Antibody-mediated Autoimmune Myocarditis Depends on Genetically Determined Target Organ Sensitivity

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Summary

Injury to cardiac myocytes often leads to the production of anti-myosin antibodies. While these antibodies are a marker of myocardial injury, their contribution to pathogenesis in diseases such as autoimmune myocarditis or rheumatic fever is much less clear. We demonstrate in this report that monoclonal anti-myosin antibodies can mediate myocarditis in a susceptible mouse strain. Additionally, we show disease susceptibility depends on the presence of myosin or a myosin-like molecule in cardiac extracellular matrix. This study demonstrates that susceptibility to autoimmune heart disease depends not only on the activation of self-reactive lymphocytes but also on genetically determined target organ sensitivity to autoantibodies.

Myocardial injury associated with myocyte necrosis leads to the transient activation of heart-reactive immune cells (1-3), including anti-myosin–producing B cells (1-3). In several pathologic conditions, anti-myosin antibodies have been considered a marker for myocyte damage rather than a contributor to ongoing tissue injury (1-3). Whereas a direct role of these autoantibodies in the pathogenesis of rheumatic fever has been proven (4-6), there has been some controversy regarding their role in the pathogenesis of autoimmune myocarditis. Autoimmune myocarditis can be induced in mice by infecting susceptible strains with Coxsackie B_3 (CB_3) virus (7-9) or by immunization with cardiac myosin (10). After viral infection, all mouse strains develop an early viral myocarditis, but some strains progress to a chronic inflammatory cell infiltrate and myocyte necrosis associated with the induction of heart-reactive antibodies and T cells (10-13). A dominant self-antigen in this response is cardiac myosin heavy chain alpha (myhca)[1] (11). Neu et al. (14) showed that the myocarditis developed by susceptible mice is histologically similar whether induced as postviral autoimmune myocarditis or after immunization with cardiac myosin. Whereas it has been possible to demonstrate that mice that develop myocarditis have high titers of anti-myosin antibody, it has not been possible to transfer disease to naive mice by transferring serum from diseased animals (14, 15). In addition, there are no reports of attempts to induce disease with passive transfer of monoclonal anti-myosin antibodies.

Both DBA/2 and BALB/c mice develop autoimmune myocarditis after CB_3 virus infection or immunization with cardiac myosin, but recent studies suggest that the mode of tissue damage may differ between these two strains (16, 17). Whereas anti-myosin antibodies are present at high titer in BALB/c mice, they do not appear to contribute significantly to pathogenesis (15-18). In fact, T cells alone can transfer myocardial inflammation and necrosis to BALB/c mice. In contrast, complement depletion in DBA/2 mice causes a marked diminution of inflammation and necrosis (16, 17), suggesting that antibody-mediated tissue damage contributes to the disease process.

In an effort to explore why anti-myosin antibodies contribute to pathogenesis only in DBA/2 mice, we have studied the serum anti-myosin response in both DBA/2 and BALB/c mice and have examined the effect of administration of monoclonal anti-myosin antibodies in both mouse strains.

Materials and Methods

Animals

All animals were treated according to institutional guidelines. BALB/c, DBA/2, A.CA, and CAJ/F_1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained at the Albert Einstein College of Medicine animal facility. SCID mice were obtained from the breeding colony maintained at the Albert Einstein College of Medicine.

Antigens

Mouse cardiac myosin was purified from BALB/c mouse hearts according to the method of Pollack et al. (18). The purity of the preparations was determined by SDS-PAGE. The myosin concentration was determined spectrophotometrically using an extinction coefficient of 5.4. Myosin was dissolved in 50 mM sodium pyrophosphate buffer, pH 7.2, and stored at $-70°C$.

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[1] Abbreviations used in this paper: myhca, cardiac myosin heavy chain alpha; TBM, antitubular basement membrane.

Immunization Protocol

A.CA, BALB/c, and DBA/2 mice was injected subcutaneously with 100 μg cardiac myosin emulsified in CFA and boosted with the same 1 wk later. Mice with the highest titer of anti-myosin reactivity were chosen for B cell fusion. These mice received a final intravenous or intrasplenic injection of 100 μg of cardiac myosin in PBS and 3 d later, the spleen cells were harvested for fusion.

ELISAs

Serum was obtained by retro-orbital puncture of metafane-anesthetized mice and stored at −70°C. For analysis by ELISA, sera were diluted in PBS-0.5% BSA (Boehringer Mannheim, Indianapolis, IN). Sera from immunized mice and hybridomas supernatants were tested for IgG antibodies to mouse cardiac myosin by ELISA as described by Neu et al. (10). Briefly, 100 μg/ml of myosin in coating buffer (sodium carbonate, sodium bicarbonate buffer, and sodium pyrophosphate, pH 9.6) was adsorbed to microtiter plates (Falcon, Lincoln Park, NJ), blocked with PBS-2% BSA, and then incubated with serial dilutions of sera from immunized mice or with culture supernatant. Anti-myosin antibodies were detected with peroxidase-conjugated anti–mouse IgG diluted 1:1,000 (Sigma Chemical Co., St. Louis, MO) and substrate, 2,2,-, azino-di-3-ethylbenzthiazoline sulfonate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Optical density was read at 405 nm on an ELISA reader. Isotype-specific peroxidase conjugated anti–mouse IgG (Sigma Chemical Co.) was used in some assays.

