2) ischemia during exercise testing; 3) systemic blood pressure ≥150/90 mm Hg; 4) concomitant systemic or endocrine diseases that are known to cause left ventricular impairment; or 5) excessive alcohol consumption, defined as a daily intake of at least 8 oz (0.24 l) of hard liquor or 2 qt (1.9 l) of beer for the preceding 10 years. 9 At the time of examination, nine patients were in New York Heart Association functional class I, 11 in class II, and six in class III. In all DCM patients, the assessment included right and left heart catheterization, selective coronary arteriography, left ventriculography, and two-dimensional echocardiography. Left ventricular end-diastolic and end-systolic echocardiographic dimensions were 49–78 mm (mean, 65±8 mm) and 39–72 mm (mean, 55±10 mm), respectively. Fractional shortening was 5–30% (mean, 16±8%), left atrial internal dimension was 17–57 mm (mean, 40±11 mm), and ejection fraction was 10–50% (mean, 31±13%).

Heart failure controls. In our previous study, 6 we reported that none of 41 patients with chronic heart failure not caused by DCM had anti-heart antibodies of the organ-specific type and only one of 41 (2%) of the cross-reactive type. Sera from 12 of these heart failure patients were randomly selected and included in the present study as controls. They were all cardiac antibody-negative and aged 47±12 years; seven were men and 5 women. Five of the 12 control patients had suffered a documented myocardial infarct 12 months to 10 years (median, 2 years) previously, and four had undergone mitral and aortic valve replacement for rheumatic heart disease. Ten patients were in New York Heart Association class III and two in class IV. In all 12 heart failure control patients, right and left heart catheterization, selective coronary arteriography, and left ventriculography were performed. Ejection fraction was 16–44% (mean, 30±7%).

Healthy blood donors. Sera from 13 healthy blood donors who were cardiac antibody-negative were also studied. They were aged 35±11 years; nine were men and four women.

Immunoblotting

Tissue sources. Atrial and ventricular tissues were either from the explanted hearts of transplant recipients with valvular or ischemic heart disease or normal atrial tissue specimens from the donor heart and were obtained at the time of heart transplantation. Histological examination of all tissues revealed no specific heart muscle disease, necrosis, or inflammation. Whole homogenates, myofibrillar extracts, and myosin were obtained from the atrial and ventricular specimens and used as antigens. Human skeletal muscle samples from vastus lateralis were obtained at the time of open heart surgery and were subsequently used as the source for myofibrillar extracts. All tissues used for whole heart homogenates and for crude myofibrillar extracts were snap-frozen in liquid nitrogen at −180°C and stored at −80°C. Samples used for myosin preparations were stored at −20°C in an ice-cold solution containing 50% glycerol, pepstatin 200 μg/l, 5 mM EGTA, and phenylmethylsulfonyl fluoride (PMSF) 200 μg/l until used.

Preparation of antigens. Whole heart homogenates in Laemmlli’s 10 buffer were prepared with a Dounce glass homogenizer. Myofibrillar extracts were prepared as previously described. 11 Briefly, tissues were homogenized in a low-salt buffer solution (LSB) containing 20 mM KCl, 2 mM K2HPO4, and 1 mM EGTA at pH 6.8; they were washed twice in LSB and then centrifuged at 5,000 rpm for 10 minutes at 4°C. The pellet was resuspended in high-salt pyrophosphate buffer solution (HSB) containing 40 mM Na4P2O7, 1 mM MgCl2, and 1 mM EGTA at pH 9.5, left for 30 minutes at 4°C, and then centrifuged for 15 minutes at 15,000 rpm. The supernatant, containing myosin and other myofibrillar proteins, was transferred to glass vials, and protein concentration was evaluated spectrophotometrically (Bradford 12 method). The preparations were then diluted in Laemmlli’s 10 buffer, boiled for 3 minutes, centrifuged for 10 minutes at 12,000 rpm to eliminate aggregates, and stored at −20°C.

