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Genetic control of Coxsackievirus B3-induced heart-specific autoantibodies associated with chronic myocarditis

M. D. TRAYSTMAN & K. W. BEISEL *Department of Pathology and Microbiology, The University of Nebraska Medical Center, Omaha, NE, USA*

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SUMMARY

Cardiac-specific autoantibodies to sarcolemmal and cardiac myosin antigens observed during the chronic phase of Coxsackievirus B3-induced myocarditis appear to be under autosomal recessive control. This observation is based on examination of F_1 hybrids bred from A/J mice which develop chronic myocarditis and C57BL/6J mice which resolve the virus-induced lesions. Previous mouse studies demonstrated that the prevalence of heart-specific autoantibodies varied with the H-2 complex. However, in 25 H-2 congenic mouse strains the strain background was the predominant determinant of autoantibody presence. Recently, we extended our genetic evaluation of the chromosomal locations governing autoantibody responses by examining 25 AXB and BXA recombinant inbred strains. Two populations of heart-specific autoantibodies were demonstrated against sarcolemmal and cardiac myosin antigens. Analyses of the AXB/BXA strain distribution patterns for these two traits revealed that the anti-sarcolemmal response was controlled by a gene(s) linked to *Np-2* and *Tcrα* loci on chromosome 14. Linkage could not be assigned for the anti-cardiac myosin response.

Keywords autoimmunity autoimmune heart disease autoantibodies viral myocarditis

INTRODUCTION

Myocarditis may occur as a consequence of myocardial damage by toxins or infectious agents, and pericarditis as a result of ischaemic or traumatic injury of the heart. In many cases the presence of circulating autoantibodies reactive with the heart reflects an ongoing, chronic inflammatory process. Numerous clinical studies have implicated immunopathologic mechanisms in the genesis of idiopathic dilated cardiomyopathy, idiopathic myocarditis, post-infarction and post-pericardiotomy syndromes, and rheumatic heart disease (Maisch *et al.*, 1982, 1983; Schultheiss & Bolte, 1985; DeScheerder *et al.*, 1985, 1987; Sargent *et al.*, 1987; McManus, Gauntt & Cassling, 1988; Cunningham *et al.*, 1989). Indeed, human idiopathic myocarditis is postulated to be an autoimmune disease. Heart-specific autoantibodies and anti-myocardial cell-mediated responses observed in patients with idiopathic inflammatory heart disease and idiopathic dilated cardiomyopathy, and in mice with chronic viral myocarditis (Maisch *et al.*, 1982, 1983; Schultheiss & Bolte, 1985; Wolfgram, Beisel & Rose, 1985), support this hypothesis (McManus *et al.*, 1988). These autoantibodies may recognize both muscle-reactive or heart-specific antigenic specificities. By indirect immunofluorescence assays (IFA) these autoantibodies stain several components of heart cells, including sarcolemma/myolemma, contractile elements, and mitochondria (Maisch *et al.*, 1982, 1983; Schultheiss & Bolte, 1985). The cardiac myosin heavy chain (*Myhc*), mitochondrial adenine nucleotide translocator and a protein component of the calcium channel are identified by these approaches as potential autoantigens (Schultheiss & Bolte, 1985; Alvarez *et al.*, 1987; Neu *et al.*, 1987b; Schultheiss *et al.*, 1988).

Humoral immunity as a mediator of myocarditis following Coxsackie virus B3 (CVB3) infection has been established indirectly in A/J and DBA/2J strains wherein cardiac IgG autoantibodies correlate with the presence of morphological disease (Huber & Lodge, 1986; Lodge, Herzum & Huber, 1987). Additionally, in DBA/2J mice, chronic viral myocarditis is attributed to IgG autoantibodies. This view is supported by the observation that the severity of disease significantly decreased following treatment with cobra venom factor which interferes with complement activity (Huber & Lodge, 1986). Recently, CVB3-induced myocarditis in DBA/2J mice was also found to be mediated by CD4 lymphocytes (Blay *et al.*, 1989). In contrast, in the BALB/cCUM mice, CVB3-induced autoimmune myocarditis is caused primarily by cytotoxic CD8 lymphocyte-mediated mechanisms (Huber & Lodge, 1984, 1986; Lodge *et al.*, 1987; Blay *et al.*, 1989). However, both humoral and cellular autoimmune mechanisms of cardiac injury occur in the A/J mouse strain (Lodge *et al.*, 1987).

Correspondence: Dr Kirk W. Beisel, Department of Pathology and Microbiology, University of Nebraska Medical Center, 600 South 42nd Street, Omaha, NE 68198-6495, USA.

