

The Clinical and Diagnostic Significance of Anti-myosin Autoantibodies in Cardiac Disease

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Abstract Autoimmunity is influenced by genetic, immune, hormonal, and environmental factors. Viral infections may trigger autoimmunity. It has been established that autoimmunity may be a contributing factor in the pathogenesis of heart disease. Anti-heart autoantibodies have been identified in the sera of patients with heart diseases, as well as in low titers in certain healthy individuals. Nevertheless, the role of humoral immunity in the development of autoimmune heart disease has not been fully established. Anti-myosin autoantibodies appear in several heart diseases such as myocarditis, dilated cardiomyopathy, Chagas' heart disease, Kawasaki disease, rheumatic fever, and ischemic myocardium. The pathogenic role of anti-myosin autoantibodies in heart disease is not fully understood. Moreover, little is known concerning the clinical implications of anti-myosin autoantibodies in heart disease and its prognostic significance. Anti-cardiac myosin autoantibodies were found to cross-react with the β -adrenergic receptor. Studies have reported the

effective use of the anti-myosin directed immune-modulating approach in animals with heart disease, although no specific anti-myosin autoantibody therapeutic approach has been attempted in humans. Herein, we review the current knowledge of anti-myosin autoantibodies and the use of targeted immune-modulating therapy in different heart diseases.

Keywords Myosin · Anti-myosin autoantibodies · Myocarditis · Dilated cardiomyopathy · Ischemic cardiomyopathy · Autoimmunity

Introduction

An autoimmune response follows an abnormal activation of the immune system components against self-antigens and tissues. This reaction may be limited to an organ or have a systemic influence, which may ultimately result in tissue damage and organ dysfunction [1, 2]. It has been established that autoimmunity may be a contributing factor in the pathogenesis of several heart diseases such as myocarditis, dilated cardiomyopathy (DCM), heart failure, rheumatic fever, atherosclerosis, and others [3–6]. However, little is known of the factors causing specific individuals to become more susceptible in developing these autoimmune diseases. As to genetic susceptibility, Portig et al. recently reported an association between the presence of HLA DQB1*0303 with myocarditis without cardiac dysfunction [7]. Liu et al. found a link between susceptibility of developing idiopathic DCM and HLA-DR4, HLA-DR15, HLA-DPB1*0601 alleles, and the possible protective role of the DPB1*0101 allele against DCM involvement [8]. Liu et al. also reported an association between the HLA-DQA1*0501 alleles and idiopathic DCM [9]. Osa et al. found a link between B-15 and DQ3 and DCM [10]. In

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addition, Hernández-Pacheco et al. reported an association between the HLA-DR16 (DRB1*1602) allele, the DR16-DQA1*0501-DQB1*0301 haplotype, and the risk of rheumatic heart disease (RHD) [11]. An association between rheumatic fever and specific tumor necrosis factor (TNF)-alpha alleles was also described [12]. The association between other genetic markers and susceptibility for developing rheumatic fever was described in detail elsewhere [12].

Infections may trigger autoimmunity due to tissue damage, exposure to autoantigens, molecular mimicry, bystander activation of autoreactive immune T cells, and virus infection persistence [13]. The association between some infectious agents and cardiac disease is well established. For instance, the Coxsackie B virus is known to be responsible for approximately 30% of myocarditis cases [14–16]. Molecular mimicry causes the immune response to emerge towards self-antigens, sharing similarities with immunologic epitopes found in the infectious agent. For instance, in rheumatic fever, cross-reactivity between group A streptococcal antigens and myocardial antigens triggers an autoimmune response.

Induction of autoimmunity is influenced by genetic, immune, hormonal, and environmental factors. We refer to this phenomenon as *the mosaic of autoimmunity* [1, 2, 17, 18]. Myocardial damage and exposure of self-antigens may also be caused by toxins or other cardiotoxic factors, ischemia, or inflammation [3, 19–23].

Autoantibodies (AABs) are the hallmark of most autoimmune diseases and are capable of causing direct tissue damage by induction of apoptosis, activation of the complement system, or trigger cell-mediated cytotoxicity [24–26]. Furthermore, certain AABs have prognostic significance and may predict the appearance of an autoimmune disease, or be used as markers for monitoring disease activity [24, 27]. Anti-heart AABs (AHA) have been identified in the sera of patients with several heart diseases, in relatives of patients with certain heart diseases, and in low titers in some healthy individuals [28, 29].

