

The Diagnostic and Clinical Significance of Anti-Muscarinic Receptor Autoantibodies

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Abstract The role of autoimmunity in cardiovascular diseases has become one of the focal points of research studies. Autoimmune response and autoreactive autoantibodies have been found in dilated cardiomyopathy, heart failure, rheumatic fever, myocarditis, atherosclerosis, and other diseases. Autoantibodies may appear due to tissue injury and exposure of autoantigens, in addition to molecular mimicry and cross-reactivity with antigens found in infectious agents in predisposed individuals. In the early 1990s, autoantibodies reacting with the M2 muscarinic receptor were found in patients with dilated cardiomyopathy and subsequently, in patients with Chagas heart disease and arrhythmic disorders. Immunization of animals with the corresponding antigen triggered cardiac abnormalities also appearing in dilated cardiomyopathy of humans. It has been suggested that antibodies against M2 muscarinic receptors play a role in the pathogenesis of cardiac diseases and may also alter the electrophysiological properties of cardiac tissue. Herein, we review the current knowledge of antibodies against M2 muscarinic receptors and the possible use of a targeted therapy against these autoantibodies.

Keywords Autoantibodies · Anti-M2 muscarinic receptor autoantibodies · Dilated cardiomyopathy · Chagas heart disease · Autoimmunity

Introduction

An autoimmune response may mediate the pathogenesis of various diseases such as Addison's disease, diabetes mellitus type 1, autoimmune hepatitis, autoimmune hemolytic anemia, and many others. During the past few years, autoimmune mechanisms in heart disease have moved to the forefront of research studies. Autoimmunity has been observed in several cardiovascular illnesses such as dilated cardiomyopathy (DCM), heart failure, rheumatic fever, myocarditis, atherosclerosis, and others [1–5]. It is unclear why certain individuals are more susceptible in developing an autoimmune response, although it is believed that genetic, immune, hormonal, and environmental factors influence the susceptibility. Predisposition for developing autoimmunity has been linked to several immune-regulatory and tissue-specific genes. Molecular mimicry and cross-reactivity between group A streptococcal α -helical M-protein and group A carbohydrates, and myocardial antigens (e.g., cardiac myosin chains, laminin, tropomyosin, vimentin, and keratin) cause rheumatic fever.

Cross-reactive autoantibodies (AAbs) usually react with evolutionarily conserved molecules, abundant in cardiac tissue. Infective agents may also amplify the autoimmune response by causing exaggerated cytokine excretion, thereby activating T cells [6]. Myocardial damage may result following various injuries such as infections, inflammation, ischemia, or toxins [2, 6–11]. AAbs may contribute to the process of autoimmune-

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Table 1 Summary of the current literature relating to the prevalence of anti-M2R AAbs in controls ($n \geq 10$)

Inclusion criteria	Included patients (n)	M/F	Mean age (years)	Anti-M2R measurement technique	Serum dilution	Results	Selected abnormal value	Percentage of abnormal results, n (%)	Ref.
Healthy controls	10	5:5	Mean 45.5	Functional assay	NA	Anti-M2R AAbs were not detected in either patient.	Addition of patients' IgG led to a significant reduction in the rate (compared with the control IgG).	0	[55]
Voluntary healthy donors	10	NA	29.5 $\pm$ 3.2	Contractility bioassay	1:100	None of the controls were sero-positive.	Isoproterenol (10 nmol/L) served as a positive control. IgG from healthy donors served as a negative control.	0	[71]
Healthy controls	14	9:5	40 $\pm$ 12	ELISA	1:800	1.98 median OD	Known positive control	NA	[59]
Healthy blood donors	34	NA	NA	ELISA using whole serum or purified IgG	From 1:20 to 1:50	NA	Above 2 standard deviations from ODs at 405 nm of the sera from healthy donors	1 (2.9%)	[72]
Healthy blood donors	40	24:16	37 $\pm$ 14	ELISA	From 1:20 to 1:160	NA	2.5 times the background optical density	3 (7.5%)	[28]
Healthy blood donors	49	38:11	50 $\pm$ 11.1 in 29 Japanese controls and 47 $\pm$ 12 in 20 Swedish controls	ELISA	From 1:20 to 1:160	NA	2 times the background optical density	4 (8.2%)	[31]
Healthy volunteers	50	NA	NA	ELISA	NA	None of the controls were sero-positive.	Values of O.D. above two SD of those of normal individuals	0	[40]
Healthy volunteers	70	35:35	35 $\pm$ 4.2	ELISA	NA	None of the controls were sero-positive.	OD=0.240	0	[45]
Healthy controls	104	82:22	56 $\pm$ 15	ELISA using whole serum or purified IgG	Beginning at 1:20	Whole sera titer 47.5 $\pm$ 28.2, purified IgG titer 50.0 $\pm$ 26.2	Optical density that was 2.5 times the background density	8 (8%) both in whole sera and purified IgG	[33]
Healthy blood donors	408	231:177	36.6 $\pm$ 21.5	ELISA	Graded dilutions from 1:20	Geometric mean of Ab titer in Abs-positive subjects 1:51 $\pm$ 2.0	P/N>2.1	46 (11.3%)	[35]