We have developed a genetically based murine model of CVB3-induced autoimmune myocarditis wherein late development of a diffuse chronic myocarditis is observed concurrently with heart-specific IgG autoantibodies (Wolfgram *et al.*, 1985, 1986; Herskowitz *et al.*, 1987; Neu *et al.*, 1987a). Wolfgram *et al.* (1985, 1986) and Alvarez *et al.* (1987) showed that these heart-specific IgG sarcolemmal antibodies were indicators of chronic myocarditis and the presence of these autoantibodies correlated highly with late disease. Analysis of normal, uninfected tissues by IFA utilizing post-infection, autoimmune sera defined the tissue reactivity pattern of these autoantibodies (Wolfgram *et al.*, 1985, 1986). The predominant reactivity is against *Myhc* (Alvarez *et al.*, 1987; Neu *et al.*, 1987a). Absorption and inhibition analyses using purified murine cardiac and skeletal (contractile) and brain (non-contractile) myosins by ELISA demonstrated that the cardiac myosin-specific IgG response differentiated the mouse strains with chronic autoimmune-based myocarditis from those which had resolved virus-induced cardiac lesions (Neu *et al.*, 1987a). In this investigation, our goal was to determine the inheritance pattern, the number of gene(s) and the chromosomal location of the gene(s) controlling the heart-specific sarcolemmal and cardiac myosin-specific autoantibodies. This was done by examining a panel of H-2 congenic mouse strains, as well as F_1 hybrids and recombinant inbred (RI) lines derived from the A/J mouse, which develops chronic myocarditis, and the C57BL/6J strain, which does not develop myocarditis.

MATERIALS AND METHODS

Animals

A panel of H-2 congenic mice was obtained originally from The Jackson Laboratories (Bar Harbor, ME) or from Dr Chella S. David (Rochester, MN). Breeding stocks of the RI strains, AXB and BXA, were obtained from Dr Muriel N. Nesbitt (La Jolla, CA). These mice were raised and maintained in microisolator cages. Mice were supplied with autoclaved Purina low fat mouse chow and acid water (pH 2·3–2·5) *ad libitum*. CVB3-infected animals were housed in a separate room from the breeding colony in microisolator caging. Both male and female mice were used in these studies.

Infection and necropsy

All animals were inoculated i.p. at 2 weeks of age with 10^5 $TCID_{50}$ CVB3 (Nancy) as described by Wolfgram *et al.* (1986). Uninfected control animals were injected with Vero cell lysates. The original stock of virus used in these studies was obtained from Dr A. Martin Lerner (Wayne State University School of Medicine, Detroit, MI) and was prepared as previously described (Wolfgram *et al.*, 1986). Mouse sera were individually collected 21 days after infection and frozen at −70° C until further analyses. Hearts were also harvested at 21 days post-inoculation for further histologic examination (Wolfgram *et al.*, 1986; Herskowitz *et al.*, 1987).

Histology

Hearts were arrested in diastole in a cold 30 mM KCL/PBS, pH 7·4, solution, coded, and processed as previously described (Wolfgram *et al.*, 1986). Lesions were assessed by light microscopy for frequency, size, location and type of cellular involvement, and scored semi-quantitatively according to published protocols (Wolfgram *et al.*, 1986; Herskowitz *et al.*, 1987). The presence of autoimmune-based myocardial disease was determined by the presence of myocardial necrosis and/or a diffuse mononuclear cell infiltrate.

Serological analyses

Indirect immunofluorescence assays were done according to Wolfgram *et al.* (1985) using FITC-conjugated goat anti-mouse immunoglobulin (Southern Biotechnology, Birmingham, AL). Normal mouse heart, skeletal muscle (gastrocnemius), kidney and liver, obtained from uninfected A.BY/SnJ mice, were used in this assay. To assure that adequate and uniform samples of heart tissue were used, each heart was cut transversely at the basal ventricular region before mounting and sectioning. Only those heart sections that had the largest area were selected for IFA. The presence of autoantibodies to sarcolemmal/myolemmal membranes and contractile elements was individually semi-quantified. Consistency in visual quantification was obtained by the comparison of the test sample to the same negative control, normal mouse serum, and a low and high titered autoimmune sera, previously quantified as 1 and 4, respectively. A grading score representing a combination of three fluorescence parameters was used in which colour intensity was determined on a 0–4 scale, amount of tissue stained on a scale of 0–2 and uniformity of staining on a scale of 0–2. For colour intensity, a grade of 0 represented no reaction above background; a + was a reaction $\leq 5\%$ above background and a 1, 2, 3 or 4 grade showed an increase in brilliance by 25, 50, 75 or 100%, respectively over background. For the amount or area of tissue stained, a grade of 0 was given when none of the tissue reacted; a + indicated an irregular scattered staining pattern involving $\leq 5\%$ of the tissue; 1 was given if $< 25\%$ of the tissue had staining of the appropriate cellular structure and 2 was given if $\geq 25\%$ of the tissue had a specific reaction pattern. Finally, for uniformity of fluorescence staining of the sarcolemmal/myolemmal or contractile elements a grade of 0 indicated that no consistency in staining was evident; a + indicated that $\leq 5\%$ of the tissue that showed reaction had an irregular fluorescent staining; 1 indicated that $< 25\%$ of the specific staining pattern had a homogeneous fluorescence and 2 showed that $\geq 25\%$ of the tissue evaluated had a uniform fluorescence staining. The additive total of these three scores determined the reactivity for each staining pattern such that a combined score of > 5 was considered positive.