AHA may react with diverse antigens such as contractile proteins, receptors, and other cellular or mitochondrial antigens [3]. It has been suggested that AHA might be used as an early marker in the development of future cardiac diseases [30]. Also, a decline in AHA during therapy might predict a favorable response in some patients [31]. Nevertheless, there is uncertainty regarding the role of some AHA in the pathogenesis of heart disease and their importance as disease markers.

Anti-myosin AABs

Actin, myosin, and troponin are sarcolemmal proteins involved in the muscular contraction process. Myosin

heavy chain is a protein with a molecular weight of 200 kD, most consistent with repetitive sequences [32]. Cardiac myosin has a coiled-coil α helical conformation [33]. The human cardiac myosin consists of several proteolytic fragments: light meromyosin (LMM; residue 1,295–1,936), subfragment-1 (S1; residue 1–842), and subfragment-2 (S2; residue 596–1,295), and heavy meromyosin (HMM; residue 1–1,295) [34]. There are eight LMM peptide groups consisting of six consecutive, overlapping peptides [34]. S1 contains the globular portion of the molecule, while S2 and LMM together encompass the α -helical coiled-coil rod region [35]. In humans, the β -myosin heavy chain isoform is expressed both in ventricular myocytes and slow skeletal muscle fibers, whereas the α -myosin isoform is found exclusively in atrial myocytes [36, 37]. Therefore, many experiments attempting to quantify a selective anti-heart humoral response has focused on anti- α -myosin-AABs [32].

Myosin exists inside cells, and although there is proof that some AABs might undergo internalization and intracellular translocation [38], it remains uncertain as to how anti-myosin AABs might interact with intracellular cardiac myosin in intact cells. Also, studies with immunofluorescence methods have failed to demonstrate internalization of anti-myosin AABs into rat ventricular cardiomyocytes [39]. It has been speculated that cardiomyocytes may present myosin or myosin-like determinants on their outer membranes, thereby making them vulnerable to an immune reaction, mediated by anti-myosin AABs [40]. It has also been suggested that anti-myosin AABs might react with other membranous proteins indirectly affecting the intracellular environment [39]. Liao et al. [41] demonstrated that susceptibility of mice in developing myocarditis following an anti-cardiac myosin immune response depended on the presence of myosin or myosin-like molecules in a cardiac extracellular matrix. It remains to be explored whether susceptibility in humans is mediated via similar mechanisms. Deposition of anti-myosin AABs was observed in damaged myocardial tissue following exposure of intracellular myosin but also in non-injured heart tissue following myosin immunization [33]. The tendency of some anti-myosin AABs (e.g., mAb 2 G4) to accumulate in damaged myocardial tissue can be used for specific delivery of genes to ischemic or injured areas [42].

Anti-myosin AABs from patients with cardiomyopathies and myocarditis were found to be targeted against epitopes in the S2 hinge region of the cardiac myosin [43]. Additionally, anti-cardiac myosin AABs were found to cross-react with the β -adrenergic receptor (β -AR) and trigger cAMP-dependent protein kinase A (PKA) signaling [33, 43]. PKA activation through β -AR is capable of inducing cell apoptosis in certain patients [33] and therefore has potential clinical significance in inducing cardiomyo-

pathic changes. Cross-reactivity is inherent in 25–30% identity and 56–57% homology between cardiac myosin and β -AR in different protein sequence alignments [33]. The S2-16 epitope of the myosin peptide (which was able to induce anti-myosin AAbs cross-reactivity with β -AR following immunization) had even higher rates of identity (34–44%) and 67–78% homology compared with β -AR in different protein sequence alignments [33].

Affinity-purified anti-myosin AAbs reduced the capacity of isolated myocytes to respond to field stimulation in a manner that was time- and concentration-dependent [39]. Peak systolic and peak diastolic intracellular Ca^{2+} were higher due to the effect of anti-myosin AAbs taken from DCM patients but not following the use of anti-myosin AAbs from patients with ischemic heart disease, compared with controls [39]. The effect was not mediated by L-type Ca^{2+} currents.

Warraich et al. [39] raised the possibility that the effect was induced by the sarcoplasmic reticulum due to an intracellular mechanism and impaired reuptake of Ca^{2+} . The discrepancy between the increased intracellular Ca^{2+} and reduced contractility might indicate altered sensitivity of the myofilaments to Ca^{2+} at the higher stimulating frequency [39].

