American Journal of Pathology, Vol. 138, No. 3, March 1991

Susceptibility to Coxsackievirus B3-induced Chronic Myocarditis Maps Near the Murine *Tcrα* and *Myhcα* Loci on Chromosome 14

Monica D. Traystman,* Lawrence H. Chow,* Bruce M. McManus,* Ahvie Herskowitz,† Muriel N. Nesbitt,‡ and Kirk W. Beisel*

From the Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska; the Department of Immunology and Infectious Diseases,† The Johns Hopkins Medical Institutions, Baltimore, Maryland; and the Department of Biology,‡ University of California at San Diego, La Jolla, California*

This study was undertaken to determine the genetic control of host susceptibility to coxsackievirus B3 (CVB3)-induced chronic myocarditis in a mouse model. An autosomal recessive autoimmune myocardial disease (amd) gene (possibly more than one gene), which determined susceptibility to CVB3-induced chronic myocarditis in the A/J and DBA/2J inbred mouse strains, was mapped to a segment of chromosome 14. Data from both the AXB/BXA recombinant inbred (RI) strains and the B10.D2(57N) H-8^b congenic mice supported this linkage relationship. Analysis of the AXB/BXA RI strain distribution patterns suggested that amd maps distal to the Np-2, Tcrα, and Myhcα loci. (Am J Pathol 1991, 138:721–726)

Many of the picornaviruses, especially the coxsackieviruses, are implicated as causative agents in viral myocarditis in humans.[1,2] In mice, the course of coxsackievirus B3 (CVB3)-induced myocarditis appears to be biphasic.[3,4] The early acute disease, which peaks 5 to 7 days after infection, is suggested to be caused by direct virus-mediated tissue injury or by the ensuing inflammatory response.[3] The second phase, occurring after viral clearance and peaking 15 to 21 days after infection, termed chronic myocarditis, is associated with heart-specific autoantibodies.[3–6] Histologically chronic myocarditis is characterized by mononuclear cells within fibrocalcific foci and in the adjacent interstitial spaces.[7] Both cell-mediated and humoral autoimmune mechanisms have been suggested as the major pathogenetic factors in chronic viral myocarditis.[2–8]

Susceptibility of mice to CVB3-induced chronic (autoimmune) heart disease was shown initially in our laboratory[5,9] to be multigenic and involve the major histocompatibility complex (*Mhc*) on chromosome 17. Furthermore we demonstrated that a non-*Mhc* gene(s) is involved in susceptibility to CVB3-induced chronic myocarditis, while a *Mhc* gene(s) modulates the intensity of the heart-specific autoantibody response. This provided the basis for the hypothesis that chronic myocarditis is mediated by autoimmune responses that are determined by *Ir*, *Igh*, *Igκ*, *Igλ*, and/or *Tcr* genes. Thus our primary goal was to define precisely the non-*Mhc* locus (or loci) controlling the chronic or autoimmune phase of CVB3-induced myocarditis. The data indicate that a segment of chromosome 14 containing the *Tcrα* and *Myhcα* genes strongly influences the development of the disease.

Materials and Methods

Animals

Twenty-five AXB or BXA recombinant inbred strains, derived from the A/J and C57BL/6J (B6) parental mouse strains, were obtained from the laboratory of Muriel Nesbitt. Congenic and standard inbred strains were purchased from Jackson Laboratories, Bar Harbor, ME. Both male and female animals were used. The CVB3-inoculated animals were housed in microisolation cages (Lab Products, Inc., Maywood, NJ) in a separate room.

Supported in part by the American Heart Association, Maryland and Nebraska affiliates, and by USPHS grants HL-30144 and HL-38276.

Accepted for publication November 7, 1990.

Address reprint requests to Kirk W. Beisel, Department of Pathology and Microbiology, University of Nebraska Medical Center, 600 South 42nd St., Omaha, NE 68198-6495.

Infection and Necropsy

All experimental animals were inoculated with 10^4 $TCID_{50}$ of virus at 2 weeks of age, as previously described.[5] Individual sera and organ samples were collected 21 days after inoculation. Hearts were arrested in diastole with ice cold 30 millimolar KCl in phosphate-buffered saline, pH 7.2, and 3- to 5-mm–thick transverse slices of the heart were fixed for 24 hours in 4% formaldehyde/phosphate-buffered saline solution, pH 7.4, and then prepared for histologic evaluation.