ELISA was used to assess the titre of antibodies to heart or skeletal muscle myosin. This assay was performed as described by Neu *et al.* (1987a), except that 2,2′-azino-di-ethylbenzthiazoline sulphonate was used as the detection substrate. Myosin IgG autoantibody was detected by a horseradish peroxidase-conjugated goat anti-mouse IgG antibody (Southern Biotechnology, Birmingham, AL). Serum was diluted 1:10 in phosphate-buffered saline (PBS)/0·1% bovine serum albumin (BSA) followed by serial 2-fold dilutions ranging from 2^1 to 2^{11}. Both normal mouse serum and a PBS/1% BSA solution were used as negative controls. A cardiac myosin-specific monoclonal antibody, CCM52 (Clark *et al.*, 1980), was used as a positive control and was the generous gift of Dr Radovan Zak (Chicago, IL).

Double absorptions using skeletal and then cardiac myosin were performed sequentially as previously described by Neu *et al.* (1987a) on all the post-infection sera from the H-2 congenic and AXB/BXA RI lines to demonstrate cardiac-specific auto-

Table 1. Inheritance pattern of heart-specific anti-cardiac sarcolemmal antibody (ASA) in (A/J × C57BL/6J)F_1 mice

Strain	ASA prevalence*	Histologic phenotype†
Parent		
A/J	22/76 (30)	Chronic myocarditis
C57BL/6J	0/103 (0)	Resolved lesions
F_1 hybrid		
(A/J × C57BL/6J)	0/45 (0)	Resolved lesions
(C57BL/6J × A/J)	0/43 (0)	Resolved lesions

* Expressed as the number of positives over the total number examined. The per cent prevalence is given in parentheses.

† Summarized from Traystman *et al.* (1991) where ventricular sections obtained at sacrifice were stained with H&E and assessed for chronic myocarditis using criteria previously described (Wolfgram *et al.*, 1986; Herskowitz *et al.*, 1987).

antibodies to myosin and sarcolemmal membranes, respectively. Briefly, the myosin-bound Sepharose was prepared according to the Pharmacia protocol at a ratio of 1 mg protein/ml Sepharose. A 1 : 10 serum dilution containing 1% BSA in PBS was added to an equal volume of packed beads and incubated for 1 h at room temperature. The absorbed sera were then tested by ELISA. Results were expressed as the per cent of remaining anti-cardiac myosin activity. The optical density values were corrected for background.

Myosin purification

Cardiac and skeletal muscle myosins were prepared as described by Neu *et al.* (1987a, b). The purified proteins were stored in 50 mM sodium pyrophosphate, pH 7·4, at 4 C.

Linkage analysis

Recombinant inbred lines were phenotyped for the presence of heart-specific sarcolemmal/myolemmal autoantibodies, cardiac myosin-specific autoantibodies and chronic myocarditis were categorized as being A/J-like or C57BL/6J-like. The RI strain distribution pattern (SDP) for each of these three traits was compared with the SDP of other loci which have been previously typed in the AXB/BXA RI lines (Nesbitt & Skamene, 1984). Concordance, linkage distances, and 95% confidence intervals (CI) were determined using the formula given by Taylor (1978).

RESULTS

Anti-cardiac sarcolemmal antibody (ASA) responses

F_1 hybrids derived from reciprocal crosses between A/J and C57BL/6J were examined to determine the inheritance pattern for development of the ASA during CVB3-induced chronic myocarditis. Table 1 shows that the (A/J × C57BL/6J)F_1 animals on day 21 did not develop autoantibodies. Furthermore, neither sex linkage nor sex influences were observed (data not shown). Both A/J and C57BL/6J control strains injected with a Vero lysate did not produce autoantibodies nor did they develop myocarditis. These data suggest that the presence of ASA was controlled by an autosomal recessive allele(s).