There is cumulative evidence that anti-myosin AAbs might appear both in heart disease and healthy individuals

(Tables 1 and 2). In 1992, a study that included 13 healthy individuals found that none had AAbs reactive with a myosin heavy chain [36]. Subsequently, Lauer et al. [44] reported that the prevalence of anti-myosin AAbs in healthy blood donors ($n = 39$) was 2.5%. In another study, conducted on 203 healthy controls, four patients (2%) were anti- α -myosin AAbs-positive [37]. Warraich et al. [32] included 59 controls and reported a 3.4% frequency of positivity for anti- α -myosin–AAbs, as well as 3.4% anti- β -myosin AAbs-positive. Nevertheless, the levels of anti-myosin AAbs found in normal individuals were low compared with the levels in cardiac diseases [45].

Circulating anti-myosin AAbs were reported in patients with myocarditis, DCM, Chagas heart disease (ChHD), Kawasaki disease, rheumatic fever, and ischemic myocardial damage. Nevertheless, in different heart diseases, anti-cardiac myosin AAbs display different antigen reactivity, which might be used for distinguishing one disease from the other [46].

Anti-myosin AAbs in Myocarditis

Myocarditis is an inflammatory process of the myocardium linked to several viral agents and may be a precursor of

Table 1 Summary of the current literature relating to the prevalence of anti-myosin AAbs in controls

Inclusion criteria	Included patients (n)	M/F	Mean age (years)	Anti-myosin measurement technique	Serum dilution	Results	Selected abnormal value	Percentage of abnormal results, n (%)	Ref.
Apparently healthy subjects with no evidence of heart disease or symptoms suggestive of cardiac arrhythmia	10	9:1	48 $\pm$ 12	Immunoblotting	1:20	NA	A band with an apparent molecular weight of 205 kD	1 (10%)	[88]
Apparently healthy nurses and laboratory personnel	20	NA	32 mean	ELISA	1:200	Enzyme activity of 192 $\pm$ 48 OD	NA	NA	[89]
Healthy donors	20	NA	NA	ELISA	NA	NA	NA	0	[74]
Healthy blood donors	28	NA	NA	ELISA	1:50	NA	Median 0.92 AU	NA	[90]
Healthy blood donors	41	NA	NA	Solid-phase radioimmunoassay	NA	1.24 $\pm$ 0.58 ^a	NA	5 (12.2%)	[91]
Healthy blood donors	43	18:25	37.5 mean	ELISA	1:80	NA	IgM positivity at 1:80 dilution	(5%)	[92]
HIV negative patients at low risk of HIV infection	52	31:21	45 $\pm$ 16	ELISA	1:320	0.179 $\pm$ 0.071	0.32	1 (1.9%)	[93]
Healthy blood donors	59	36:23	40 $\pm$ 9.6	ELISA	1:100	0.739 $\pm$ 0.04 ^b	Above 2 standard deviations from the mean of the normal controls	2 (3.4%)	[32]
Healthy blood donors	203	100:103	45 $\pm$ 16	ELISA	1:320	0.17 $\pm$ 0.01 ^b	Above 2 standard deviations from the mean of the normal controls	4 (2%)	[30, 37]

Values are given in mean $\pm$ standard deviation

NA not available, UA arbitrary units

^a Expressed as an antibody index

^b $\pm$ Standard error

Table 2 Summary of the current literature relating to the prevalence of anti-myosin AABs in various clinical conditions ($n > 50$ patients)

Clinical condition	Inclusion criteria	Included patients (n)	M/F	Mean age (years)	EF (%)	Anti-myosin measurement technique	Serum dilution	Results	Selected abnormal value	Percentage of abnormal results, n (%)	Ref.
DCM	Fulfillment of WHO and National Heart, Lung and Blood Institute criteria; absence of ischemic heart disease	123	93:30	42 $\pm$ 14	24 $\pm$ 11	ELISA	1:320	0.27 $\pm$ 0.02 ^a	Above 2 standard deviations from the mean of the normal controls	25 (20.3%)	[37]
	Criteria fulfillment and need for heart failure and arrhythmia treatments	57	NA	Randomly chosen from a group of 110 DCM patients aged 44 $\pm$ 13 years	NA	ELISA	1:320	0.3 $\pm$ 0.02 ^a	Above 2 standard deviations from the mean of the normal controls	14 (24.5%)	[30]
	NYHA III/IV	82 (68 adults)	57:11 adults, 8:6 children	43 $\pm$ 12 adults, 9 $\pm$ 4 children	33 $\pm$ 11 & for adults	ELISA	1:100	1.075 $\pm$ 0.06 ^a for the entire group; 0.975 $\pm$ 0.12 ^a for children	Above 2 standard deviations from the mean of the normal controls	19 (23.2%), 16 of which are adults	[32]
IHD	Admitted to the coronary care unit with unstable angina	92	65:27	63 $\pm$ 11	NA	ELISA	1:320	0.2 $\pm$ 0.01 ^a	Above 2 standard deviations from the mean of the normal controls	4 (4.3%)	[37]
	A week following acute myocardial infarction	67	49:18	61.6 $\pm$ 11	42.5 $\pm$ 15.33 for the anti-myosin-positive group	ELISA	NA	NA	NA	18 (26.9%)	[74]
	End-stage ischemic heart disease; history of myocardial infarction or angiographically proven coronary disease	62	52:10	52 $\pm$ 8	40 $\pm$ 12.4 ^b	ELISA	1:100	0.950 $\pm$ 0.06 ^a	Above 2 standard deviations from the mean of the normal controls	10 (16.1%)	[32]
Myocarditis	Clinical diagnosis of myocarditis	53; only 24/53 had positive Dallas criteria	35:18	42 $\pm$ 15	25 $\pm$ 10 in the Dallas-positive patients	ELISA	1:320	0.33 $\pm$ 0.06 ^a in Dallas-positive patients, and 0.21 $\pm$ 0.02 ^a in Dallas-negative patients	Above 2 standard deviations from the mean of the normal controls	9 (17%)	[58]