Histology

Preparation and processing were performed as previously described.[5] Areas of necrosis, mononuclear cell infiltration, fibrosis, and dystrophic mineralization were evaluated and quantitated microscopically using a modified grading system established in previous studies.[7]

Virus

Vero African green monkey kidney cells (American Type Tissue Culture, Bethesda, MD) were used to grow and assay the virus.[5] CVB3 (Nancy) originally was isolated by Dr. Joseph Melnick from a patient who had a minor febrile illness. The stock virus used in these studies originally was obtained from Dr. Lerner, Wayne State University School of Medicine (Detroit, MI), whose stock was derived from Dr. Grodums, University of Saskatchewan (Saskatoon, Canada).

Results

Initially we studied the inheritance pattern of CVB3-induced chronic myocarditis by determining the disease phenotype of the F_1 progeny from susceptible A/J and resistant B6 mice. Reciprocal crosses were used to examine X and Y chromosome linkage. As shown in Table 1, the parental B6, (A/J × B6) F_1 or (B6 × A/J) F_1 mice did not develop CVB3-induced chronic myocarditis, whereas the A/J mice did. Neither a sex linkage nor a sex influence, as determined by comparison of disease susceptibility in male *versus* female animals, was observed (data not shown). Control mice injected with a Vero lysate did not develop disease. These data indicate that susceptibility to CVB3-induced murine chronic myocarditis is an autosomal recessive trait.

Linkage analyses of susceptibility to CVB3-induced chronic myocarditis in the AXB/BXA recombinant inbred (RI) strains was carried out to map a putative chromosomal location. Twenty-five randomly chosen AXB/BXA RI strains were examined for their development of CVB3-induced autoimmune disease. Seven to thirty animals were studied for each of the 25 groups (Table 2). Control animals included the parental A/J (*H-2*^{a}) and B6 (*H-2*^{b}) and their *Mhc* congenics, A.B.Y/SnJ (*H-2*^{b}) and B10.A/SgSf (*H-2*^{a}), respectively. As expected, these mice showed a susceptibility pattern that was independent of their *Mhc* haplotype. Of the 25 RI strains, 10 were found to be susceptible and 15 were resistant to chronic myocarditis, as determined by Chi-square analyses of the prevalence data. In general, as previously observed,[5] there was a direct relationship between the severity of fibrocalcific lesions and the presence of chronic myocarditis in these RI strains.

Based on a one-gene hypothesis, we examined the strain distribution pattern for autoimmune myocardial disease (*amd*). Table 3 shows the strain distribution pattern for *amd* and reveals a linkage of *amd* to the nucleoside phosphorylase-2 (*Np-2*) and the *Tcr* genes on chromosome 14 (Figure 1). Map distances of 13 and 11 cM (centamorgans), respectively, were found. The *Tcr*δ also resides within the *Tcr* complex.[10] These data indicate that a gene or a group of tightly linked genes on chromosome 14 determine murine susceptibility to CVB3-induced chronic myocarditis.

To substantiate the presence of the *amd* gene(s) on chromosome 14, we also examined two *H-8* congenic strains, B6.C-*H-8*^{c} and B10.D2(57N)/Sn (*H-8*^{b}), which carry a segment of chromosome 14 that encodes for the

Table 1. *Inheritance Pattern of CVB3-induced Chronic Myocarditis in Mice*

Mouse strain	*H-2* Haplotype	Fibrocalcific lesions Prevalence*	Fibrocalcific lesions PI†	Chronic myocarditis Prevalence*	Chronic myocarditis Phenotype‡
A/J	a	15/19 (79)	2.0 ± 0.3	8/19 (42)	s
B6	b	6/39 (15)	0.2 ± 0.1	0/39 (0)	r
(A/J × B6)F_1	a/b	5/24 (21)	0.2 ± 0.1	0/24 (0)	r
(B6 × A/J)F_1	b/a	10/37 (27)	0.2 ± 0.1	0/37 (0)	r

* Expressed as the number of positive animals/total number and the percentage of animals showing pathology is given in parentheses.
† The pathologic index (PI) is a mean score based on a scale of 0 to 4. Pathology scores were given by two or three independent observers.
‡ Strains were classified as susceptible (s) or resistant (r) to chronic myocarditis as described in Table 2.