To determine the H-2 influence on this trait, 24 H-2 congenic strains were examined for production of ASA (see Table 2). Prevalence rates of ASA among the four A/J and three BALB H-2 congenics and the DBA/2J mouse strain ranged from 12 to 46%, and all of these strains were previously phenotyped histologically as having chronic myocarditis (Rose *et al.*, 1988). Of the five C3H H-2 congenics, only C3H.SW/SnJ, C3H.JK/SnJ and C3H.Q/Sf had ASA as well as chronic myocarditis; the remaining two strains had neither cardiac lesions nor ASA. The C57BL/6J strain and the 10 B10 H-2 congenic strains lacked the heart-specific ASA and all were previously classified as having resolved virus-induced lesions (Rose *et al.*, 1988). In these latter strains only three animals had low titered, cross-reactive, anti-muscle antibodies as detected by IFA. These results suggest that the presence of ASA is always associated with chronic myocarditis and thus can be used as a marker of disease.

Table 2. Induction of anti-cardiac sarcolemmal antibody (ASA) and chronic myocarditis in Coxsackievirus B3-infected H-2 congenic strains

Strain	Haplotype	ASA prevalence*	Histologic phenotype†
A/J	a	39/91 (43)	Chronic myocarditis
A.BY/SnJ	b	35/160 (22)	Chronic myocarditis
A.CA/SnJ	f	11/39 (32)	Chronic myocarditis
A.SW/SnJ	s	23/73 (32)	Chronic myocarditis
BALB.B/Kh	b	4/23 (17)	Chronic myocarditis
BALB/cByJ	d	5/39 (13)	Chronic myocarditis
BALB.K/Kh	k	3/25 (12)	Chronic myocarditis
C57BL/6J	b	0/118 (0)	Resolved lesions
B10.A/SgJ	a	0/95 (0)	Resolved lesions
C57BL/10SnJ	b	0/51 (0)	Resolved lesions
B10.D2/nSnJ	d	0/21 (0)	Resolved lesions
B10.M/SnJ	f	0/18 (0)	Resolved lesions
B10.WB/SnJ	j	0/26 (0)	Resolved lesions
B10.BR/SnJ	k	0/27 (0)	Resolved lesions
B10.Q/SgSf	q	0/20 (0)	Resolved lesions
B10.RIII/SgSf	r	0/13 (0)	Resolved lesions
B10.PL/SgSf	u	0/17 (0)	Resolved lesions
B10.SM/SgSf	v	0/20 (0)	Resolved lesions
C3H.SW/SnJ	b	7/22 (32)	Chronic myocarditis
C3H.JK/SnJ	j	6/13 (46)	Chronic myocarditis
C3H/HeSn	k	0/19 (0)	Resolved lesions
C3H.NB/SnJ	p	0/21 (0)	Resolved lesions
C3H.Q/Sf	q	5/21 (24)	Chronic myocarditis
DBA/2J	d	12/26 (46)	Chronic myocarditis

* Expressed as the number of positives over the total number examined. The per cent prevalence is given in parentheses.

† Summarized from Rose *et al.* (1988) where ventricular sections obtained at sacrifice were stained with H&E and assessed for chronic myocarditis using criteria previously described (Wolfgram *et al.*, 1986; Herskowitz *et al.*, 1987).

The number of non-H-2 gene(s) involved in the ASA trait and their chromosomal location were explored by examining post-infection serum from a panel of 25 AXB and BXA RI strains (see Table 3). As predicted (Wolfgram *et al.*, 1986), the ASA were found in the A/J and A.BY/SnJ strains and were absent in B10.A/SgSf and C57BL/6J controls. Of the 25 RI lines, 14 expressed the 'A' phenotype of the A/J parental strain as defined by the presence of ASA. These ASA sera had a tissue reactivity of sarcolemmal staining (Fig. 1a) and, in some cases, fibrillary fluorescence (Fig. 1b) in the heart was also noted. In

Table 3. Induction of anti-cardiac sarcolemmal antibody (ASA) in Coxsackievirus B3-infected AXB/BXA recombinant inbred lines

Strain	Prevalence*	Phenotype†
A/J	33/91 (36)	A
A.BY/SnJ	35/160 (22)	A
B10.A/SgSf	0/95 (0)	B
C57BL/6J	0/118 (0)	B
AXB 1	1/11 (9)	—
2	10/21 (48)	A
5	0/29 (0)	B
6	7/11 (64)	A
7	0/18 (0)	B
8	1/11 (9)	—
10	0/10 (0)	B
15	9/19 (47)	A
17	5/30 (17)	B‡
18	4/22 (18)	B‡
19	0/21 (0)	B
20	0/19 (0)	B
21	0/14 (0)	B
23	4/9 (44)	A
25	3/26 (12)	B‡
BXA 6	5/10 (50)	A
8	3/10 (30)	A
10	0/16 (0)	B
11	0/17 (0)	B
12	1/17 (6)	—
19	3/19 (16)	A
22	8/11 (73)	A
23	5/8 (63)	A
24	3/17 (18)	A
25	4/24 (17)	A

* Expressed as the number of positives over the total number examined. The per cent prevalence is given in parentheses.