Values are given in mean $\pm$ standard deviation

NA not available, CHF congestive heart failure, IHD ischemic heart disease

^a $\pm$ Standard error^b Angiographic measurement

DCM. It is suspected that a secondary autoimmune response may evolve following initial myocardial injury and presentation of autoantigens into the immune system [3]. Nevertheless, the pathogenesis of myocarditis remains unclear [47]. Neu et al. proposed that involvement of myocarditis might occur more frequently in individuals with genetic susceptibility [40]. Certain strains of mice (i.e., A.CA, A.SW, and A.BY mice) were found to be more susceptible in developing myocarditis following a viral infection with Coxsackie virus B3 (CVB3) compared with other strains [48].

One of the major autoantigens in mice, genetically predisposed to develop CVB3-induced autoimmune myocarditis, was found to be a heavy chain of myosin [49, 50]. Infection of A.CA and A.SW mice with CVB3 resulted in elevated circulating levels of two AAbs that reacted with myosin: an IgG which was specific for myocardial myosin and an IgM autoreactive for heart, skeletal muscle, and brain myosins [48]. In contrast, CVB3 infection of BIO.PL/SgSf and BIO.A/SgSf mice (which were not susceptible in developing myocarditis) resulted in an elevation of the IgM population but not the IgG population, with specific autoreactivity to cardiac myosin [48]. The lack of pathogenicity of anti-myosin IgM in the DBA/2 mouse strain has been supported by other studies as well [51].

Neu et al. [48] suggested that the presence of IgG AAbs targeted against cardiac myosin could be used as markers in the development of myocarditis following viral infections in animal models. Immunization of predisposed mice (A/J, A.SW/SnJ, and A.CA/SnJ mice) with murine cardiac myosin, but not with skeletal muscle myosin, resulted in autoimmune myocarditis, similar to that observed following CVB3 infection [40].

In one experiment, anti-myosin AAbs extracted from serum obtained from A.CA mice that developed myocarditis following CVB3 infection, or the use of a whole serum following immunization with myosin, did not significantly react with CVB3 antigens [50]. Therefore, it was speculated that anti-myosin AAbs do not cross-react with CVB3 and that a CVB3 infection in mice does not trigger production of anti-myosin AAbs due to molecular mimicry, but possibly due to tissue damage and exposure of autoantigens. Nevertheless, other studies, applying different investigative techniques demonstrated cross-reactivity between monoclonal Abs against CVB3, Coxsackie virus B4, and myosin [52, 53].

Subsequently, Gauntt et al. [54] reported that several neutralizing Abs against a highly myocarditic CVB3 virus (CVB3m) were able to induce cardiopathologic changes in CD-1 or C3H/HeJ mice, possibly due to cross-reactivity. Nevertheless, it remains controversial whether molecular mimicry between Coxsackie viruses and cardiac myosin plays a role in the pathogenesis of myocarditis [35].

Penninger et al. [55] confirmed molecular mimicry between myosin heavy chain and *Chlamydia* proteins. Moreover, immunization of mice with *Chlamydia*-driven peptides resulted in pericardial and perivascular immune infiltration, and histological myocarditis similar to changes observed following immunization with cardiac myosin [55].