Table 2. *AXB/BXA RI Strain Distribution Pattern for Susceptibility to CVB3-induced Chronic Myocarditis in Mice*

Mouse strain		*H-2* Haplotype	Fibrocalcific lesions Prevalence*	Fibrocalcific lesions PI†	Chronic myocarditis Prevalence*	Chronic myocarditis Phenotype‡
Congenics						
A/J		a	20/50 (40)	1.5 ± 0.2	9/50 (18)	s
A.BY/SnJ		b	50/122 (41)	1.2 ± 0.1	19/122 (16)	s
B6		b	13/91 (14)	0.2 ± 0.1	1/91 (1)	r
B10.A/SgSf		a	7/61 (11)	0.1 ± 0.1	0/61 (0)	r
RI						
AXB	1	a	1/11 (9)	0.1 ± 0.1	0/11 (0)	r
	2	a	15/21 (71)	1.7 ± 0.3	10/21 (48)	s
	5	a	14/29 (48)	0.8 ± 0.2	0/29 (0)	r
	6	b	14/15 (93)	2.2 ± 0.3	7/15 (47)	s
	7	a	8/18 (44)	0.5 ± 0.2	0/18 (0)	r
	8	a	0/11 (0)	0 ± 0	0/11 (0)	r
	10	b	6/11 (55)	1.0 ± 0.4	4/11 (36)	s
	15	a	16/19 (84)	1.8 ± 0.3	3/19 (16)	s
	17	a	6/30 (20)	0.3 ± 0.1	0/30 (0)	r
	18	b	1/23 (4)	0.2 ± 0.2	0/23 (0)	r
	19	b	5/25 (20)	0.4 ± 0.2	0/25 (0)	r
	20	•§	0/19 (0)	0 ± 0	0/19 (0)	r
	21	a	12/25 (48)	1.0 ± 0.2	7/25 (28)	s
	23	b	10/10 (100)	2.4 ± 0.4	4/10 (40)	s
	25	a	11/22 (50)	0.6 ± 0.1	0/22 (0)	r
BXA	6	a	3/10 (30)	0.4 ± 0.2	3/10 (30)	s
	8	b	0/7 (0)	0 ± 0	0/7 (0)	r
	10	b	3/17 (18)	0.2 ± 0.1	0/17 (0)	r
	11	b	8/16 (50)	1.1 ± 0.3	2/16 (13)	s
	12	a	0/18 (0)	0 ± 0	0/18 (0)	r
	19	b	9/28 (32)	0.5 ± 0.2	0/28 (0)	r
	22	b	11/20 (55)	0.9 ± 0.2	0/20 (0)	r
	23	a	11/20 (55)	1.4 ± 0.4	5/20 (25)	s
	24	•	3/13 (23)	0.8 ± 0.4	2/13 (15)	s
	25	a	3/28 (11)	0.2 ± 0.1	1/28 (4)	s

* Expressed as the number of positive animals/total number and the percentage of animals showing pathology is given in parentheses.
† The PI is a mean score based on a scale of 0 to 4. Pathology scores were given by two or three independent observers.
‡ Strains were classified as susceptible (s) or resistant (r) to chronic myocarditis as determined by Chi-square analyses ($P = 0.05$) using the prevalence of disease observed in the *H-2* haplotype-matched parental strains as the expected values.
§ Not determined.

H-8, *Np-2*, *Tcrα*, alpha cardiac myosin heavy chain (*Myhcα*), and pancreatic ribonuclease-1 (*Rib-1*) genes, as derived from BALB/cBy (*H-8*^{c}) and DBA/2J (*H-8*^{b}) mice, respectively. The remainder of the genome was derived from the *H-8*^{a}, B6, and C57BL/10 (B10) mice, respectively.[12,13] The B10.D2/nSnJ strain is similar to B10.D2(57N)/Sn strain except for lacking the DBA/2J-derived segment of chromosome 14. The B6, B10, and B10.D2/nSnJ strains are resistant to CVB3-induced chronic myocarditis, while both the BALB/c and DBA/2

Table 3. *Mouse Strain Distribution Pattern of Susceptibility to CVB3-Induced Chronic Myocarditis with Other Loci on Chromosome 14*

Locus	AXB	1	2	5	6	7	8	10	15	17	18	19	20	21	23	25
		Strains														
Np-2		b*	a	•†	a	a	b	b	b	b	b	b	b	b	a	•
Tcrα‡		b	a	b	a	a	b	b	b	b	b	b	b	a	a	b
amd		b	a	b	a	b	b	a	a	b	b	b	b	a	a	b
	BXA	6	8	10	11	12	19	22	23	24	25					
Np-2		a	a	a	•	b	b	a	a	•	a					
Tcrα		a	a	a	b	b	b	a	a	a	a					
amd		a	b	b	a	b	b	b	a	a	a					

* Genotype of the strains, where *a* is derived from A/J and *b* is derived from B6.
† • not determined.
‡ The genotype of the *Tcrα* locus was provided by E. Palmer and U. Apple and was determined by RFLP analysis using a constant-region and several variable-region probes.