Values are given in mean $\pm$ standard deviation, NA not available

Table 2 Summary of the current literature relating to the prevalence of anti-M2R AAbs in various clinical conditions ($n > 20$)

Clinical condition	Inclusion criteria	Included patients (n)	M:F	Mean age (years)	EF (%)	Anti-M2R measurement technique	Serum dilution	Results, Abs titer	Selected abnormal value	Percentage of abnormal results, n (%)	Ref.
DCM	Idiopathic	32	NA	59.4±8.6	37.8±15.4	ELISA	From 1:20 to 1:160	NA	2 times the background optical density	6 (18.75%)	[31]
	Idiopathic. Lack of ischemic heart disease, valvular disease, and hypertrophic cardiomyopathy. Fulfillment of WHO/ISFC criteria for DCM.	36	22:14	54±16	27±11	ELISA	From 1:20 to 1:160	NA	2.5 times the background optical density	14 (38.8%)	[28]
		53	34:19	55.7±15.5	31.9±12.2	ELISA	Graded dilutions from 1:20	Geometric mean of Abs titer in Abs-positive subjects 1:212.2±2.7	P/N>2.1	27 (50.9%)	[35]
	Clinically stable using conventional therapy. EF<45%. Lack of >75% stenosis of the major coronary arteries, myocarditis or known specific CM.	104	82:22	56±14	31±10	ELISA using whole serum or purified IgG	Beginning at 1:20	Whole sera AAbs titer 154.6±71.3, purified IgG anti-M2R AAbs titer 184.8±89.6	Optical density that was 2.5 times the background density	42 (40%) using whole sera, 37 (36%) using purified IgG.	[33]
Chagas disease	Positive <i>T. cruzi</i> serology, asymptomatic patients, normal ECG and chest X-ray, with autonomic nervous system dysfunction	36	NA	NA	NA	ELISA	NA	Most patients were sero-positive.	Values of O.D. above two SD of those of normal individuals	32 (88.8%)	[40]
	Positive <i>T. cruzi</i> serology, asymptomatic patients with normal autonomic nervous system response	48	26:22	37±6.2	67±6.5	ELISA	NA	Sero-positivity rates were lower than those of patients with dysautonomia	OD=0.240	15 (31.5%)	[45]
	Positive <i>T. cruzi</i> serology, asymptomatic patients, normal ECG and chest X-ray, with normal autonomic nervous system response	49	NA	NA	NA	ELISA	NA	Sero-positivity rates were lower than those of patients with dysautonomia	Values of O.D. above two SD of those of normal individuals	13 (26.5%)	[40]
	Positive <i>T. cruzi</i> serology, asymptomatic patients with autonomic nervous system dysfunction	65	34:31	34±7.1	69±7.1	ELISA	NA	Almost all patients were sero-positive.	OD=0.240	64 (98.46%)	[45]
	Chronically infected patients	66	NA	NA	NA	ELISA using whole serum or purified IgG	From 1:20 to 1:50	NA	Above 2 standard deviations from ODs at	62 (93.9%)	[72]