† A denotes the A/J parental trait and B denotes the trait of C57BL/6J.

‡ Phenotype of strains whose serum lost ASA activity after adsorption with cardiac myosin.

—, Not determined.

most cases, the anti-sarcolemmal pattern was also observed in the skeletal muscle of those same animals. Eight of the 25 strains had the phenotype of the C57BL/6J parental strain although a few animals exhibited autoantibodies reacting only with skeletal muscle tissue. The remaining three RI lines, AXB 1, AXB 8 and BXA 12, could not be phenotyped, since all three strains each had a single animal with ASA. Since anti-cardiac myosin antibodies also have a sarcolemmal staining pattern in IFA (Wolfgram *et al.*, 1985), adsorption of the sera from all these strains was done. In three strains, AXB 17, 18 and 25, the ASA activity could be removed by adsorption with cardiac myosin and these were thus categorized as having a 'B' phenotype.

Prediction of the number of genes involved in expression of ASA trait can be determined by using classical Mendelian segregation ratios. The gene(s) controlling this trait was designated *asa* for *a*nti-*s*arcolemmal *a*utoantibodies. Using a one-gene hypothesis, a 1:1 ratio of responders to non-responders would be expected. The segregation pattern of 11:8 of responders (58%) to non-responders (42%) for the ASA is comparable to a 1:1 ratio. Using χ^2 analysis the one-gene hypothesis can be accepted ($0{\cdot}50 < P < 0{\cdot}30$). Analysis of the SDP for the other >150 traits defined in the AXB/BXA lines was then done. The SDP was similar to loci previously mapped to chromosome 14 (Dembic *et al.*, 1985; Elliot *et al.*, 1986; Traystman *et al.*, 1991). The map distances are 3 cM (95% CI-18) and 7 cM (95% CI-32) for *Np-2* and *Tcrα*, respectively (see Fig. 2).

Genetic control of cardiac myosin antibody response

Adsorption studies by Alvarez *et al.* (1987) and Neu *et al.* (1987a) have shown that most of the heart-specific autoantibodies are directed against cardiac myosin. Adsorption studies were done to characterize the development of cardiac myosin-specific autoantibodies in 19 of the AXB/BXA RI lines. As shown in Table 4, following adsorption with skeletal muscle myosin, cardiac myosin-specific autoantibodies were found in the post-infection autoimmune sera from A/J and A.BY mice, whereas these autoantibodies were not detected in the B10.A and C57BL/6 strains. Nine of the 19 (47%) AXB/BXA examined had cardiac myosin-specific autoantibodies. These lines were AXB 2, 6, 17, 18, 23, 25 and BXA 23, 24 and 25. The remaining lines (AXB 1, 5, 15, 21 and BXA 6, 8, 10, 12, 19 and 22) lacked these autoantibodies.

Our finding of a segregation ratio of 9:10 (presence *versus* absence) is approximately a 1:1 ratio. This finding suggests that at least one gene (i.e. designated cardiac myosin autoantibody, *cma*) controls the autoantibody response to cardiac myosin, as determined by χ^2 analysis ($P > 0{\cdot}9$). The SDP of the cardiac myosin autoantibody response did not correlate with any of the known >150 genetic markers so far identified and thus could not be assigned a chromosomal location. In addition, there was no apparent genetic association between the H-2 complex and the presence of cardiac myosin-specific antibodies.

Comparison of strain distribution pattern of ASA, cardiac myosin autoantibodies and chronic myocarditis

Important genetic relationships between the two types of cardiac-specific autoantibodies and the development of myocarditis were observed. Table 5 summarizes the SDP data obtained from the IFA, adsorption and our published histopathological (Traystman *et al.*, 1991) studies. The autoimmune response to cardiac myosin is not always present in association with the appearance of the cardiac-specific sarcolemmal autoantibodies. In addition, six of 14 RI lines had ASA and no cardiac myosin autoantibodies. Second, there was no association between the cardiac myosin-specific autoantibodies and autoimmune myocarditis since three of eight lines had these autoantibodies but no disease and four of 10 lines with disease lacked the cardiac myosin-specific autoantibodies. Therefore, the gene controlling the cardiac myosin autoimmune response is not linked with either the ASA or chronic myocarditis traits. These data support a chromosome 14 linkage between ASA and chronic myocarditis. The presence of ASA was not found to be strictly related to myocarditis, since RI lines were identified which had disease without ASA (AXB 10, 21, BXA 11) or ASA only (AXB 17, BXA 8, 10, 19, 20).