To determine epitope specificity of serum anti-myosin antibodies, 10 μg/ml of mouse cardiac myosin fragments in coating buffer was adsorbed to microtiter plates (Falcon) at 37°C for 1 h. The plates were dried at room temperature overnight. After a blocking step with PBS-5% milk (Bio-Rad Laboratories, Richmond, CA) at 37°C for 1 h, the wells were incubated with sera (1:500 in PBS-0.5% BSA) at 37°C for 1 h. After washing, peroxidase-coupled anti–mouse IgG (1:1,000; Sigma Chemical Co.) was added at 37°C for 1 h. Plates were washed and substrate, 2,2-,azino-di-3-ethyl-benzthiazoline sulfonate was added. Optical density was read at 405 nm on an ELISA reader.

Cell Fusion

B cell hybridomas were obtained by fusion of spleen cells to the nonproducing myeloma cell line NSO using standard fusion technology (19). Cells were cultured at 37°C in HAT medium at 2 × 10⁵ myeloma cells per ml. Cells from wells with anti-myosin reactivity were cloned on soft agar and individual colonies picked and rescreened for production of anti-myosin antibody.

Characterization of Monoclonal Antibodies

Isotype of Anti-myosin Antibodies. Goat anti–mouse Ig specific for different classes and subclasses (1:1,000 in PBS; Fisher Biotech, Pittsburgh, PA) were adsorbed to 96-well microtiter plates at 37°C for 2 h. After blocking with PBS-2% BSA at 4°C overnight, hybridoma supernatants were incubated in the wells for 2 h at 37°C and washed. Anti-class– or subclass-specific goat anti–mouse antibodies were then conjugated to alkaline phosphatase (1:1,000; Sigma Chemical Co.) at 37°C for 1 h. After washing, the phosphatase substrate was added and the assay read at 405 nm on an ELISA reader (Bio-Rad Laboratories).

Epitope Mapping on Anti-myosin Antibodies. Fragments of BALB/c mouse myhca were displayed in a 5% SDS-PAGE minigel. Proteins were transferred to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH). The membranes were blocked with PBS-5% milk for 1 h

at room temperature. Strips were incubated with monoclonal anti-myosin antibodies (5–10 μg/ml in PBS) overnight at 4°C and washed with PBS-0.05% Tween 20. Immune complexes were developed using a peroxidase-labeled goat anti–mouse IgG (Sigma Chemical Co.) at 37°C for 1 h (or 2 h at room temperature). After washing, the blot was developed with substrate (ECL Western blotting detection system, Amersham International, Amersham, Bucks, UK).

Pathogenicity of Anti-myosin Antibodies

Purification of Monoclonal Anti-myosin Antibodies. CAF1/J mice were injected intraperitoneally with 0.5 ml 2,6,10,14 Tetramethylpentadecane 98% (Aldrich Chemical Co., Inc., Milwaukee, WI). 1 wk later, the mice were again injected with 2,6,10,14 Tetramethylpentadecane. 2 d later, each mouse was injected with 10⁷ monoclonal anti-myosin antibody secreting hybridoma cells in saline. For hybridomas generated from A.CA and DBA/2 mice, mice were irradiated with 600 rad. The anti-myosin antibody–containing ascites was collected and stored at −20°C.

Anti-myosin antibodies of the IgG isotype were purified from ascites fluid by 50% ammonium sulfate (pH 7.2) precipitation at 4°C overnight. The precipitated IgG was dissolved in 5–10 ml PBS, then passed (0.8 ml/min) through a protein G–Sepharose column in a fast performance liquid chromatography (FPLC) system. Bound protein was eluted with 0.1 M glycine, pH 2.7, at 1 ml/min and collected in a neutralizing buffer (2 M Tris-HCl, pH 8.0).

The purity of IgG antibodies was checked by SDS-PAGE under reduced and nonreduced conditions. The concentrations of anti-myosin antibodies were measured using an ELISA for IgG.

Induction of Myocarditis by Anti-myosin Antibodies. Each mouse was injected intraperitoneally with purified anti-myosin antibody, 100 μg in 200 μl PBS daily, 5 d a week for 3 wk. Mice were killed after 3 wk and the hearts were rapidly excised and placed into 10% buffered formalin.

Histologic Analysis

Formalin-fixed hearts were embedded in paraffin according to standard procedures. Sections were prepared from hearts dissected in the atrial-apical axis, thereby producing two halves in which all four chambers could be seen. Three levels, each ∼5 μm in thickness, were prepared and stained with hematoxylin-eosin. Sections were evaluated from all hearts by S. Factor who was blinded to the immunization status of each animal. Each heart section was examined for active myocarditis, its extent and localization. The diagnosis of myocarditis is an inflammatory infiltrate associated with myocyte necrosis or damage. The presence of interstitial myocardial inflammation without necrosis, so-called borderline myocarditis, was also noted.

Immunocytochemistry

Serially cut, 5-μM-thick sections of formaldehyde-fixed, paraffin-embedded tissue were deparaffinized and rehydrated, then blocked with 2% horse serum (Vecta stain ABS Kit; Vector Laboratories Inc., Burlingame, CA) in PBS in moist chambers. After blocking, the sections were incubated with biotinylated antibody against mouse IgG (1:800 for 1 h at room temperature). After washing, sections were incubated with alkaline phosphate–labeled ABC reagent (Vecta stain ABC Kit), washed, and developed with substrate for streptavidin-alkaline phosphatase (BCIP [5 bromo-4 chloro-3 indolyl phosphate p-toluidine salt] and NBT [nitroblue tetrazolium chloride]; GIBCO BRL, Gaithersburg, MD). The color development was stopped by the addition of distilled water. The

sections were dehydrated in alcohol and mounted on coverslips with cytosol mounting medium (Stephens Scientific, Riverdale, NJ).