Table 2 (continued)

Clinical condition	Inclusion criteria	Included patients (n)	M:F	Mean age (years)	EF (%)	Anti-M2R measurement technique	Serum dilution	Results, Abs titer	Selected abnormal value	Percentage of abnormal results, n (%)	Ref.
									405 nm of the sera from healthy donors		
	At least 2 different tests confirming <i>T. cruzi</i> infection.	75 patients: 45 normal LV function. 30 LV dysfunction	39:36	41±8 for normal LV group, 44±10 for LV dysfunction group	64±5 for normal LV group, 51±15 for LV dysfunction group	ELISA	1:800	2.76 median OD for normal LV group, 2.72 median OD for LV dysfunction group	Known positive control	NA	[59]
HCM	Echocardiographic diagnosis	23	22:1	56.8±17.6	78.5±8.7	ELISA	From 1:20 to 1:160	NA	2 times the background optical density	4 (17.4%)	[31]
SVA	Graves' hyper-thyroidism with AF	17	8:9	60.9±12.7	51.6±16.1	Contractility bioassay	1:100	NA	Isoproterenol (10 nmol/L) served as positive controls. IgG from healthy donors served as negative controls.	15 (88%)	[71]
	Idiopathic AF	104	82:22	57±14	NA	ELISA using whole serum or purified IgG	beginning at 1:20	Whole sera titer 78.3±30.0, purified IgG titer 83.3±31.0	Optical density that was 2.5 times the background dens	24 (23%) using whole sera, 24 (23%) using purified IgG.	[33]
SND	Primary (n=4) or caused by Chagas heart disease (n=9)	13	4:9	Mean 45.4	NA	Functional assay	NA	NA	Addition of patients' IgG led to a significant reduction in the cultured cardio-myocytes beating rate (compared with the control IgG) and the effect was antagonized by atropine	10 (76.9%)	[55]

Values are given in mean±standard deviation

NA is not-available, DCM dilated cardiomyopathy, SVA supraventricular arrhythmias, SND sinus node dysfunction, AF atrial fibrillation, HCM hypertrophic cardiomyopathy, and LV left ventricular.

mediated tissue damage by the induction of apoptosis, complement activation, or triggering of cell-mediated cytotoxicity. The presence of AAbs is the hallmark of most autoimmune diseases, although the functionality of some AAbs remains unknown [12, 13]. AAbs may be associated with autoimmune disease activity [14]. Anti-heart autoantibodies (AHA) can be extracted from the sera of patients with various heart diseases, although low titers have been found in healthy individuals as well [15]. AHA reacts with different myocardial tissue components such as receptors, contractile proteins, and other intracellular antigens [2, 16, 17]. Nevertheless, the role of AHA in the pathogenesis of cardiac diseases remains unknown [18]. M2 muscarinic acetylcholine receptor (M2R) is the main muscarinic receptor present on myocardial tissue [19], belonging to a family of G protein-coupled receptors [20]. Muscarinic receptors were identified on the sarcolemma and T-tubules of rat cardiomyocytes [21]. Activation of the M2R results in cAMP-dependent and cAMP-independent intracellular mechanisms: activation of K^+ channels, decrease of cAMP production and increase in cGMP production, as well as stimulation of phosphatidylinositol turnover [22–24].

In 1990, Paaerez Leiros et al. reported that the IgG fraction from animals with experimental myocarditis caused an effect similar to that of cholinergic agonists, diminished the reactivity of exogenous cholinergic agonists and inhibited radioligand binding to membranous muscarinic receptors [25]. It was also reported that an interaction between IgG from *Trypanosoma cruzi*-infected mice or the corresponding F(ab')₂ fragments and cardiac cholinergic receptors resulted in cholinergic signal activation and reduction of atrial contractility [26, 27]. In 1993, Fu et al. were the first to report the presence of autoreactive AAbs versus a peptide corresponding to the sequence of the second extracellular loop of the human M2R in DCM patients [28]. They found that 38.8% of sera taken from patients with DCM reacted with the peptide, compared with 7.5% in healthy controls [28]. The sequence of amino acids 169–193 of the second extracellular loop were chosen for sera screening because of its suggested immunological and functional properties [28].