Transfer of anti-cardiac myosin AAbs in mice was not associated with myocarditis-like cardiomyopathic changes. It was suggested that autoreactive T helper cells were more important factors in causing immune-mediated tissue damage in mice [46]. Smith et al. [56] reported that the transfer of purified T cells, but not high-titer anti-myosin AAbs serum from C.B-17 mice to SCID mice, resulted in myocarditis induction, further supporting the theory that myosin-induced myocarditis is a T cell-mediated disease. In contrast, other studies have found that the transfer of anti-myosin AAbs to certain strains of mice did induce myocarditis [33]. It has been proposed that the infusion of anti-myosin AAbs induced myocarditis in DBA/2 but not in BALB/c mice, since myosin or a similar protein was present in the extracellular matrix of DBA/2 mice [34]. However, it was also possible that differences in the function of the immune system may have contributed to this variance. Overall, it seems that both humoral and cellular response against cardiac myosin may involve the pathogenesis of myocarditis and subsequent dilated cardiomyopathy [33].

AAbs were detected in the cardiac tissue of myocardial biopsies taken from patients with myocarditis [43]. Anti-myosin AAbs were detected in 42–52% of the patients with myocarditis [37, 44, 57] and in about 42–52% with chronic myocarditis [44, 57]. Patients with clinical myocarditis but with negative Dallas criteria of myocarditis on histology had significantly higher abnormal anti- α -myosin AAbs cases (17%) compared with controls [58]. Lauer et al. [57] reported that, of the 33 patients with biopsy proven chronic myocarditis, 17 were initially positive for anti-myosin AAbs; 13 were positive at the end of the 6-month follow-up. It was also found that anti-myosin AAbs-negative patients tended to significantly improve symptoms as well as stroke volume, ejection function, and myocardial stiffness parameters compared with anti-myosin AAbs seropositive patients who did not [57]. Thus, anti-myosin AAbs may serve as a prognostic index in some patients, although the results of such small-scale studies should be addressed with caution.

Anti-myosin AAbs in Dilated Cardiomyopathy

DCM is characterized by cardiac dilation and a poorly contracting left and/or right ventricle. DCM is the common

cause of non-ischemic heart failure [59], with an annual incidence of approximately 20,000 newly diagnosed cases of idiopathic DCM in the USA [46]. Approximately 25% of DCM cases are familial [37, 60]. Toxins, alcohol, infections, inflammatory conditions, metabolic abnormalities, nutritional deficiencies, and neuromuscular diseases can also cause cardiac dysfunction and DCM [28, 61]. Unknown etiology may account for up to 65–80% of DCM cases [59]. Some studies have found that certain idiopathic cases are caused by autoimmune mechanisms, triggered by viral infections [3, 61]. Several AHA have been detected in DCM [3, 19, 61]. It has been suggested that some of these AAbs might be used in the follow-up of disease severity [46].

In 1992, Caforio et al. [36] reported 14 out of 26 DCM patients with circulating AHA; 12 out of 14 AHA-positive DCM patients had AAbs directed against both α - and β -MHC (11 were cardiac specific, and one was also reactive with skeletal muscle). The authors reported that all 12 of the included patients with heart failure due to ischemic or valvular lesions were negative for AHA according to immunofluorescence, while only one (8%) had AAbs reactive with both α - and β -MHC in immunoblotting [36].

In 1995, Goldman et al. [37] tested the presence of anti- α -myosin-AAbs in DCM patients and found that 25 out of 123 tested positively (20.3%). In addition, 41 out of 252 DCM asymptomatic relatives (16%) were positive for anti- α -myosin-AAbs [37].

Anti-cardiac myosin AAbs in patients with cardiomyopathies were found to be of the IgG2/IgG3 subclasses, suggesting a Th1 immune response [46]. Also, IgG3 levels were higher in DCM patients compared with patients with end-stage ischemic heart disease and normal individuals [32]. Levels of IgG2 were similar in patients with DCM and ischemic cardiomyopathy, and both were significantly higher than the controls [32]. IgG3 was demonstrated to be more effective in inducing complement activation via C1q binding and in inducing antibody-dependent cellular cytotoxicity [32].

Caforio et al. [30] also reported that during the DCM course, frequency of AHA decreased from 25% to 10% of patients during 14 ± 12 (mean, SD) months of follow-up. The decrease in AAbs is also apparent in other unrelated autoimmune conditions such as diabetes mellitus type 1 [30]. Anti- α -myosin-AAbs titer also decreased during the disease course [30]. The significance of these findings is not fully understood.