Myosin preparations were obtained as previously described. 13 Briefly, samples were homogenized and washed four times with 40 mM KCl at 4°C. Twenty milliliter of an extracting solution at pH 6.5, which contained 300 mM KCl, 0.1 mM dithiothreitol (DTT), 150 mM phosphate buffer, and 5 mM MgATP, was added to each sample. After 30 minutes, the insoluble residues were removed from the extract by centrifugation at 27,000g for 15 minutes. The supernatant was then dialyzed overnight against 300–400 volumes of solution containing 10 mM KCl, 0.1 mM DTT, and 1 mM Tris-HCl at pH 7.2. Protein concentration was determined according to Lowry et al’s 14 method using albumin as standard.

Electrophoresis and immunoblotting. Cardiac and skeletal muscle proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmlli. 10 Whole homogenate preparations were resolved with 10% polyacrylamide gels. The α- (atrial-specific) and β- (ventricular and slow skeletal) myosin heavy chain isoforms were separated by 4% SDS-PAGE as described. 15 With this method, 15 myofibrillar extracts from a mixture of normal atrial and ventricular tissue gave two closely spaced bands because of the two isoforms; the ventric-
ular β–myosin heavy chain exhibits a higher electrophoretic mobility than the atrial α–myosin heavy chain. The same two bands caused by the two isomers are seen when hypertrophied atrial tissue is used because of the well-recognized phenomenon of increased expression of the β–myosin heavy chain in pressure-overloaded atria. To assess the reactivity of the positive sera for both α– and β–myosin heavy chain isomers, we used myofibrillar extracts from human hypertrophied left atrium obtained from patients with chronic valve disease and raised intracardiac pressure. The atrial tissue used contained both α– and β–myosin heavy chain, as assessed by densitometric analysis. Because β–myosin heavy chain expressed in hypertrophied atrial tissue is identical to that present in normal ventricular tissue, we used hypertrophied atrium instead of a mixture of normal atrial and ventricular tissue because of greater tissue availability. Six sera were also tested on myofibrillar extract from vastus pectoralis muscle. This preparation contained equal proportions of type 2 and type 1 (β)–myosin heavy chain as assessed on 4% SDS-PAGE and confirmed by immunoblotting with monoclonal antibodies specific for type 1 (β)–myosin heavy chain.

Electrophoretic resolution of myosin light chain atrial and ventricular isotypes (MLC-1a and MLC-2a and MLC-1v and MLC-2v, respectively) was obtained with 12.5% SDS-PAGE. A single reference well loaded with prestained molecular weight (MW) markers (Amersham) was included in each gel. The proteins were electrophoretically transferred to nitrocellulose sheets (Biorad) according to the method of Towbin et al. To determine optimal blotting conditions, preliminary experiments were performed as previously described. In particular, the following steps were optimized: transfer conditions, composition of the transfer buffer, saturation time of the blots, serum dilution, dilution of peroxidase-labeled secondary antibody, and incubation time. Titration experiments were also performed on two representative sera (one positive, one negative); reproducibility of the results was monitored by introducing in every assay these two sera chosen as standards. The intensity of the bands given by all sera was read by two independent observers who were blind to clinical and immunological data.