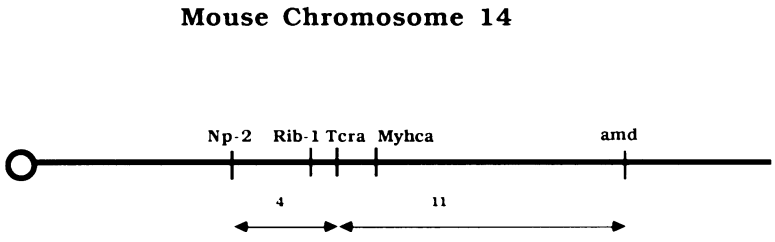

Figure 1. *Linkage relationship of* amd *with other loci on chromosome 14. The* amd *locus has been assigned provisionarily to a map location distal to the* Np-2, Rib-1, Myhcα, *and* Tcrα *loci (Ceci et al[11]; unpublished data; Dr. Nancy Jenkins, personal communications). Map distances are given in centaMorgans.*

develop autoimmune myocarditis as a sequela to CVB3 infection (Table 4).

Discussion

Several genes that are thought to play a part in autoimmune-based chronic myocarditis map to chromosome 14.[11,12] Results of our current studies suggest that the autosomal recessive gene(s) *amd* also is located on chromosome 14. It is well recognized that a trimolecular interaction between the T-cell receptor, the Mhc class II, and the antigenic peptide is required to elicit an immune response. In viral autoimmune myocarditis, the Mhc component has been suggested through the Mhc association with heart-specific autoimmune responses[5] and its role in immunopathogenesis.[14] A gene(s) for the T-cell receptor alpha chain, which is located on chromosome 14, may govern an autoimmune response to heart antigens that are elicited. In addition, the alpha and beta heavy chain genes of cardiac myosin are linked to the *Tcrα* and *Np-2* loci.[13] These latter findings are provocative because cardiac myosin is a major autoantigen and immunogen in cardiac autoimmunity.[15,16]

The B10.D2(57N)/Sn *H-8*^{b} mice carry a segment of chromosome 14 derived from susceptible DBA/2J mice. In as much as the B10.D2(57N)/Sn also develops chronic disease, the linkage of *amd* to chromosome 14 is significantly strengthened. The absence of chronic disease in B6.C-*H-8*^{c}/By mice suggests several possibilities. 1) The gene(s) controlling CVB3-induced chronic myocarditis in BALB/c mice may differ from DBA/2J mice. This conclusion is supported by the differences in immunopathologic mechanisms responsible for producing acute myocarditis in these two strains[17] 2) The site of recombination in the B6.C-*H-8*^{c}/By mice excludes the BALB/cBy susceptibility *amd* allele. 3) More than one gene controls chronic myocarditis. Our data suggest that B6.C-*H-8*^{c}/By mice lack at least one of these putative genes.

Both the *Tcrα* and *Myhcα* loci are linked on chromosome 14 in humans.[18] Jarcho et al[19] used 120 DNA probes with restriction fragment length polymorphisms in a large kindred to identify the locus (or loci) linked to a gene causing familial hypertrophic cardiomyopathy. Their multipoint linkage analyses showed that probe CR1-L436 identified the D14S26 locus on chromosome 14, which is genetically linked to familial hypertrophic cardiomyopathy. This assigned locus, 14q1, is approximately 20 cM from the locus for the *Tcrα* gene. Because this segment of chromosome 14 is homologous in both mice and humans, the studies of Jarcho et al[19] and our present observations indicate that a segment of chromosome 14 plays a significant role in the pathogenesis of familial hypertrophic cardiomyopathy and in chronic murine myocarditis.