Transmission of autoreactive T cells from mice with myocarditis to SCID mice resulted in cardiomyopathic changes [3]. Nevertheless, in other human studies, a lack of exaggerated peripheral T lymphocytic cell response (collected from DCM patients and their relatives) to cardiac myosin compared with controls was found [46, 60],

implying that, in humans, an anti-myosin immune response might cause tissue damage in DCM, with an AAbs-mediated mechanism [46]. However, in that particular study, peripheral immune cells, not specifically T cells, were investigated and extracted from cardiac tissue.

Transfusing rats with anti-myosin IgG induced cardiomyopathic changes resembling DCM, i.e., enlargement of the left cardiac chamber and thinning of the septum and posterior wall, compared with controls [33]. These findings further support the theory that the anti-myosin immune response contributes to the pathogenesis of DCM.

It has been suggested that chronic over-stimulation of the β -adrenergic receptor by cross-reactive anti-myosin AAbs might cause myocarditis to progress to DCM [43]. Activation of cAMP-dependent PKA pathways may ultimately favor apoptosis, similar to the mechanism of catecholamine-induced cardiotoxicity [62].

Anti-myosin AAbs in Chagas Heart Disease

Infection by the protozoan parasite *Trypanosoma cruzi* results in ChHD. Subsequently, 25–30% of infected individuals will develop ChHD, 5–30 years following the infection [63, 64]. Persistence of parasitic infection, autoimmune reaction, and microvascular dysfunction may all contribute to the pathogenesis of ChHD [65]. Several studies have suggested that autoimmunity plays a central role in the pathogenesis [66, 67]. Leon et al. [67] found that there is an anti-myosin-specific autoimmune response both in humans with ChHD and in animal models. Immunization of mice with *T. cruzi* cruzipain or with protein extract of *T. cruzi* emulsified in complete Freund's adjuvant induced a myosin-specific immune response [67]. An anti-myosin autoimmune reaction appeared early in the acute infection [68]. It was found that *T. cruzi*'s B13 protein had cross-reactive epitopes with human cardiac myosin and that CD4⁺ T cells in the hearts of ChHD patients recognized both [63]. Additionally, immunization of mice with cardiac myosin induced *T. cruzi*-specific cellular and humoral immune responses [67]; therefore, the immunologic cross-reactivity between *T. cruzi* and myosin was bidirectional. It was also found that cardiac damage and release of self-myosin from cells are not essential in inducing an anti-myosin immune reaction [67]. Injection of myosin-coupled splenocytes to mice prior to infection with *T. cruzi* suppressed myosin-specific delayed-type hypersensitivity and suppressed cellular myosin autoimmunity but did not affect either the production of anti-myosin AAbs nor myocarditis [69]. Therefore, it seems that the cellular anti-myosin autoimmune response is not essential to the production of acute cardiomyopathic changes following *T. cruzi* infection in mice, although not excluding its role in the pathogenesis.

Ballinas-Vedugo et al. [64] reported that, in 11% of 26 enrolled patients with ChHD, anti-myosin AAbs appeared. Nevertheless, the role of anti-myosin AAbs in the pathogenesis of ChHD requires further investigation.

Anti-myosin AAbs in Rheumatic Carditis

RHD is caused by an autoimmune response against β -hemolytic streptococcal infection [3]. Acute rheumatic fever (ARF) and Kawasaki disease (KD) are the leading causes of acquired heart diseases in childhood [45]. As mentioned above, molecular mimicry was found between streptococcal α -helical M-protein, and group A carbohydrates to α/β cardiac myosin chains, tropomyosin, keratin, vimentin, and laminin cause autoimmunity in ARF [3]. AAbs in patients with RHD were deposited in the myocardium and the valvular endothelium [35].

Krisher et al. [70] were the first to report that myosin sharing of immunodeterminants with a component of *Streptococcus pyogenes*, in addition to Abs against streptococcal proteins reacting with myosin. Also, anti-myosin AAbs recognized the group A streptococcal M protein antigen [45]. Anti-myosin AAbs appeared to be directed to an epitope contained in the amino acid sequence 184–188 (GLN-LYS-SER-LYS-GLN) [71].

Anti-myosin AAbs cross-react with laminin found in the basement membrane of the cardiac valve and secreted by endothelial cells [34]. Specific epitopes of human cardiac myosin are capable of inducing valvulitis in Lewis rats. Valvulitis can also be induced by immunization with cardiac myosin, cardiac myosin subfragments, HMM, or S2, and LMM regions. Peptide fragments from S2 and from HMM caused both valvulitis and myocarditis [34]. Histologically, valvulitis following myosin immunization was similar to the changes observed in rheumatic fever (e.g., presence of CD4⁺ and CD8⁺ T cells) [34].