Two different protocols were used to study high (>70 kd) and low (≤70 kd) MW proteins; transfer was performed at 0.2 A overnight for the former and at 0.4 A for 3 hours for the latter. After SDS-PAGE was performed, one lane of the gel was cut and stained with Coomassie blue, whereas the remaining part of the gel was used for transfer. After transfer, the nitrocellulose was washed with Ponceau red to monitor transfer efficiency and to visualize the proteins present on each lane. The paper was then cut into strips, each corresponding to one lane. Ponceau red staining was then removed by washing in distilled water, and the single lanes were soaked in 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS) (Tris-HCl, 50 mM; CaCl₂, 2 mM; NaCl, 85 mM; pH 8.0) for 2 hours at 37°C. The blots were then incubated in sera from patients or controls at 1:100 dilution in TBS containing 3% BSA for 2 hours. Separate blots incubated with mouse monoclonal antibodies (MoAbs) or rabbit polyclonal antibodies against different contractile or cytoskeletal components were also used as controls to identify the bands recognized by the human sera. In particular, the following MoAbs were used: anti-α– and anti-β–myosin heavy chain (clones BA-D5, BF-32), anti-cardiac and skeletal myosin heavy chain (IV8F9, unpublished clone), anti-β–cardiac and skeletal myosin heavy chain (clone BF-35), anti-cardiac troponin T (clone RV-C2), anti-troponin I (clone TI-1), and anti-α-actinin (clone BM 75.2, Sigma, St. Louis, Mo.). A polyclonal rabbit anti-desmin antibody (Sclavo, Siena, Italy) was also used. After washing with TBS, the blots were incubated for 1 hour at room temperature with the appropriate peroxidase-labeled secondary antibody: rabbit anti-human Ig for the blots incubated with patients’ sera, goat anti-mouse Ig for the blots incubated with mouse MoAbs, and goat anti-rabbit Ig (Dakopatts, Denmark) for those incubated with rabbit anti-desmin antibody. All of the peroxidase-labeled secondary antibodies were titrated with known positive control sera to determine optimal dilutions. The 1:1,000 dilution (in TBS/3% BSA) was chosen as optimal for the anti-Ig antiserum and for all of the other antigens used in our assay. The reaction was developed with 3,3’-diaminobenzidine (Sigma) in the presence of imidazole as previously described. For each assay, at least one blot was incubated with the peroxidase-labeled second antibody in the absence of human serum as control for nonspecific binding. Preliminary experiments were performed with anti-IgG antisera instead of mixed Ig as second antibody; nonspecific background did not change, however, and mixed Ig was then used throughout the study. A MW standard curve based on the log acrylamide concentration and log MW of each pre-stained marker was determined for each gel and was used to estimate the apparent MW for bands of immunoreactivity detected with the human sera.

**Data analysis.** χ² test or Fisher’s exact test was used as appropriate to compare the positive immunoblotting results in DCM versus controls and to assess the association of the immunoblotting results with those obtained by immunofluorescence.

**Results**

**Immunoblotting**

**Homogenate preparations.** All of the 51 sera were first screened on human heart homogenate preparations. Twelve of the 14 DCM sera (86%) containing anti-heart antibodies by immunofluorescence (11 of the organ-specific type and one of the cross-reactive type) reacted with a band having an apparent MW of 200 kd. This band was identified as myosin heavy chain by the four specific MoAbs used. None of the 13 normal sera (p = 0.0001), none of the 12 antibody-negative DCM sera, and only one of the 12 heart failure immunofluorescence-negative control sera (one of 24 versus 12 of 14, p = 0.0001) contained antibodies against myosin heavy chain by immunoblotting (Table 1). None of the 51 sera reacted with tropomyosin, actin, or troponin T, I, or C.

Several bands not caused by such contractile components were also detected (Figure 1). These bands were more common in DCM-positive sera (12 of 14, 86%) than in heart failure–negative control sera (two of 12, 17%, p = 0.002), DCM antibody–negative sera (zero of
TABLE 1. Prevalence of Antibodies to Human Heart Proteins in Sera From DCM Patients and Controls by Immunoblotting

<table>
<thead>
<tr>
<th>Heart Protein</th>
<th>Cardiac antibody–positive by immunofluorescence (DCM)</th>
<th>Cardiac antibody–negative by immunofluorescence (DCM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=11)</td>
<td>Cross-reactive (n=3)</td>
</tr>
<tr>
<td>MHC (α and β)</td>
<td>11%</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>(78.5)</td>
<td>(33)</td>
</tr>
<tr>
<td>MLC-1v</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>(43)§</td>
<td>(66)§</td>
</tr>
<tr>
<td>Nonmyofibrillar proteins</td>
<td>9%</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>(64)‡‡</td>
<td>(100)‡‡</td>
</tr>
</tbody>
</table>

DCM, dilated cardiomyopathy; HF, heart failure; MHC, myosin heavy chain; MLC-1v, myosin light chain 1-ventricular.