The genetic studies and concepts regarding autoimmune myocarditis reported herein have biologic parallels in other models. Using the same experimental approach as we used, the response to H-Y antigen[20] and the demyelination induced by Theiler's encephalomyocarditis

Table 4. *Examination of* H-8 *Congenic Mouse Strains for CVB3-induced Chronic Myocarditis*

	H-2	H-8	Fibrocalcific lesions		Chronic myocarditis	
Strain	Haplotype	Genotype	Prevalence*	PI†	Prevalence*	Phenotype†
A/J	a	•§	18/30 (60)	1.2 ± 0.2	6/30 (20)	s
B6	b	a	5/27 (19)	0.2 ± 0.1	1/27 (4)	r
B10/SnJ	b	a	8/16 (13)	0.1 ± 0.1	0/61 (0)	r
B10.D2/nSnJ	d	a	7/21 (33)	0.6 ± 0.2	1/21 (5)	r
BALB/cByJ	d	c	13/23 (57)	1.3 ± 0.3	5/23 (22)	s
DBA/2J	d	b	17/23 (74)	1.7 ± 0.3	6/23 (26)	s
B6.C-*H-8*^{c}/By	b	c	2/31 (6)	0.1 ± 0.1	1/31 (3)	r
B10.D2(57N)/Sn	d	b	11/18 (61)	1.6 ± 0.3	4/18 (22)	s

* Expressed as the number of positive animals/total number and the percentage of animals showing pathology is given in parentheses.
† The PI is a mean score based on a scale of 0 to 4. Pathology scores were given by two or three independent observers.
‡ Strains were classified as susceptible (s) or resistant (r) to chronic myocarditis as described in Table 2.
§ Neither a nor b.

virus[21] have been linked genetically with the murine *Tcrα* and *Tcrβ* 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 (reviewed in Kumar et al[22]). In the case of CVB3-induced chronic myocarditis, sequence characterization and structure function analyses of a *Tcrα* variable-region gene as being *amd* would establish more firmly the concept that chronic myocarditis is an organ-specific autoimmune disease.

Recently the two rat cardiac myosin heavy chain isoforms, alpha and beta, have been sequenced[23–25] and comparisons between the two predicted amino acid sequences demonstrated that these differences occurred in clusters, termed divergent regions. There is a 99% amino acid sequence homology between rat and mouse *Myhcα* (KW Beisel, unpublished data). In the mouse, the *Myhcα* is found exclusively in the heart, while the *Myhcβ* isoform serves primarily as the slow skeletal muscle isoform.[26] Because the cardiac-specific myosin response is observed in murine virus- and cardiac myosin-induced autoimmune myocarditis,[15,16] we would predict that the alpha isoform-specific divergent regions contain the autoimmune epitopes. Restriction fragment length polymorphism studies demonstrated that at least three *Myhcα* alleles are present in the mouse[13] and may relate to strain differences in *Myhcα* immunogenicity as well as in the potential to develop autoimmune myocarditis.

Additional experiments are required to define more precisely the functional relationship and the location of the *amd* gene with regard to the *Tcrα* variable region and the *Myhcα and β* genes. Subsequently the functional relationship of these genetic determinants can be assessed.

Acknowledgments

The authors thank Dr. Noel R. Rose for helpful discussions and S. Schonlaw for laboratory assistance.