There have also been reports of interaction between chronic chagasic patients' AAbs (which may contain anti-M2R-AAbs) and the central part of the third intracellular loop of M2R [11, 29]. It was found that anti-M2R-AAbs do not interact with the ligand-binding site of M2R [30] and suggested that an initial autoimmune response is triggered against the second extracellular loop of the M2R (and possibly against other myocardial antigens as well). The subsequent tissue damage causes exposure of other antigens previously hidden from the immune system (such as the central part of the third

intracellular loop of M2R) [11] and autoimmune response against such antigens.

Anti-M2R-AAbs have been identified in different cardiovascular conditions such as DCM, Chagas heart disease (ChHD), various arrhythmic disorders, as well as in healthy individuals (Tables 1 and 2). Nevertheless, Fu et al. found that patients with malignant essential and malignant secondary hypertension did not have circulating anti-M2R-AAbs [31]. It has been reported that anti-M2R-AAbs rates may be present in 0–11.3% of healthy individuals [28, 32–35]. Liu et al. included the biggest cohort of healthy individuals ($n=408$) and found that anti-M2R-AAbs were present in low titers in 11.3% of the controls [35]. The significance of the presence of anti-M2R-AAbs remains to be investigated.

Effects of Anti-M2 Muscarinic Receptor Autoantibodies on Cardiomyocytes

Anti-M2R-AAbs behave as positive allosteric modulators of M2R in vivo, with partial agonist activity [20, 36, 37]. Purified anti-M2R-AAbs extracted from sera of patients with DCM had a negative chronotropic effect on incubated neonatal rat cardiomyocytes' contraction rate by means of a dose-dependent relationship [38]. These findings were also supported by in vivo rat experimentation [39]. The negative chronotropic effect was rapidly reversed after 1 μ M atropine was added to the medium [38]. In contrast to other muscarinic agonists (i.e., carbachol), the effect of anti-M2R-AAbs is not subjected to relatively rapid desensitization and does not cause internalization of the muscarinic receptors in a short period of time [38]. Nevertheless, these results were contradicted by other studies in which desensitization of M2R was achieved following chagasic IgGs administration (which frequently contains anti-M2R-AAbs), following prolonged receptor stimulation [20, 30]. Immunization of rabbits with M2R resulted in muscarinic receptor up-regulation but without a change in the equilibrium dissociation constants [29]. Moreover, the AAbs diminished the effects of exogenous carbachol. It has been suggested that chronic interaction between M2R and AAbs might cause a blockage of the cardiac parasympathetic modulation [40]. In contrast to the last statement, in vivo experimentation with mice immunized against the N terminus part of the second extracellular loop of the M2R, who developed anti-M2R-AAbs was conducted. It was found that the immunized mice had a significantly higher degree of bradycardia following carbachol administration [20]. This discrepancy is not easily explained and may be due to methodological differences (in vivo in the later study [20] compared with in vitro [40] and the source of anti-M2R-AAbs: immunization with M2R peptide [20] vs.

using a purified IgG fraction of human chagasic patients [40]). Interestingly, it was also demonstrated that the negative chronotropic effects of anti-M2R-AAbs can be aborted following administration of peptide corresponding to the second extracellular loop of M2R [41].

The negative chronotropic effects of anti-M2R-AAbs results from the inhibitory effect on L-type calcium current. An increase of outward acetylcholine (ACh)-regulated potassium current ($I_{K,ACh}$) due to anti-M2R-AAbs, causes hyperpolarization as well as shortening of the action potential duration and effective refractory period [19, 36, 42].