Extracellular Matrix Protein Preparation

BALB/c and DBA/2 heart tissue extracellular biomatrix proteins were prepared according to the method described by Rojkind et al. (21). The protein concentration was assayed using a dye-binding assay of protein quantitation (22) and stored at $-20°C$ in glass tubes.

Samples of extracellular matrix proteins were neutralized with Tris and heated for 10 min at 90°C in sample buffer in the presence of dithiothreitol (Calbiochem-Novabiochem Corp., La Jolla, CA). 10 μg of each sample were loaded in a 5% SDS-PAGE minigel and run at 35 V for the stacking gel, 105 V for the running gel. SDS-PAGE gels were treated for 15 min in cold transfer buffer (2 mM Tris-HCl, 200 mM glycine, and 20% methanol). The extracellular matrix proteins were transferred to nitrocellulose (Schleicher & Schuell, Inc.) at 35 V overnight at 4°C using a miniprotein transfer chamber (Bio-Rad Laboratories). The membrane was blocked with PBS-2% milk for 1 h at room temperature. The strips were incubated with different monoclonal anti-myosin antibodies at 37°C for 2 h and washed with PBS-0.05% Tween 20. Immune complexes were developed using a peroxidase-labeled goat anti-mouse IgG (Sigma Chemical Co.) and substrate (ECL Western blotting detection system).

In each experiment, the gels were also stained wtih 0.035% Coomassie brilliant blue, 45% methanol, and 10% acetic acid overnight and destained with 5% acetic acetic acid, 7% methanol.

Expression of Mouse Cardiac Myosin Heavy Chain Fragments

The BALB/c myhca coding region was amplified in four segments by reverse transcriptase PCR from cardiac RNA. The following primer pairs were used: Fragment, ATGACGGATGCC-CAGATG and AGAAGTGGGCTTCCT (encoding amino acids 1-562); fragment 2, AGGAAGCCCACTTCT and TTCTGCAGC-TGAAG (encoding amino acids 562-1102); fragment 3, CTTCAG-CTGCAGAA and TCATGCTCCAGGGAG (encoding amino acids

1102-1542); and fragment 4, CTCCCTGGAGCATGA and CAC-GATGAGGAATAA (encoding amino acids 1542-1972).

Each of the PCR products was engineered to contain an in-frame NcoI site at the 5' end, a termination codon at the 3' end and a HindIII site. The fragments were cloned into the expression vector pMW172 at the NcoI and HindIII sites (23). For purification of the fragments, after isopropyl thiogalacto-pyranoside IPTG induction, 1-3 liters of bacterial culture was collected by centrifugation and lysed in the French press as previously described (23). For fragments 1 and 2, the insoluble pellet after lysis was solubilized in a Sarkosyl extraction buffer (24) and further purified by preparative acrylamide gel electrophoresis. For fragments 3 and 4, the soluble fraction after lysis was subjected to boiling. Myosin rod fragments will renature after this treatment, resulting in substantial purification (23).

Results

Serum Anti-myosin Response. To determine if there is a difference in the serum antibody response to myosin between BALB/c and DBA/2 mice, we examined titers of anti-myosin antibodies in serum of myosin-immunized mice. Anti-myosin reactivity was similar in myosin-immunized DBA/2 and BALB/c mice. Fig. 1 shows that there was no significant difference in titers of IgG anti-myosin antibodies in these two strains. Whereas antibodies of the IgG1, IgG2a, and IgG2b isotypes were present in both BALB/c and DBA/2 mice after immunization with autologous cardiac myosin (Fig. 2), BALB/c mice showed a significantly higher level of IgG1 anti-myosin antibodies.