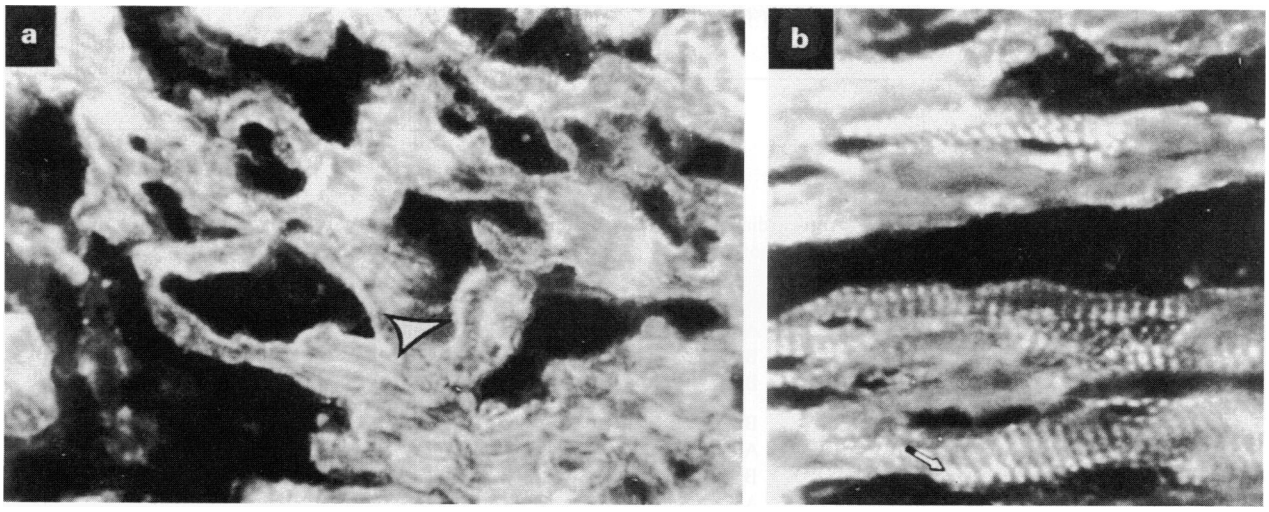

Fig. 1. Indirect immunofluorescence assay of sera from an A/J mouse with chronic myocarditis. (a) Tangential cut of myocytes highlighting dense fluorescence staining of sarcolemmal surface (→) of all myocytes. Magnification × 200. (b) Longitudinally oriented myocytes with fluorescence-positive linear bands running perpendicular to the long axis of the myocytes. These linear bands (→) represent staining of contractile proteins. Magnification × 360.

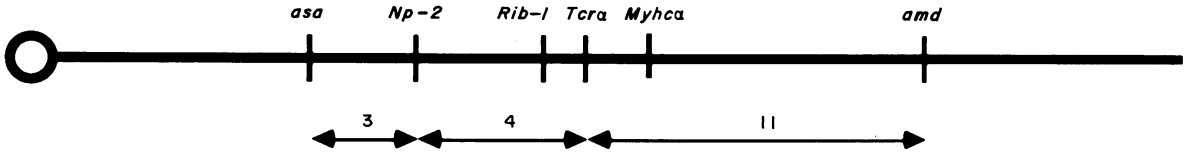

Fig. 2. Linkage relationship of *asa* with other loci on mouse chromosome 14. The putative *asa* locus has been provisionally assigned to a map location proximal to the *Np-2*, *Rib-1*, *Tcrα*, *Myhcα*, and *amd* loci (Traystman *et al.*, 1991). Map distances are given in centaMorgans (cM).

DISCUSSION

Herein are documented two types of heart-specific autoantibodies, cardiac myosin and sarcolemmal. Results from studies of the AXB/BXA RI strains demonstrated a strong genetic linkage of *asa*, which controls production of heart-specific ASA, to *Np-2* and *Tcrα* on chromosome 14 and to development of autoimmune myocardial disease (*amd*). In addition, these studies suggest that the autosomal recessive gene(s), *asa*, is present on a segment of chromosome 14 proximal to the centromere from the *Np-2*, the *Tcrα* complex, the cardiac myosin heavy chain alpha (*Myhcα*) and beta (*Myhcβ*) genes and the putative locus for *amd* (see Fig. 2). Thus, we speculate that one or more of these linked genes on chromosome 14 plays a part in autoimmune-based chronic myocarditis. For example, a gene(s) for the *Tcrα* variable region may govern an autoimmune response to heart antigens. Since cardiac myosin is a putative autoantigen and immunogen in cardiac autoimmunity (Alvarez *et al.*, 1987; Neu *et al.*, 1987a, b), the proximity of *asa* to the *Myhcα* and *Myhcβ* is provocative (Beisel & Booth, 1989; Ceci *et al.*, 1990; Traystman *et al.*, 1991). Moreover, the *Tcrα* and *Myhcα* loci are linked on chromosome 14 in man (Matsuoka *et al.*, 1989).