Anti-myosin AAbs in Kawasaki Disease

KD is also known as a mucocutaneous lymph node disease and is characterized by acute febrile vasculitis of childhood that may result in coronary artery aneurysms and life-threatening cardiac complications. Etiology remains unknown. Over 50% of patients with KD will develop myocarditis, which may be complicated by a depressed contractile function and cardiac dilatation, independent of the coronary involvement [45]. The pathogenesis of myocardial involvement in KD is unknown.

In one study, sera of 13 children with acute KD were compared with 12 matched febrile controls and ten normal adults for the presence and titer of anti-cardiac myosin

AAbs. Patients with KD had significantly higher anti-myosin IgM AAbs titers compared with the other groups [45]. However, acute KD was not associated with elevated anti-myosin IgG AAbs, which may be encountered in rheumatic carditis [45]. Also, the anti-cardiac myosin AAbs in KD had a different reactivity pattern with several LMM peptides of human cardiac myosin, compared with patients with rheumatic fever [45]. It therefore seems that the humoral responses in KD and in ARF are specific and targeted against different LMM epitopes.

KD is an established FDA-approved absolute indication for Intravenous immunoglobulin (IVIG) therapy. When given within 10 days following onset of fever, IVIG therapy in KD results in a significant decrease of coronary artery aneurysms [72]. Cunningham et al. [45] demonstrated that IVIG was beneficial in KD patients with cardiac contractile abnormalities but without coronary involvement. It is possible the anti-idiotypic activity of IVIG against anti-myosin AAbs contributes to improved myocardial function.

Anti-myosin AAbs in Myocardial Infarction and Ischemic Cardiomyopathy

Ischemic heart diseases are a major cause of morbidity and mortality as well as a financial burden worldwide. In recent decades, vast amounts of data have been collected regarding conventional cardiovascular risk factors (i.e., obesity, smoking, diabetes, dyslipidemia). However, little is known of the role of autoimmunity in patients with myocardial infarction (MI) and ischemic cardiomyopathy (ICM). The immune system may involve the cardiac remodeling process following MI [73]. Ischemia may cause myocardial cell death and release of cardiac myosin, subsequently causing autoimmunity.

Moraru et al. [73] tested anti-myosin immune system reactivity in 18 patients who had previously undergone MI (2 weeks to 4 months) and 18 controls. They found that the peripheral blood mononuclear cells had a proliferative response to cardiac myosin in a third of the MI patients. Nevertheless, although cellular autoimmune reactions to cardiac myosin can be detected in some patients post-MI, no difference in the serum levels of anti-myosin IgG AAbs was detected [73]. The authors did not assess whether an autoimmune response against cardiac myosin in patients following MI was associated with an adverse outcome.

In another study of 92 patients with ischemic heart disease, four were anti- α -myosin-AAbs-positive (~4%) [37]. Additionally, anti- β -myosin AAbs were detected in 16% of the patients following an acute MI [37]. Reactivity to β -myosin was higher in advanced ischemic heart disease stages compared with mild ICM and controls [32].

In a study by Pang et al. [74], out of 67 patients evaluated following MI, 18 were tested for anti-myosin AAbs (26.87%). Significant increases were found in the presence of decreased wall motion and ventricular aneurysm in the anti-myosin AAbs-positive patients (92.85%) compared with negative patients (37.5%). During a 6-month follow-up, mortality was significantly higher in the anti-myosin AAbs-positive group (38.89% vs 10.20%) [74]. Interestingly, anti-cardiac myosin AAbs from ICM patients differed from AAbs in DCM patients in their ability to alter contractility, presumably due to differences in an Ig subclass isotype [39].