References

1. Woodruff JF: Viral myocarditis: A review. Am J Pathol 1980, 101:425–579
2. McManus BM, Gauntt CJ, Cassling RS: Immunopathologic basis of myocardial injury. Cardiovasc Clin 1986, 18:163–184
3. Rose NR, Wolfgram LJ, Herskowitz A, Beisel KB: Postinfectious autoimmunity: Two distinct phases of coxsackievirus B3-induced myocarditis. Ann NY Acad Sci 1986, 475:146–156
4. Lodge PA, Herzum M, Huber SA: 1987. Coxsackievirus B-3 induced myocarditis: Acute and chronic forms of the disease. Am J Pathol 1987, 128:455–463
5. Wolfgram LJ, Beisel KW, Herskowitz A, Rose NR: Variations in the susceptibility to coxsackievirus-B3-induced myocarditis among different strains of mice. J Immunol 1986, 136:1846–1852
6. Wolfgram LJ, Beisel KW, Rose NR: Heart-specific autoantibodies following coxsackievirus B3 myocarditis. J Exp Med 1985, 161:1112–1121
7. Herskowitz A, Wolfgram LJ, Rose NR, Beisel KW: Coxsackievirus B3 murine myocarditis—Histopathologic spectrum in genetically defined inbred strains. J Am Coll Cardiol 1987, 9:1311–1319
8. Maisch B, Trostel-Soeder R, Stechemesser E, Berg PA, Kochsiek K: Diagnostic relevance of humoral and cell-mediated reactions in patients with acute viral myocarditis. Clin Exp Immunol 1982, 48:533–545
9. Beisel K, Wolfgram L, Herskowitz A, Rose N: Differences in severity of coxsackievirus B3-induced myocarditis among *H-2* congenic mouse strains. Prog Leuk Biol 1985, 3:195–205
10. Chien Y, Iwashima M, Kaplan KB, Elliot JF, Davis MM: A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. Nature 1987, 327:677–682
11. Ceci JD, Kingsley DM, Silan KB, Copeland NG, Jenkins NA: An interspecific backcross linkage map of the proximal half of mouse chromosome 14. Genomics 1990, 6:673–678.
12. Dembić Z, Bannwarth W, Taylor BA, Steinmetz M: The gene encoding the T-cell receptorα-chain maps close to the *Np-2* locus on mouse chromosome 14. Nature 1985, 314:271–273
13. Beisel KW, Booth BJM: Identification of three alleles for *Mus musculus* at the *Myhc-a* locus which co-segregates with *Np-2, Tcra* and *Rib-1*. Nucleic Acids Res 1989, 17:10519
14. Blay R, Simpson K, Leslie K, Huber S: Coxsackievirus-induced disease CD4+ cells initiate both myocarditis and pancreatitis in DBA/2 mice. Am J Pathol 1989; 135:899–907
15. Neu N, Beisel KW, Traystman MD, Rose NR, Craig SW: Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to coxsackievirus B3-induced myocarditis. J Immunol 1987, 138:2488–2492
16. Neu N, Rose NR, Beisel KW, Herskowitz A, Gurri-Glass G, Craig SE: Cardiac myosin induces myocarditis in genetically predisposed mice. J Immunol 1987, 139:3630–3636
17. Huber SA, Lodge PA: Coxsackievirus B3 myocarditis. Identification of different pathogenic mechanisms in DBA/2 and Balb/c mice. Am J Pathol 1986, 122:284–291
18. Matsuoka R, Yoshida MC, Kanda N, Kimura M, Ozasa H, Takao A: Human cardiac myosin heavy chain gene mapped within chromosome region 14q11.2→q13. Am J Med Genet 1989, 32:279–284
19. Jarcho JA, McKenna W, Pare P, Solomon SD, Holcombe RF, Dickie S, Levi T, Donis-Keller H, Seidman JG, Seidman CE: Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. N Engl J Med 1989, 321:1372–1378

20. Epstein R, Sham G, Womack H, Yägue J, Palmer E, Cohen M: The cytotoxic T cell response to the male-specific histocompatibility antigen (H-Y) is controlled by two dominant immune response genes, one in the MHC, the other in the *Tar*α-locus. J Exp Med 1986, 163:759–773
21. Melvold RW, Jokinen DM, Knobler RL, Lipton HL: Variations in genetic control of susceptibility to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease I. Differences between susceptible SJL/J and resistant BALB/c strains map near the T cell α-chain constant gene on chromosome 6. J Immunol 1987, 138:1429–1433
22. Kumar V, Kono DH, Urban JL, Hood L: T cell receptor repertoire and autoimmune diseases. Ann Rev Immunol 1989, 7:657–682
23. McNally EM, Gianola K, Leinwand LA: Complete nucleotide sequence of full length cDNA for rat cardiac myosin heavy chain. Nucleic Acids Res 1989, 17:7527–7528.
24. Kraft R, Bravo-Zehnder M, Taylor DA, Leinwand LA: Complete nucleotide sequence of full length cDNA for rat cardiac myosin heavy chain. Nucleic Acids Res 1989, 17:7529–7530.
25. McNally EM, Kraft R, Bravo-Zehnder M, Taylor DA, Leinwand LA: Full-length alpha and beta cardiac myosin heavy chain sequences Comparisons suggest a molecular basis for functional differences. J Mol Biol 1989, 210:665–671.
26. Clark WA, Everett AW, Chizzonite RA, Eisenberg BR, Zak R: Classification and characterization of cardiac isomyosins. Eur Heart J 1984, 5[Suppl F]:69–75