A dominant gene causing familial hypertrophic cardiomyopathy has been mapped to a locus at chromosome 14ql which is approximately 20 cM from the locus for the *Tcrα* gene (Jarcho *et al.*, 1989). Recently, genetic lesions in the *Myhc* have been implicated in familial hypertrophic cardiomyopathy; these are a hybrid *Myhc α/β* (Tanigawa *et al.*, 1990) and a missense mutation in the evolutionarily conserved Arg to Gln residue at residue 403 (Geisterfer-Lowrance *et al.*, 1990). Myocarditis is suggested to be a common step in the development of the end-stage cardiomyopathies (Kaplan & Frengley, 1969). Since this segment of chromosome 14 is homologous between mouse and man, and *Myhc* products may be involved in cardiomyopathy and myocarditis, our studies implicate a significant role for this segment of chromosome 14 in a variety of heart diseases.

The genetic relationship of the H-2 complex to autoantibody prevalence can be seen in the C3H H-2 congenic strains in which variation in disease pattern is more marked than in the other four congenic groups. Both quantitative and qualitative differences in ASA prevalence were observed only in C3H congenics. A non-H-2 genetic influence upon ASA prevalence could also be confirmed by comparing strains with the *b* and *d* haplotypes. Thus, the A.BY/SnJ (22%), BALB.B/Kh (17%), C57BL/10J (0%) and C3H.SW/SnJ (32%) strains, all of which carried the *b* haplotype, showed variation in the prevalence of ASA. Similarly, the BALB/cByJ (13%), B10.D2/nSnJ (%) and DBA/2J (46%) strains carried the *d* haplotype and varied in the prevalence of ASA.

Maisch *et al.* (1982) have shown the presence of heart-specific autoantibodies following viral myocarditis in humans. The antibodies bind complement and can apparently lyse rat cardiocytes. Indirect immunofluorescence assay studies demonstrated that these antibodies were directed toward the myolemmal membrane suggesting that they may act as an etiological agent in chronic inflammatory heart disease. Indeed, heart-reactive autoantibodies have long been suspected as a patho-

Table 4. Production of cardiac myosin autoantibodies in Coxsackie virus B3-infected AXB/BXA mice

Strain	Cardiac myosin autoantibody titre*	% residual activity after adsorption†		Anti-cardiac myosin phenotype‡
		Heart	Skeletal	
A/J	640	27	0	A
A.BY/SnJ	1280	38	0	A
B10.A/SgSf	40	0	0	B
C57BL/6J	80	0	0	B
AXB 1	160	0	0	B
2	320	44	0	A
5	160	0	0	B
6	320	21	0	A
7	80	—	—	—
10	80	—	—	—
15	160	0	0	B
17	320	26	0	A
18	160	13	0	A
19	80	—	—	—
20	320	—	—	—
21	160	0	0	B
23	320	25	0	A
25	640	32	0	A
BXA 6	320	0	0	B
8	320	0	0	B
10	320	0	0	B
11	160	—	—	—
12	160	0	0	B
19	160	0	0	B
22	160	0	0	B
23	160	27	0	A
24	320	36	0	A
25	160	36	0	A

* Values determined using a myosin ELISA.

† Sera were adsorbed with skeletal muscle myosin.

‡ A denotes the A/J parental trait and B denotes the trait of C57BL/6J.

—, Not determined.

Table 5. Genetic relationship between AXB/BXA recombinant inbred strains for anti-cardiac sarcolemmal antibody (ASA), cardiac myosin-specific autoantibodies and Coxsackievirus B3-induced chronic myocarditis

Strain	H-2	Trait		
		Anti-cardiac myosin	ASA	Myocarditis*
A/J	a	A†	A	A
A.BY/SnJ	b	A	A	A
B10.A/SgSf	a	B	B	B
C57BL/6J	b	B	B	B
AXB 1	a	B	—	B
2	a	A	A	A
5	a	B	B	B
6	b	B	A	A
7	a	—	B	B
8	a	—	—	B
10	b	—	B	A
15	a	B	A	A
17	a	A	A	B
18	b	A	B	B
19	b	—	B	B
20	—	—	B	B
21	a	B	B	A
23	b	A	A	A
25	a	A	B	B
BXA 6	a	B	A	A
8	b	B	A	B
10	b	B	A	B
11	b	—	B	A
12	a	B	—	B
19	b	B	A	B
20	b	B	A	B
23	a	A	A	A
24	—	A	A	A
25	a	A	A	A

* From Traystman *et al.* (1991).

† A denotes the A/J parental trait and B denotes the trait of C57BL/6J.