*p=0.0001 vs. normals.
†p=0.0001 vs. DCM antibody negative.
‡p=0.0001 vs. heart failure controls.
§p=0.0002 vs. DCM antibody negative.
¶p=0.03 vs. DCM antibody negative.
#p=0.02 vs. heart failure controls.
**p=0.01 vs. normals.
***p=0.02 vs. heart failure controls.

12, 0%, p=0.0001), or in normal sera (zero of 13, 0%, p=0.0001) (Table 1). Of the 12 DCM sera that reacted with such unknown bands, nine had organ-specific and three had cross-reactive heart antibodies by immunofluorescence. The bands recognized by DCM-positive sera had apparent MW of 30–35 kd in seven patients (50%), 55–60 kd in three (21%), 100 kd in two (14%), and 130–150 kd in two (14%). The bands seen in the two heart failure–negative control sera had apparent MW of 30–35 kd in one patient (8%) and 55–60 kd in another patient (8%). All of the human sera and the peroxidase-conjugated secondary antibody (either mixed Ig or IgG secondary antibody) alone reacted with a 45-kd band (Figure 1). This band was caused by the binding of the second antibody to the immunoglobulin heavy chain contained.
in the antigenic mixture, as it is well recognized that serum immunoglobulins are contained in tissue homogenate preparations. This band was never observed with myofibrillar extracts (Figures 2 and 3) or with myosin preparations.

Myofibrillar and myosin preparations. All sera that reacted with myosin heavy chain on whole heart homogenate preparations were also found to be positive for this antigen on myofibrillar extracts, whereas all sera that did not react with myosin heavy chain on homogenate preparations were negative on myofibrillar extracts (Figure 2). All positive sera reacted both with the α- and β-cardiac myosin heavy chain isoforms (lane 3b, Figure 3). Six representative positive and negative sera were also tested on myofibrillar extract from skeletal muscle. Positive sera for the β-cardiac isoform (of which three were classified as organ-specific and one as cross-reactive by immunofluorescence) also reacted with β-skeletal myosin heavy chain, whereas sera that did not react with cardiac myosin were also unreactive on skeletal muscle preparations (data not shown).

All positive and negative findings for cardiac myosin heavy chain were also confirmed with purified myosin preparations. In addition, a proportion of the sera, when tested with purified myosin preparations, contained antibodies against the ventricular isoform 1 of myosin light chains (MLC-1v) (Figure 4). Antibodies to MLC-1v were more commonly found in the DCM sera, which contained cardiac antibodies by immunofluorescence (eight of 14, 57%), than in DCM antibody-negative controls (one of 12, 8%, p=0.03). Such antibodies were also slightly but not significantly more common in the DCM sera, which contained cardiac antibodies by immunofluorescence (eight of 14, 57%), than in heart failure-negative control sera (three of 12, 25%) or normals (two of 13, 15%, p=NS) (Table 1). Six of the eight DCM sera positive for MLC-1v had cardiac antibodies of the organ-specific type and two of the cross-reactive type.

Relation of Immunoblotting Results and Immunofluorescence Data

Significant associations were found between positive immunofluorescence results and positivity by immunoblotting for myosin heavy chain (12 of 14 versus one of 37, p=0.0001), for noncontractile proteins (12 of 14 versus two of 37, p=0.006), and for MLC-1v (eight of 14 versus six of 37, p=0.01) (Table 1). Discussion

During the past 30 years, an autoimmune pathogenesis has been recognized in several disorders that had been
classified as idiopathic. The discovery of circulating autoantibodies to the corresponding target organs or to ubiquitous subcellular components (e.g., mitochondria) has only recently led to the identification of the corresponding autoantigen, e.g., thyroperoxidase in thyroid autoimmunity, mitochondrial lipoyl dehydrogenase in primary biliary cirrhosis, the acid-producing ATPase of the gastric parietal cell in pernicious anemia, and glutamic acid decarboxylase in insulin-dependent diabetes mellitus.