Fine specificity differences in anti-myosin antibodies between these two strains were assessed by assaying sera for reactivity to four genetically engineered fragments of BALB/c mouse myhca that span the molecule. As shown in Fig. 3, both BALB/c and DBA/2 sera showed reactivity to the entire length of the molecule, including fragment 2 (amino acids
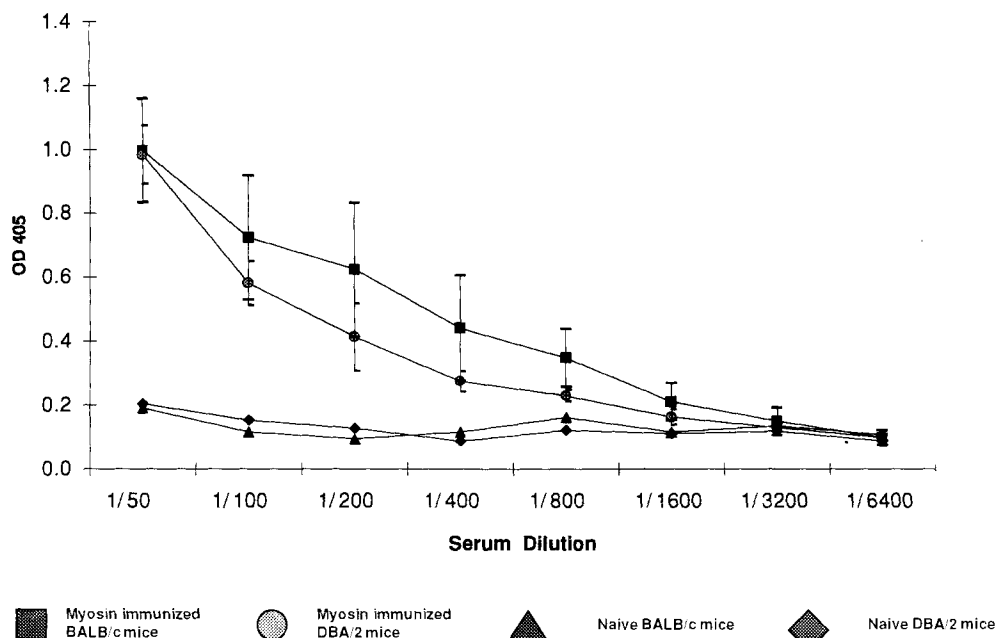
Figure 1. BALB/c and DBA/2 mice were injected subcutaneously at four sites with 100 μg BALB/c cardiac myosin emulsified in CFA and boosted with the same immunogen 7 d later. Serum samples were obtained 2 wk after the first immunization. Anti-myosin antibodies were assayed by ELISA. Briefly, 10 μg/ml of cardiac myosin was adsorbed to microtiter plates, blocked with PBS-2% BSA, and then incubated with serial dilutions of sera from immunized mice. Anti-myosin reactivity was detected with peroxidase-conjugated anti-mouse IgG diluted 1:1,000 (Sigma Chemical Co.) and substrate, 2,2,-, azino-di-3-ethylbenzthiazoline sulfonate. Optical density was read at 405 nm on an ELISA reader. Each line represents an average of five mice.
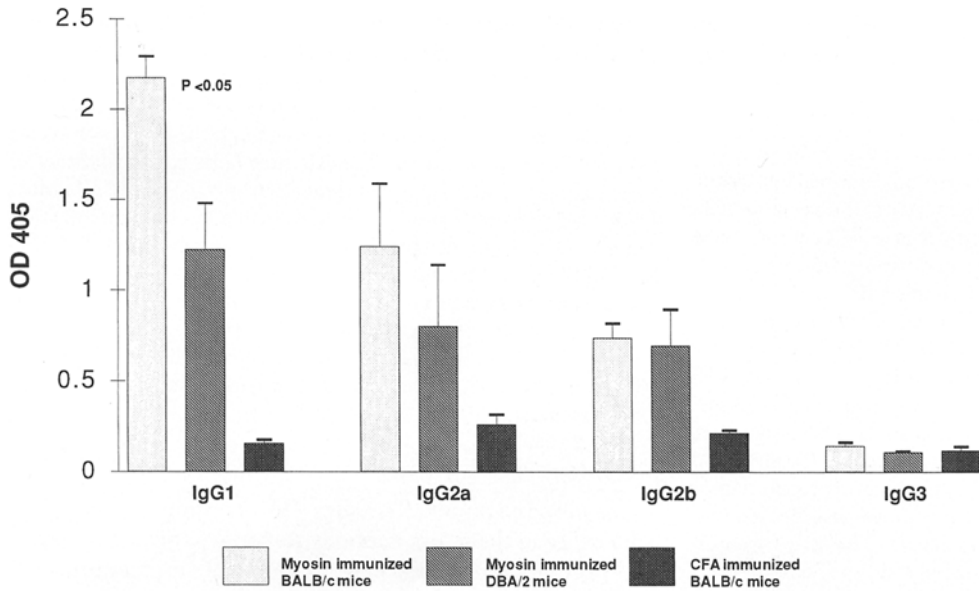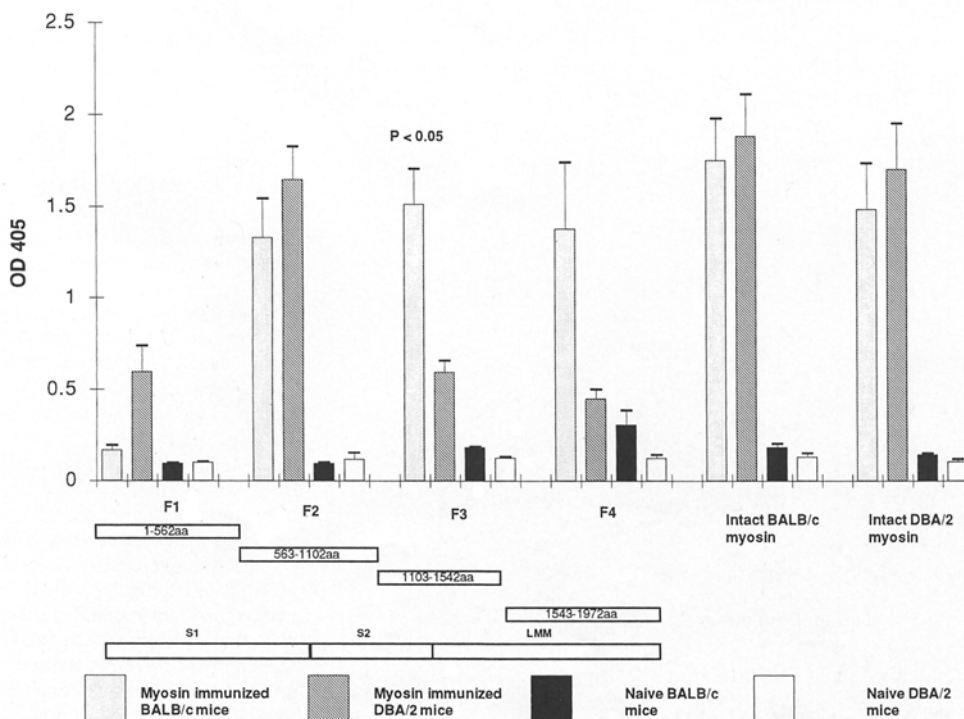
Figure 2. BALB/c and DBA/2 mice were immunized with BALB/c cardiac myosin as described in Fig. 1. Serum samples, obtained 2 wk after the first immunization, were assayed for subclass of anti-myosin antibodies. Each bar represents an average of five mice.