—, Not determined.

genic agent in inflammatory heart disease. The strong correlation between the presence of heart-specific autoantibodies and morphological disease suggests that these autoantibodies are not epiphenomena but pathogenic agents of the disease (Wolfgram *et al.*, 1986; Blay *et al.*, 1989). While the precise pathogenic role of these autoantibodies has not been determined, our data suggest that heart-specific autoantibodies may not be necessary to initiate disease in all hosts.

Circulating autoantibodies are found in patients with post-pericardiotomy and post-infarction syndromes (DeSheerder *et al.*, 1985, 1987) and are assocaited with interstitial inflammation and immune complexes. The release of heart tissue antigens resulting from mechanical trauma or physiological impairment is probably responsible for these autoantibodies. Indirect immunofluorescence assay studies have shown these antibodies to be directed toward sarcolemmal/subsarcolemmal and fibrillary myocyte structures (Maisch *et al.*, 1982, 1983; Wolfgram *et al.*, 1985). In addition, elevated titres to anti-actin and anti-myosin have also been reported following surgical trauma (DeScheerder *et al.*, 1987). Bound antibodies have been reported in rheumatic fever patients (Kaplan & Frengley, 1969) and Chagas' disease patients (O'Connell *et al.*, 1986) which may indirectly support a humorally-mediated etiological basis for idiopathic myocarditis and dilated cardiomyopathy. These antibodies have been shown to bind to sarcolemmal membranes, a variety of intra-myocyte proteins and adjacent cytostructures. In addition, heart autoantibodies associated with immune complexes were observed in patients with cardiomyopathy (Bolte *et al.*, 1980).

A summary of the clinical studies of idiopathic cardiomyopathy and presence of antibodies have shown that these patients have a higher prevalence (10–55%) of myocardial bound autoantibodies than other types of cardiomyopathies or normal patients; however, the relationship between the presence of autoantibody and severity of clinical disease remains unresolved

(Bolte *et al.*, 1980; Maisch *et al.*, 1982, 1983; DeScheerder *et al.*, 1987). Future studies to determine and characterize the target antigen of these ASA will be important in clarifying their physiologic and pathologic role in heart disease.

Studies by Neu *et al.* (1987b) support the postulate that cardiac myosin may be a 'pertinent' autoantigen, since immunization of susceptible mouse strains with cardiac myosin induces myocarditis. In contrast, our study provides indirect evidence that the cardiac myosin-specific autoantibodies did not play a primary role in myocarditis. First, data demonstrated that three RI lines, AXB 17, 18 and 25, did not develop late phase disease, but produced cardiac myosin-specific autoantibodies. The presence of ASA in the sera of these animals was thought to be due to anti-myosin antibodies known to produce IFA staining (Alvarez *et al.*, 1987). Other strains that produced ASA with cardiac myosin-specific autoantibodies developed more severe myocarditis than those without cardiac myosin-specific responses, suggesting that cardiac myosin antibodies enhanced disease. Second, the B10 H-2 congenics did not produce autoantibody at day 21 and are resistant to late disease. However, three strains in this group, B10.D2/nSnJ, B10.BR/SgJ and B10.Q/SgSf, do produce transient cardiac myosin-specific autoantibodies and myocarditis around day 9 or 10 (unpublished data). These data support the hypothesis that cardiac myosin autoantibody is not critical to development of late myocarditis, but may contribute to inflammation in less defined ways. In addition, the observed strain variation in the cardiac myosin response may reflect a combination of factors. Mouse strain polymorphism in the *Myhcα* itself could influence the serologic and myocarditogenic epitopes recognized by the anti-cardiac myosin immune response. Also, pathogenic cardiac myosin autoantibodies may be produced in a subgroup of mouse strains which develop autoantibodies to cardiac myosin. Of course, the cardiac myosin immune responses would be determined by specific alleles of the H-2 class I and/or II and *Tcr* genes.

The genetic studies and concepts regarding autoimmune myocarditis reported herein may have broad biological implications. The response to H-Y antigen (Epstein *et al.*, 1986), the demyelination induced by Theiler's encephalomyocarditis virus (Melvold *et al.*, 1987), and susceptibility to collagen-induced arthritis (Banerjee *et al.*, 1988) have been genetically linked with the murine *Tcrα* and *Tcrδ* loci, respectively. A similar association of *Tcr V* region genes and autoimmune diseases, such as experimental allergic encephalomyelitis and collagen-induced arthritis, has been demonstrated by the finding of limited and selective variable region gene usage in autoantigen-specific T cell clones (Kumar *et al.*, 1989). Additional experiments are required to define more precisely the genetic relationship of the *asa* and *amd* genes with regard to the *Tcrα V* region genes. In the case of CVB3-induced chronic myocarditis, the putative identification of a *Tcrα* variable region gene as controlling ASA and/or autoimmune myocarditis would more firmly establish the concept that chronic myocarditis is an organ-specific autoimmune disease.

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