This study demonstrates that α- and β-myosin heavy chain isoforms are relevant autoantigens recognized by the heart-specific antibodies detected by immunofluorescence in DCM. This is consistent with a recent experimental model of autoimmune heart disease in which genetically predisposed mice developed cardiomegaly and produced heart-specific antibodies after immunization with cardiac myosin.

Western blotting is a powerful tool for analyzing and characterizing autoantigens and autoantibodies in autoimmune disease. Variations in the intensity of the bands can be a limitation of the Western blotting technique. In this study, the experimental procedures were optimized and then standardized as recommended. The reproducibility of the results was monitored by introducing in each assay the same positive and negative sera as standards. Immunoblotting on whole tissue homogenate preparations, as seen in our study and in previous work, may give high background staining. In the present study, all sera were first screened against whole homogenate preparations, but all positive and negative results for contractile proteins were then confirmed on myofibrillar extracts and on purified myosin preparations. Positivity on immunoblotting for myosin heavy chain and for noncontractile proteins was strongly associated with positivity by immunofluorescence. A few sera, however, were positive by immunoblotting but negative by immunofluorescence. This may reflect the greater sensitivity of the immunoblotting technique compared with immunofluorescence. In our study, the anti-heart antibody titers in the tested sera ranged from 1:10 to 1:80 by immunofluorescence, whereas all sera were positive at 1:100 dilution by immunoblotting; the end-point titer of the serum chosen as positive standard was 1:80 by immunofluorescence and 1:250 by immunoblotting. The greater sensitivity of the immunoblotting technique has been recognized previously. Neumann et al also found such greater sensitivity in their study. They used Western blotting to identify relevant autoantigens in DCM. No single band of cardiac antigen reactivity was associated with DCM or with the presence of heart-reactive antibodies by immunofluorescence, whereas, interestingly, sera from patients with myocarditis had a higher prevalence of reactivity with antigens of 190–199 kD. The specific identity of the reactive autoantigens was not defined; potential candidates in this range of MW clearly include myosin heavy chain. In the present study, the 200-kD band recognized by the DCM sera was unequivocally identified as myosin heavy chain by use of purified human myosin preparations and several well-characterized monoclonal antibodies to human myosin heavy chain; the antigen identity was also confirmed by 4% SDS-PAGE separation of myosin heavy chain before Western blotting. In addition, the use of this
FIGURE 4. Electrophoretic separation and immunoblotting of myosin preparation. Cardiac muscle proteins contained in the preparation (3.4 μg protein/lane) were separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and either stained with Coomassie blue or transferred to nitrocellulose for immunoblotting. Lanes 1–4 are the immunoblots with (lanes 1 and 2) negative control sera and (lanes 3 and 4) sera from two dilated cardiomyopathy patients containing antibodies to myosin light chain ventricular 1 (MLC-1v). Lane 5 is the Coomassie blue-staining of the antigenic preparation, containing actin (Ac), tropomyosin (TM), myosin light chain ventricular 1 (MLC-1v), and myosin light chain ventricular 2 (MLC-2v).

An improved electrophoretic procedure enabled us to identify both α- and β-isofoms as relevant autoantigens. In humans, the α–myosin heavy chain isoform is expressed exclusively in atrial myocytes, whereas the β–isoform is present both in ventricular myocytes and in slow skeletal muscle fibers.31,15,16 In the present study, all of the DCM sera positive by immunoblotting reacted with both α– and β–myosin heavy chain. Possible explanations for this finding include the presence of two distinct antibody populations, as seen in mice.31,32 One heart-specific (anti-α) and another skeletal muscle cross-reactive (anti-β), and/or the production of antibodies directed against epitopes common to both α– and β–isofoms, which are highly homologous.33 The β–cardiac isoform is identical to type 1 (slow) skeletal muscle myosin heavy chain;34 thus, it is not surprising that the positive DCM sera reacted with both cardiac and skeletal β–myosin heavy chain.