Nascimento et al. found that when monoclonal antibodies were directed against the second extracellular loop of M2R (i.e., B8E5), the isoproterenol-stimulated L-type calcium current was reduced; however, the basal current was not significantly affected [43]. Therefore, it may be concluded that anti-M2R-AAbs are capable of limiting the effectiveness of the endogenous agonist. Additionally, anti-M2R-AAbs reduced the cytoplasmic levels of the cyclic AMP induced by the β -agonist isoprenaline to neonatal rat cardiomyocytes [41]. Similar findings were reported by Wang et al., who also noted that the anti-M2R-AAbs effect was prevented by atropine [44]. Increase of the intracellular cyclic GMP was also observed due to anti-M2R-AAbs [40]. Other studies have suggested that inhibition of the β -agonist-stimulated L-type calcium current by AAbs-mediated activation of M2R is mediated via increased intracellular cyclic GMP and not via decreasing intracellular cyclic AMP [43].

Ganzinelli et al. proposed that anti-M2R-AAbs are capable of inducing positive regulation of COX-2 and iNOS mRNA and subsequent proinflammatory response with increased serum levels of PGE2 and NO in some patients [45].

Anti-M2 Muscarinic Receptor Autoantibodies in Dilated Cardiomyopathy

DCM is characterized by systolic dysfunction and chamber dilatation of the left heart or both left and right ventricles [34, 46]. DCM may be caused by various triggers such as infections, toxins, alcohol, inflammatory conditions, metabolic abnormalities, and neuromuscular diseases [4, 15]. Additionally, patients with autoimmune rheumatic disorders (e.g., systemic sclerosis, systemic lupus erythematosus, and dermatomyositis) may also develop DCM [4]. Familial DCM has been described as well [34]. Miettinen et al. estimated that approximately 50% of DCM cases remain idiopathic [47]. Autoimmune mechanisms seem to play a major role in the pathogenesis of certain DCM cases [4, 48–50].

A co-occurrence of circulating anti-M2R-AAbs and anti- β 1-adrenergic receptor autoantibodies (anti- β 1AR-AAbs) in various cardiac conditions was reported to be 25% to 80% [20, 35]. Anti- β 1AR-Abs act as allosteric regulators of receptor activity, causing agonist and partial-agonist (observed in humans) or inverse-agonist (observed in animals) effects on the adrenergic receptor by affecting ligand binding and altering receptor conformation [51–54]. It was suggested that the overall influence of these AAbs depends not only on their concentrations but also on the relative density of specific receptors on different heart tissues [55].

Anti-M2R-AAbs have been reported in 18–51% of patients with idiopathic DCM [19, 20, 29, 31, 35, 38, 41]. Anti- β 1AR-AAbs are also common in DCM and have a 100 times higher affinity to the adrenergic receptor than anti-M2R-AAbs has to the M2R [41]. Nevertheless, anti-M2R-AAbs and anti- β 1AR-AAbs have entirely different functional effects, and their long-term co-existence provides an unclear net effect [49]. It was suggested that immunological homeostasis was achieved due to anti-M2R-AAbs activity, compensating for the sympathetic drive induced by anti- β 1AR-AAbs [20]. Nevertheless, further research is necessary in order to investigate the mutual effects of both AAbs [20].

Transfer of AAbs and peripheral blood lymphocytes from rabbits immunized with combined β 1AR and M2R peptides reproduced autoimmune-mediated early stages of cardiomyopathic damage in severe combined immunodeficient mice [49]. Matsui et al. found that monthly immunization of rabbits for 1 year, with synthetic peptides corresponding to the sequence of the second extracellular loop of either the human β 1AR or M2R, induced cardiac morphological changes including significantly enlarged ventricles and thinner walls, resembling those of DCM in humans [56]. In rabbits immunized against M2R, the right ventricle was mainly affected [56]. Histologically, rabbit IgG was localized on the sarcolemma of cardiomyocytes and CD4/CD8 T-cells. Macrophages were also recognized in the myocardium [56, 57]. Furthermore, monthly immunization of rabbits with both antigens resulted in high circulating levels of anti-M2R-AAbs and anti- β 1AR-AAbs and cardiomyopathic changes [57]. The hearts of immunized rabbits exhibited thickening of the left ventricular wall and interventricular septum, as well as myocardial cell hypertrophy and a mild inflammatory reaction [57]. Of the ten immunized rabbits, two died suddenly and displayed an extensive infiltration of inflammatory cells to the myocardial tissue, cardiomyocyte degeneration, and necrosis. Nevertheless, histological changes were mild in rabbits who received simultaneous immunization with both antigens, compared with the histopathological changes observed in rabbits immunized with either antigen [57].