Conclusions, Future Prospect, and Anti-myosin AAbs-Directed Therapy

Anti-myosin response fulfills the criteria for organ-specific autoimmunity according to Witebski's postulates for autoimmune disease [75]. Various heart diseases are characterized by different immunological heterogeneity of the anti-myosin AAbs (e.g., different frequency, reactivity, and IgG subclasses) [32]. It is still unclear as to the importance of anti-myosin AAbs and its fine specificity as markers for heart diseases. Although myosin represents a major auto-antigen in animal models of myocarditis, it remains unclear as to how important the role of anti-myosin AAbs in the pathogenesis of heart diseases is in humans. Moreover, the prognostic significance of the presence of circulating anti-myosin AAbs in healthy individuals and in relatives of DCM patients is undetermined. Additionally, the presence of an anti-myosin immune response (following immunization of mice with *T. cruzi* proteins) is not necessarily associated with cardiac injury [67]. It seems that anti-myosin AAbs are pathogenic in some patients [76], although we have as yet to characterize them. There are reports that their presence might be associated with an adverse outcome [57]. The clinical importance of seroconversion from anti-myosin AAbs, positive to negative, remains to be explored. Several pathogenic mechanisms of anti-myosin AAbs have been suggested, such as chronic stimulation of the adrenergic receptor by cross-reactive anti-myosin AAbs. Nevertheless, the potential benefit of anti-myosin AAbs-directed therapy remains to be investigated. Immunosuppression cannot be utilized in cases of autoimmunity induced following infections since a persistent infectious agent can cause major complications [77]. Moreover, immunosuppression was found to be ineffective in the Myocarditis Trial [35].

Skyllouriotis et al. [46] suggested a therapeutic strategy where immunomodulating therapy changes the Th1 immune response to a Th2 immune response. Other approaches could have eliminated the anti-myosin AAbs

from the sera. It was demonstrated, in vitro, that absorption of anti-myosin AAbs with anti-human IgG or by antigen-specific inhibition abolished anti-myosin-mediated cell signaling [43]. For example, peptides containing the sequence SM5 were found to inhibit anti-myosin AAbs [71].

Immunoadsorption represents another method of eliminating AAbs from the circulation [33]. Nevertheless, a review of the literature did not yield reports of anti-myosin AAbs-specific immunoadsorption use [78]. Interestingly, the β -adrenergic blocker, carvedilol, reduced the severity of experimental autoimmune myocarditis induced by immunization of Lewis rats with porcine cardiac myosin [79], possibly providing further evidence of the role of cross-reactivity of anti-myosin AAbs with β -adrenergic receptor [43]. Carvedilol was superior to metoprolol and propranolol. Yuan et al. [79] suggested that the superiority of carvedilol was attributed to its anti-oxidative and anti-inflammatory properties. Interestingly, carvedilol seemed to be more beneficial than metoprolol in patients with heart failure or sero-positive for other AAbs (e.g., anti- β 1-adrenergic receptor antibodies) [80]. Another reported therapeutic approach was nasal administration of cardiac myosin in mice causing antigen-specific tolerance and reducing the severity of myosin-induced myocarditis [77]. Immune tolerance to cardiac myosin in myocarditis (as demonstrated in the animal model) can also be achieved by injecting an anti-CD4 monoclonal antibody, thereby reducing the circulating levels of Th1 cytokines [47]. Immune tolerance to cardiac myosin, a dramatic decrease in production of anti-myosin AAbs, and prevention of myocarditis initiation and cardiac injury following immunization of mice with myosin, were also achieved by transfusing the anti-L₃T₃ monoclonal antibody [81].

Anti-TNF neutralizing monoclonal antibodies also reduced the severity of myocarditis in mice immunized with myosin when given before immunization [82]. Treatment of mice, immunized with porcine cardiac myosin with thioredoxin-1 (a redox-regulatory protein with antioxidant and anti-inflammatory effects) was also found to significantly reduce severity of myocarditis [83]. The incidence and severity of experimental myocarditis (immunization of mice with myosin driven peptides) was also reduced following prophylactic or late treatment with a ligand epitope antigen presentation system (conjugation of residues 334–352 of cardiac myosin and J peptide), although, in some cases, it was associated with major adverse reactions, anaphylaxis, and death [84]. Nevertheless, in other experiments, an induction of humoral tolerance to the cardiac myosin did not prevent clinical autoimmune myocarditis [85], which may imply that the immuno-modulating approach should focus on more than one antigen.

Theoretically, vaccinations against infectious pathogens known to be associated with induction of heart diseases might prevent primary cardiac damage and an anti-myosin autoimmune response [13]. Rose reported on the beneficial effect of anti-viral therapy once virus-associated cardiac disease was identified [86]. On the other hand, Zimmermann et al. did not find any beneficial effect of interferon beta-1b in DCM patients [87]. Therefore, these clinical approaches require further medical research before applying to common practice.

Based on experimentation of animals, it is indicated that different immune-modulating approaches may be useful in treatment and prevention of myosin-associated myocarditis and possibly other heart diseases. Nevertheless, preferably, therapy should be initiated in early disease stages. The safety issue must be addressed before these approaches can be of practical use. Timing of treatment also presents a major problem since clinical signs in heart disease such as myocarditis emerge late in the course of the disease, when abundant cellular damage may have already been caused [35]. Detection of early stages of the subclinical disease remains a challenging task.

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