There is an apparent discrepancy between the antigen recognized by immunoblotting and the immunofluorescence staining pattern. Eleven of the 12 sera that were positive for myosin by immunoblotting gave a diffuse cytoplasmic staining on atrial and ventricular myocytes and were negative on skeletal muscle, whereas only one had a striated pattern on both heart and skeletal muscle fibers by immunofluorescence.6 This finding is intriguing: in fact, both monoclonal and polyclonal antibodies to myosin or to other contractile components give a striated pattern by immunofluorescence.35,36 It is likely that DCM sera contain several distinct antibody populations, one reacting with myosin and others with cardiac-specific cytoplasmic antigens. The diffuse cytoplasmic immunofluorescence could therefore be caused by the latter antibodies, which mask the additional striational reactivity. This possibility is supported by the detection of several unknown bands with homogenate preparation that are not caused by antibodies to myosin or to other contractile proteins. Several potential cardiac-specific cytoplasmic autoantigens have been described, including mitochondrial and soluble antigens.37,38 Characterization of such antigens by use of purified myocardial cellular fractions in the immunoblotting assay or by other techniques is now warranted and should lead to the identification of the organ-specific cytoplasmic autoantigens, as suggested by our original description of antibodies that reacted with a diffuse immunofluorescence pattern only on cardiac myocytes and that were shown to be cardiac specific by quantitative absorption with relevant tissues, including human skeletal muscle.6 The fact that the DCM sera containing organ-specific cardiac antibodies did not show a striational pattern on cardiac tissue and did not react with skeletal muscle by immunofluorescence but were positive by immunoblotting for both cardiac and skeletal β–myosin heavy chain may also relate to the greater sensitivity of the immunoblotting technique.

The low prevalence of antimyosin antibodies in our heart failure controls is consistent with previous work showing that myosin autoantibodies, as assessed by immunofluorescence and confirmed by absorption experiments with purified myosin, are rare in patients with cardiac and skeletal muscle disorders, including myasthenia gravis, myocardial infarction, and Dressler’s syndrome.39 Interestingly, in the same study,39 no DCM sera were included, but the only serum with antimyosin activity was from a patient with Coxsackie B4 viral pleuropericarditis.39 Antimyosin antibodies have been found by ELISA in patients after cardiac surgery;40 the antigen used was rabbit skeletal muscle heavy meromyosin. Heavy meromyosin contains both myosin heavy and light chains; the antimyosin antibodies detected after cardiac surgery may have been directed against myosin light chain. In the present study, although antibodies to myosin heavy chain were rare in disease controls and normal individuals, there was a relatively high prevalence of antibodies to myosin light chain. This may relate to the fact that myosin heavy chains are insoluble in isotonic media, whereas myosin light chains are soluble; after minimal myocardial cell injury, myosin light chains may circulate and lead to lymphocyte stimulation and subsequent autoantibody production (e.g., myosin light chains circulate after acute myocardial infarction41,42).

Myosin is an intracellular molecule; thus, the question arises as to how this antigen elicits an autoimmune response with antibody production in patients with DCM. Viral infection or other causes of tissue necrosis might lead to release or exposure of myosin and trigger autoimmunity in individuals with a predisposing genetic background.29 Another mechanism by which virus might...
promote autosensitization to myosin would be that of molecular mimicry. Monoclonal antibodies to Coxsackie B4 virus also react with heart muscle, and a recent report describes a monoclonal antibody to Coxsackie VP-1 capsid protein that cross-reacts with cardiac myosin heavy chain. The myosin antibodies found in the Coxsackie B3--induced, immune-mediated myocarditis in A/J congenic mice, however, did not cross-react with the virus. In addition, the myosin antibodies produced in myosin-induced autoimmune myocarditis in the same genetically predisposed mouse strains did not react with the virus. These observations argue against the likelihood that viral infection triggers autoimmunity to the heart by molecular mimicry. Further studies are needed to clarify the mechanism by which myosin, as well as other intracellular autoantigens previously identified, may trigger and perpetuate, although not directly cause, autoimmune heart disease. It also remains to be established whether the antibodies found in DCM sera have a direct pathogenic role or, like the antmyosin antibodies found in murine autoimmune myocarditis, are only markers of immune damage.

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References
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