Therefore, it is possible that the presence of both AAbs contradicts the effects caused by each type of AAb separately.

Others have attempted mice immunization by using plasmid DNA coding for the entire human β 1AR or M2R proteins [29]. Immunization resulted in a mixed Th1/Th2 immune response, elevation of AAb directed against the second extracellular loop, in contractile dysfunction, and cardiac remodeling in both groups [29].

Nevertheless, the contribution of anti- β 1AR-AAbs and anti-M2R-AAbs to the pathogenesis of DCM in humans and the influence of such AAbs on prognosis is still unclear.

Anti-M2 Muscarinic Receptor Autoantibodies in Chagas Heart Disease

ChHD appears following a parasitic infection with *T. cruzi* [41]. It is the most common cause of cardiomyopathy and cardiovascular death in young patients living in endemic regions of South and Central America [58], with 30% of infected individuals ultimately developing cardiomyopathy [59]. Ventricular tachyarrhythmia, bradyarrhythmias, and sinus node dysfunction are common in patients with ChHD [58]. Staging of ChHD is based on electrocardiographic and echocardiographic findings [11]. Several pathogenic mechanisms have been proposed, including persistent parasitic infection, autoimmune reaction, and microvascular dysfunction [60]. Nevertheless, very low levels of parasites in chronic stages and the presence of myocardial infiltration by immune cells, support the general belief that autoimmunity is the main cause of ChHD [40]. Both autoreactive mononuclear cells and AAbs have been found in ChHD [11, 45].

Anti-M2R-AAbs are present in about 40–77% of ChHD patients [19, 41]. However, in specific ChHD subgroups, AAbs-positivity rate may increase up to almost 100% [45] or decrease to 26.5% [40]. Similar to DCM, anti-M2R-AAbs may be simultaneously recognized with anti- β 1AR-AAbs in patients with ChHD [58]. Nevertheless, the titer of these AAbs does not correlate with the severity of ChHD and the extent of contractile dysfunction [60]. Unlike DCM, the affinity of anti-M2R-AAbs and anti- β 1AR-AAbs to their receptor appears to be similar in ChHD [41]. The difference in reactivity and affinity of anti- β 1AR-AAbs to β 1AR [41] may account for this disparity. Importantly, most patients with ECG abnormalities (with or without heart failure) had had circulating AAbs reactive with the third intracellular portion M2R [11]. This correlation was not observed in the majority of patients without ECG abnormalities [11]. Interestingly, antibodies against the C terminus of the parasitic TcP2 β ribosomal protein (e.g.,

anti-R13 antibodies) are capable of activating either M2R or β 1AR, thus providing evidence that the same antigen can cause an immune response against two different membranous receptors with different activities, probably due to different possible peptide conformations [58]. A correlation was found between the presence of anti-M2R-AAbs in chagasic dysautonomic patients and serum levels of PGE2 [45].

Higher levels of anti-M2R-AAbs were found in ChHD patients with chronotropic incompetence during exercise testing [60]. The presence of anti-M2R-AAbs and anti- β 1AR-AAbs with apparently similar affinity might provide an explanation for the occurrence of both ventricular tachyarrhythmias and bradyarrhythmias in ChHD patients [55].