563-1102). This region was previously shown to be important for induction of autoimmune myocarditis in BALB/c mice (25). BALB/c sera showed significantly higher reactivity to the rod region (fragments 3 and 4) of myhca. In contrast, DBA/2 mice showed higher binding to fragment 1 from the head region of myosin. Serologic studies, therefore, suggested a difference in fine specificity of anti-myosin antibodies in cardiac myosin-immunized BALB/c and DBA/2 mouse sera, but showed no difference in reactivity to the intact myosin molecule.

Characterization of Monoclonal Anti-myosin Antibodies and Antibody-mediated Disease Induction. To explore whether differences in fine antigenic specificity might account for the difference in pathogenicity, we generated monoclonal anti-myosin antibodies from three susceptible strains of mice, DBA/2, BALB/c, and A.CA. Four IgG monoclonal antibodies against different regions of myhca were selected for in vivo assays of disease induction. Characteristics of these antibodies are summarized in Table 1. Since BALB/c and DBA/2 myhca alleles differ by a single amino acid at residue 545, serine and alanine, respectively (26), we verified by ELISA that the anti-myosin antibodies bound equally well to BALB/c and DBA/2 cardiac myosin (Fig. 4). Each of these four IgG antibodies was injected intraperitoneally into naive BALB/c and DBA/2

Figure 3. Mice were immunized with BALB/c cardiac myosin as described in Fig. 1. Genetically engineered BALB/c mouse myhca fragments were used to study the fine specificity of IgG anti-myosin antibodies in BALB/c and DBA/2 mouse sera. These fragments were produced in *E. coli* as nonfusion proteins using a modified pET vector system. The overexpressed proteins were partially purified and eluted from acrylamide gels. Anti-fragment reactivity was assayed by ELISA. 10 μg/ml of each fragment was adsorbed to ELISA plates. The ELISA was performed as described in Fig. 1 using serum at a 1:500 dilution. Each bar represents an average of three mice.

Table 1. *Characterization of Monoclonal Anti-myosin Antibodies*

Anti-myosin antibody	Strain of origin	Isotype	Antigenic specificity
11C6-E3	A.CA	IgG2b	Head fragment 1
11F7-C1	A.CA	IgG1	Rod fragment 3
2D6-B1	DBA/2	IgG2a	Head fragment 2
10D4-A9	BALB/c	IgG2a	Head fragment 1

Monoclonal anti-myosin antibody–secreting hybridomas were generated from cardiac myosin–immunized A.CA, DBA/2, and BALB/c mice. Isotypes of anti-myosin antibodies were determined by ELISA. Antigenic specificity was determined using genetically engineered BALB/c mouse myhca fragments.

mice. All four monoclonal anti-myosin antibodies caused disease in DBA/2 mice (Table 2, Fig. 5) showing that antibody alone can induce myocarditis in a susceptible host. In contrast, no antibody caused disease in BALB/c hearts. Since anti-myosin antibodies against both head and rod regions of myhca can cause pathology in DBA/2 mice, it suggests that the fine specificity differences between BALB/c and DBA/2 anti-myosin responses cannot alone account for the difference in susceptibility to antibody-mediated disease seen in these two mouse strains.

We next examined sections of hearts for the deposition of antibody. As shown in Fig. 6, DBA/2 mice showed marked IgG deposition in the affected hearts whereas BALB/c mice, which do not develop myocarditis after administration of the same anti-myosin antibody, showed little or no IgG deposition. The Ig deposition seen in DBA/2 mice was not restricted to the area of necrosis and inflammation but was present throughout the heart. These studies demonstrate that there is a difference in the deposition of anti-myosin antibodies in BALB/c and DBA/2 hearts and that this difference is associated with disease.

Table 2. *Induction of Autoimmune Myocarditis in BALB/c, DBA/2, and SCID Mice by Monoclonal Anti-myosin Antibodies*

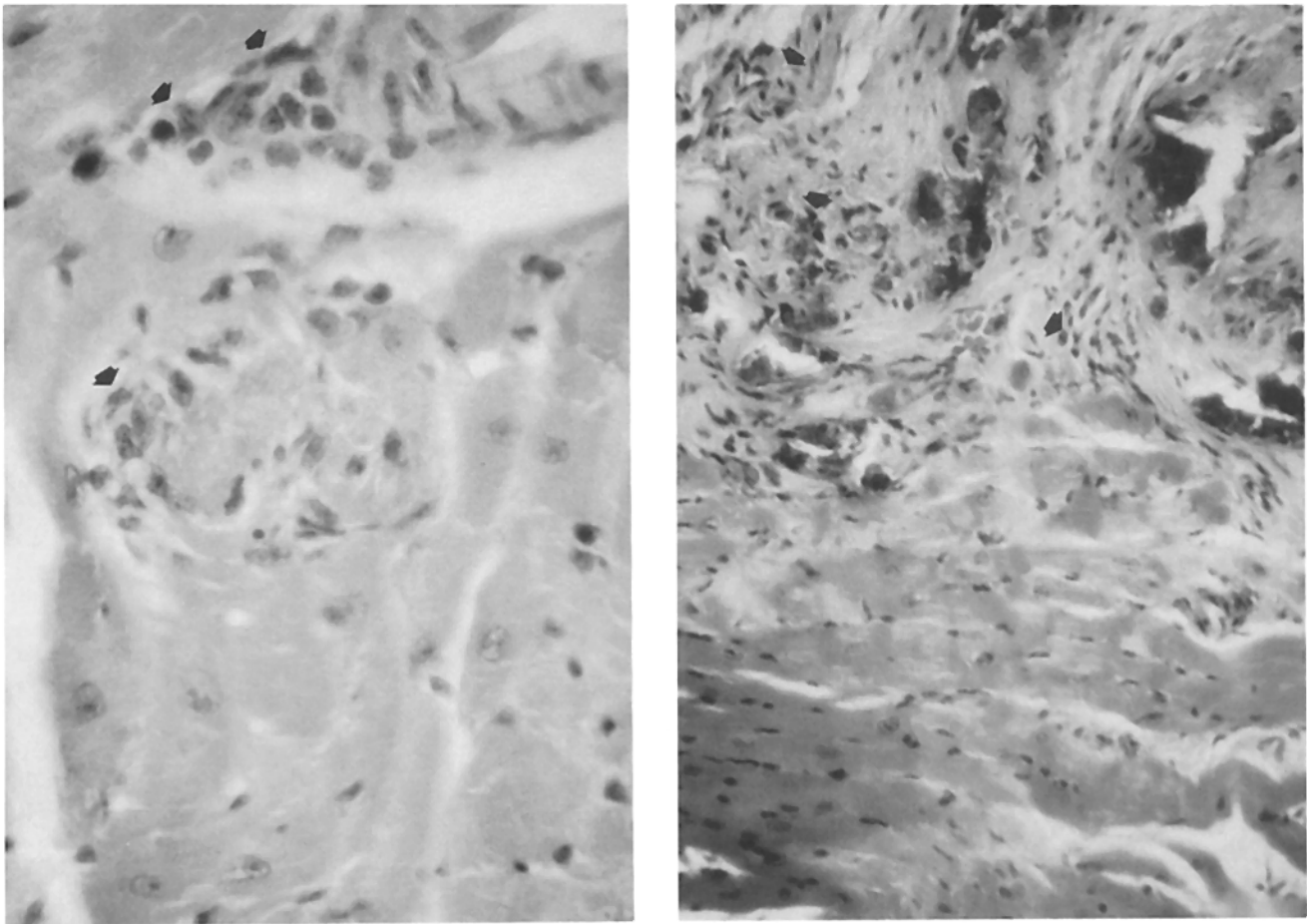| Anti-myosin antibody	Myocarditis DBA/2	Myocarditis BALB/c	Myocarditis SCID
11C6-E3	5(5)	0(4)	0(4)
11F7-C1	5(6)	0(6)	0(4)
2D6-B1	4(7)*	0(5)	0(5)
10D4-A9	3(4)	0(5)	0(6)

Hearts were scored for the presence of myocarditis (inflammation and myocyte necrosis) using the Dallas criteria (20). The numbers in parentheses represent the number of mice injected.
* The three remaining DBA/2 mice were + / – myocarditis (calcification and necrotic cells). Each mouse was injected intraperitoneally with purified anti-myosin antibody, 100 μg in 200 μl PBS daily 5 d a week for 3 wk. Mice were killed after 3 wk. Formalin-fixed heart sections were stained with hematoxylin-eosin.

To investigate whether clearance of anti-myosin antibody by anti-idiotypic antibodies in BALB/c mice could account for this difference, we employed SCID mice that have a BALB/c genetic background. SCID mice have a defect in recombinase activity that blocks the development of mature T and B lymphocytes, thereby precluding the possibility that endogenous anti-idiotypic antibodies were being elicited by the administration of anti-myosin antibody. SCID mice also showed no Ig deposition and no cardiac disease (Table 2, Fig. 6), demonstrating that the susceptibility to antibody-mediated autoimmune disease lies in the sensitivity of the heart to anti-myosin antibodies, not in the antibodies themselves.

Analysis of Cardiac Extracellular Matrix. To identify the antigen recognized by anti-myosin antibodies in DBA/2 hearts, we used monoclonal anti-myosin antibodies to probe Western blots of purified cardiac extracellular matrix proteins (21). As seen in Fig. 7, a monoclonal anti-sarcomeric myosin antibody recognizes a protein in DBA/2 extracellular matrix having the apparent molecular weight of myosin heavy chain.
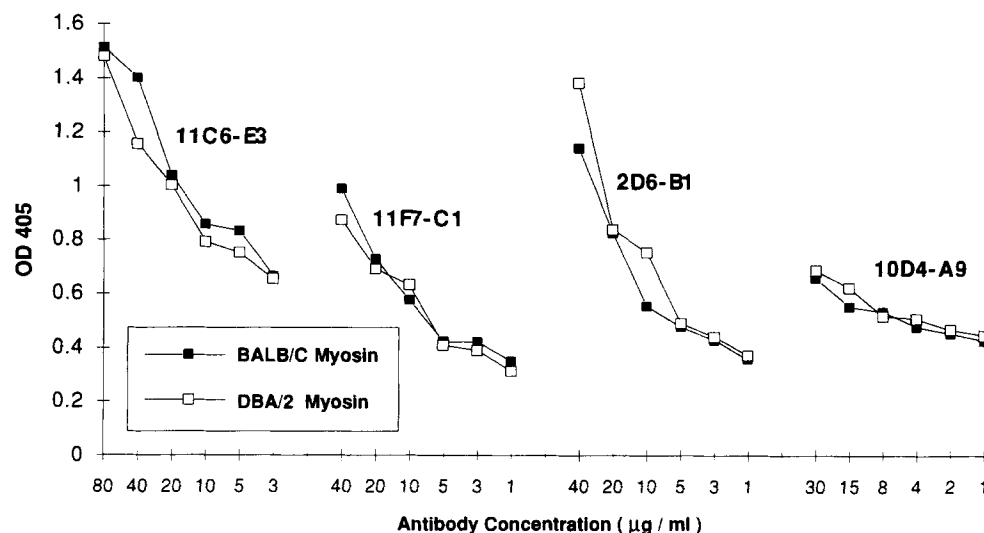

Figure 4. To compare the binding affinity of monoclonal anti-myosin antibodies to BALB/c and DBA/2 myosin, titrations of antibody were assayed by ELISA as described in Fig. 1.