Cardiac vagal dysfunction in ChHD is usually asymptomatic. Vagal impairment may occur early in patients with Chagas disease and is independent to the development of contractile dysfunction [59]. It is associated with altered heart rate variability modulation but not with the change of vagal tonus. The clinical implication of vagal dysfunction on disease progression and outcome remains unknown [61].

Anti-M2 Muscarinic Receptor Autoantibodies in Arrhythmic Disorders

AHA, in general, were found in several heart diseases associated with arrhythmic tendency [55]. Anti-M2R-AAbs, in particular, has been reported in various primary arrhythmic disorders such as idiopathic sinus node dysfunction, idiopathic atrial fibrillation, and idiopathic ventricular tachycardia [19].

Bradyarrhythmias

Anti-M2R-AAbs seems to have a direct effect on the sinus node function [33] and is rarely found in people with normal sinus node function [55]. De Oliveira et al. demonstrated that sera and IgG from rabbits infected with *T. cruzi* caused sinus bradycardia and even third degree atrioventricular (AV) block in non-infected rabbits [62]. Sera extracted from ChHD patients and complex cardiac arrhythmias caused AV conduction blocks in isolated rabbit's hearts [19, 59, 62].

A study performed in 1997 compared nine patients with sinus node dysfunction (either due to ChHD or idiopathic) with 17 patients with normal sinus node function. It was found that 7/9 patients had AAbs with a cholinergic effect compared with 2/17 in the normal sinus node function [63].

ChHD patients who also developed sinus node dysfunction were especially prone for positive anti-M2R-AAbs (about

78% were positive for anti-M2R-AAbs) [19, 55], which is highly suggestive of a cause and effect relationship.

Anti-M2R-AAbs have also been reported in up to 75% of patients with primary sinus node dysfunction in small-scale studies [19, 55].

Atrial Fibrillation

Atrial Fibrillation (AF) is one of the most common types of arrhythmia and is commonly encountered in patients with heart failure [33]. Supraventricular premature contractions appeared shortly after adding purified anti-M2R-AAbs (extracted from patients with DCM and idiopathic AF) to chick embryos [19, 33]. It was speculated that anti-M2R-AAbs, which are relatively common in DCM patients, contributes to the high occurrence of AF in the same patient population [36]. Accordingly, DCM patients, positive for anti-M2R-AAbs, developed AF more often than DCM patients without circulating anti-M2R-AAbs (40% vs. 18%, respectively) [36]. Interestingly, atrial premature contractions in chick embryos following purified anti-M2R-AAbs were no longer detected when administered atropine or peptides corresponding to the epitope of the AAbs [33].

Other studies have found that, in addition to advanced age and increased plasma levels of atrial natriuretic peptide, the presence of anti-M2R-AAbs was found to be the strongest independent risk factor for AF in DCM patients [19, 33, 36, 42]. Researchers have suggested that anti-M2R-AAbs are capable of altering the atrial electrophysiological properties.

Interestingly, anti-M2R-AAbs has been reported in about 25% of idiopathic AF patients [19, 33], which might reflect autoimmune involvement in idiopathic proarrhythmogenic disease.

It was suggested that the increase of the $I_{K_{ACh}}$ current due to anti-M2R-AAbs shortens refractoriness and increases conduction velocity in the atrium, thereby raising risk of AF [19]. It is noteworthy that the $I_{K_{ACh}}$ current is continuously active in chronic AF patients [33]. Moreover, AF was not induced following M2R stimulation in mice with knock-out $I_{K_{ACh}}$ channel gene [36], indicating the importance of ACh-regulated potassium current in inducing AF following cholinergic activation. Additional experiments were conducted on the atria of rabbits immunized against a peptide, corresponding to the second extracellular loop of the rabbit M2R. It was further demonstrated that the presence of anti-M2R-AAbs is associated with longer intra-atrial activation times, shorter atrial effective refractory periods, and significantly greater atrial arrhythmogenicity compared with control rabbits [36]. Anti-M2R-AAbs was also associated with atrial fibrosis, which further decreases the atrial conduction velocity and increases the risk for AF [36].