Figure 5. (*Left*) Heart section from a DBA/2 mouse injected with monoclonal anti-myosin antibody 2D6-B1. There is focal left ventricular myocellular necrosis and mononuclear inflammation diagnostic of active myocarditis. Arrows identify sites of inflammation. (Hematoxylin-eosin, original, ×200). (*Right*) Heart section from a DBA/2 mouse injected with monoclonal anti-myosin antibody 10D4-A9. There is a subepicardial left ventricular region of myocardial scarring with myocellular necrosis and calcification, and inflammation diagnostic of active myocarditis. Arrows identify sites of inflammation. (Hematoxylin-eosin, original, ×80).
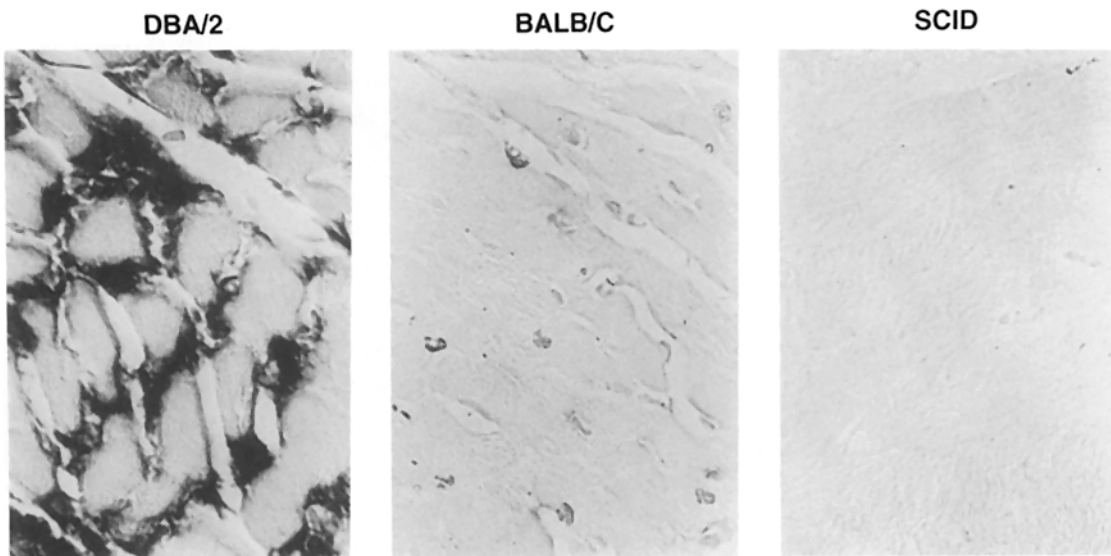
Figure 6. Ig deposition in BALB/c, DBA/2, and SCID mouse hearts. BALB/c, DBA/2, and SCID mice were injected with purified monoclonal anti-myosin antibody in PBS. Paraffin-embedded heart sections were deparaffinized and rehydrated, blocked with 2% normal goat serum (Vecta stain ABC Kit, Vector Laboratories, Inc.) in PBS in moist chambers. After blocking, the sections were incubated with biotinylated antibody against mouse IgG, alkaline phosphatase–labeled avidin, (ABC reagent, Vecta stain ABC Kit) and substrate.
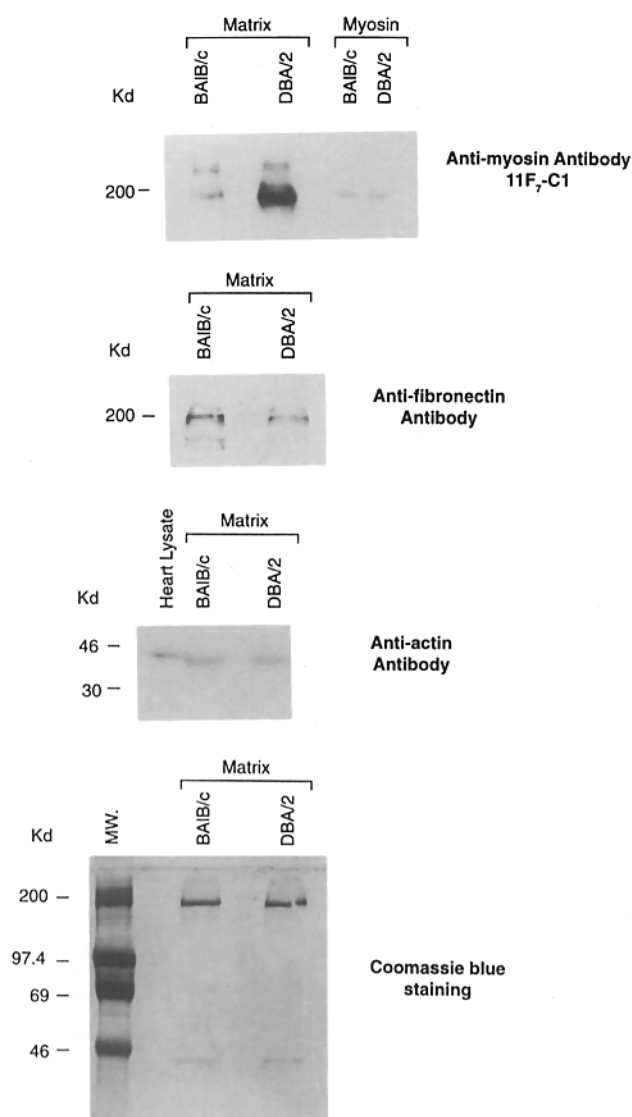
Figure 7. Cardiac extracellular matrix proteins of BALB/c and DBA/2 mouse hearts were purified according to the method described by Rojkind et al. (21). 10 μg of each sample was displayed on a 5% SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose. The nitrocellulose blots were blocked with 5% milk in PBS and incubated with anti-myosin antibody, rabbit anti-fibronectin antibody, or rabbit anti-actin antibody. After washing, blots were developed using a peroxidase-labeled goat antiserum to mouse or rabbit IgG (Sigma Chemical Co.) and substrate (ECL Western blotting detection system).