Ventricular Arrhythmias

Anti-M2R-AAbs has been reported in about 40% of idiopathic VT patients [19]. In contrast, other studies describing patients with ventricular arrhythmias (VA; 14 patients with primary VA, nine with ChHD, and five with idiopathic DCM), 75% were positive for anti- β 1AR-AAbs, while only 10.7% were positive for anti-M2R-AAbs [55]. Chiale et al. did not identify anti-M2R-AAbs in 21 patients with inappropriate sinus tachycardia (but found a high prevalence of anti- β 1AR-AAbs) [64]. It is possible that the occurrence of VA in patients positive for anti-M2R-AAbs is due to the coexistence of anti- β 1AR-AAbs in the sera or due to other unknown mechanisms.

Repolarization Abnormalities

ChHD patients positive for muscarinic activity, due to the presence of circulating anti-M2R-AAbs, had higher QT dispersion (QTd) parameters [19, 65]. QTd is an electrocardiographic marker of repolarization heterogeneity associated with higher rates of ventricular arrhythmias and sudden death in some patients [66, 67]. Additionally, sera from ChHD patients with muscarinic activity caused an increase in QT interval length in isolated rabbits' hearts [65]. The effect on QT was reversed following the addition of atropine [65].

No electrophysiological rationalization for anti-M2R-AAbs-induced QT interval prolongation and QTd prolongation is currently available [19].

Anti-M2 Muscarinic Receptor Autoantibody-Directed Therapy

In various studies, the negative chronotropic effects of anti-M2R-AAbs were reversed following the addition of atropine [38, 55]. It is therefore possible that patients with bradyarrhythmias due to anti-M2R-AAbs will benefit from cholinergic-antagonists. Nevertheless, to the best of our knowledge, no prospective studies in humans has been attempted to validate this hypothesis.

Immunoadsorption (IA) is a process where IgG Abs are adsorbed from the patient's sera. IA may play a potential role as an immune-modulating therapy in various indications and in immune-mediated heart diseases [68]. It is plausible that some of the apparent beneficial effects of IA in heart disease results from the elimination of anti-M2R-AAbs. It has been suggested that IA may be beneficial in AF patients positive for anti-M2R-AAbs [42], although no trials have as yet been conducted to test this hypothesis. Recently, Baba et al. reported that completion of IA was

beneficial in a small group of heart failure patients with circulating anti- β 1AR-AAbs and/or anti-M2R-AAbs, further supporting the need for future investigation [69].

Ganzinelli et al. demonstrated in a mice model of ChHD that infusion of M2R peptide is capable of reducing myocardial inflammation foci and improve contractile dysfunction [45]. Therefore, another possible therapeutic approach could be infusion of peptides which would bind to anti-M2R-AAbs and prevent the AAbs from interacting with the myocardial tissue. Nevertheless, at this time, this approach remains theoretical.

Intravenous immunoglobulins (IVIG) are gamma globulins, mostly consisting of IgG. IVIG is produced from the pooled plasma of thousands of healthy donors [3, 70] and has emerged as a possible therapy in many immune-mediated and non-immune indications [70]. Importantly, IVIG has anti-idiotypes properties against AAbs, also inhibiting Fc-receptor-mediated activity [3]. Nevertheless, the use of IVIG in the setting of anti-M2R-AAbs-positive patients with cardiovascular diseases remains to be investigated as well.

Conclusions and Future Perspectives

Although anti-M2R-AAbs were detected in various cardiac conditions, the reason for the development of such AAbs and their contribution to the pathophysiology in humans remains largely unknown. The consequences of anti-M2R-AAbs in patients who also develop anti- β 1AR-AAbs remains to be explored, since experimentation with animals has suggested that the presence of both anti-M2R-AAbs and anti- β 1AR-AAbs may cause immunological homeostasis. Additionally, targeted therapy against anti-M2R-AAbs is an appealing possible therapeutic approach towards treatment of AF in DCM, and treatment of sinus node dysfunction in particular. Nevertheless, it remains to be investigated who will benefit from such therapeutic strategy.

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