Monoclonal anti-myosin antibodies specific for epitopes in either the myosin head or rod recognize this protein (data not shown). Because this protein has the same apparent molecular weight as myosin heavy chain and is immunoreactive with all monoclonal anti-myosin antibodies tested, we believe this protein may be myosin heavy chain. Alternatively, it may be a structurally homologous protein containing several shared antigenic determinants in common with myosin. This protein is present, though in much smaller amounts (10-fold less), in cardiac extracellular matrix from BALB/c mice.

We next examined whether the presence of this protein was due to released intracellular proteins resulting from inadvertent destruction of myocytes during the preparation of extracellular matrix proteins. We probed the blots with antibody to actin, as the presence of actin in extracellular matrix should reflect release of this protein from intact myocytes in the course of isolation of extracellular matrix proteins. As shown in Fig. 7, the amount of actin present is the same in both strains. The increased presence of myosin in DBA/2 extracellular matrix, therefore, cannot be due to differential fragility of DBA/2 and BALB/c myocytes. In addition, the amount of fibronectin, a known extracellular matrix protein is also the same in both preparations.

Discussion

It is clear that the genetics of autoimmune diseases is complex, and that multiple genes can predispose to autoimmunity. Many of the candidate genes that have been proposed to play a role in susceptibility to autoimmune disease encode proteins of importance within the immune system, in the induction or regulation of an immune response or in the clearance of antigen-antibody complexes. It is evident, however, from studies of human diseases and of animal models, that autoreactive lymphocytes can be activated in some individuals, yet the individuals have no progression to autoimmune disease. Many studies (27, 28) have demonstrated the presence of autoantibodies in the serum of relatives of individuals with autoimmune disease. Serologic studies suggest these autoantibodies have the structural characteristics of pathogenic autoantibodies, yet they do not appear to cause disease.

We have examined the molecular basis for susceptibility to anti-myosin antibody–mediated heart disease. BALB/c mice do not develop an antibody-mediated disease, whereas DBA/2 mice do. The data demonstrate that the two strains make equivalent anti-myosin antibody responses and that antibodies from either strain will cause disease in a susceptible host and will not cause disease in a resistant host. It has similarly been shown in rats that there is strain-specific susceptibility to the nephritis caused by antitubular basement membrane (TBM) antibody (29). Brown Norway rats develop an antibody-mediated disease, whereas Lewis rats show no deposition of anti-TBM antibody in kidneys and no nephritis. Whereas there appears to be no TBM antigen exposed in vivo in kidneys of Lewis rats, trypsin solubilization of renal TBM appears to expose the target antigen (30). It is not known how the nephritogenic epitopes are concealed in the intact tissue of Lewis rats.

The critical difference in target antigen exposure between the DBA/2 and the BALB/c heart appears to be that myosin, or a myosin-like protein, is present in substantially larger quantities in the extracellular matrix of DBA/2 hearts. The presence of this protein in normal extracellular matrix may explain how antibody to a presumably intracellular antigen can cause disease in undamaged tissue. The reasons for its increased expression in DBA/2 extracellular matrix are not known. If the protein is indeed myosin, the single amino acid difference between BALB/c and DBA/2 myosin might be respon-

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sible for preferential trapping of DBA/2 myosin in extracellular matrix. Alternatively, and we believe more likely, strain-specific differences in extracellular matrix proteins or in proteases that degrade myosin may underlie the sequestration of myosin in DBA/2 hearts. Finally, it is possible that myosin is processed differently in the two strains. A recent study (31) shows that liver fat–storing cells can produce a muscle type of myosin that deposits in extracellular matrix suggesting the possibility that myosin released from dying cells is binding to an extracellular matrix protein or, less likely, that myosin is actively secreted into the extracellular matrix.

This study demonstrates that anti-myosin antibodies alone are pathogenic in a susceptible host. The basis for the differential sensitivity to anti-myosin antibodies in different mouse strains involves a genetically determined difference in target organ sensitivity to the same antibody. This observation substantially alters our understanding of the genetics of murine autoimmune myocarditis. In humans, anti-myosin antibodies have been considered a marker of myocardial injury but have not been considered to have pathogenic potential. Our data raise the possibility that in some individuals a serum anti-myosin response might contribute to cardiac injury.

It will be important to study human cardiac tissue to see whether myosin or a myosin-like molecule is deposited in extracellular matrix of individuals susceptible to cardiac injury from anti-myosin antibody. Differential target organ sensitivity might help explain the genetic predisposition of certain individuals to rheumatic fever as well as susceptibility to antibody-mediated cardiac damage after myocardial ischemia and other injuries to the heart.

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