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AUTOANTIBODIES AND CARDIOVASCULAR DYSFUNCTION: CAUSE OR CONSEQUENCE?

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Abstract

There has been a long history of the exploration into autoimmunity as possible pathogenic factor of cardiovascular diseases from unknown cause represented by dilated cardiomyopathy (DCM). Auto-antibodies (AAbs) have emerged either as humoral responses provoked by release of "self-antigens" due to tissue damage or dysregulated humoral immunity itself. The pathogenic roles of some AAbs have been suggested by the findings from basic research using *in vitro* and *in vivo* disease model as well as clinical studies including immunoadsorption studies removing AAbs from patients with DCM. In this context, the importance of AAbs belonging to IgG3 subclass has also been implicated. In this review article we summarize the findings accumulated to date regarding AAbs which have been considered to be involved in the pathology of DCM or pregnancy-related cardiovascular disease. Furthermore, we discuss the significance of AAbs as a possible cause of DCM and the potential roles as novel therapeutic target.

Keywords

autoantibody; myosin; β_1 adrenergic receptor; muscarinic M2 acetylcholine receptor; troponin I; dilated cardiomyopathy; peripartum cardiomyopathy; preeclampsia

Introduction

Dilated cardiomyopathy (DCM) is one of the most common causes of heart failure without an ischemic etiology and the most common reason for heart transplantation. In many cases, the underlying causes of DCM are unknown or "idiopathic" and may affect individuals across all ages. There has been a long-standing interest in exploring the contribution of autoimmunity towards the pathogenesis of DCM. This is based on the presence of anti-

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Compliance with Ethics Guidelines

Conflict of Interest

Yuji Nagatomo and W. H. Wilson Tang declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

cardiac auto-antibodies (AAbs) in a subset of patients with DCM. It has been postulated that myocardial tissue damage may lead to the release of intracellular proteins that can serve as "self-antigens" in order to provoke humoral responses leading to the generation of AAbs. On the other hand, dysregulated humoral immunity itself can serve as the primary driver of AAb production, directly contributing to progressive myocyte damage as observed in some systemic autoimmune disorders associated with cardiac complications (e.g. systemic lupus erythematosus).

Over the past decades, researchers have investigated the direct physiological role of AAbs via basic *in vitro* or *in vivo* experiments, as well as via modulation of their effects by removal or neutralization. In this review article, we critically examine the contemporary understanding of specific AAbs that have been mechanistically linked to the pathogenesis of DCM with emphasis on the discussion of how quantitative AAb measurements may lead to potential therapeutic implications.

Dilated Cardiomyopathy: A Possible Autoimmune Origin

Several cardiac AAbs have been consistently reported to be present in sera from patients with DCM [1–3]. However, such associations do not necessarily establish causality, especially when the acuity, time course, and localization of autoimmune responses are largely unknown. Earlier research focus was based upon establishing the association between introduction of AAbs and induction of DCM phenotypes. Indeed, immunization with non-cardiac peptides such as β_1-adrenergic receptor (β_1AR) second extracellular loop [4,5] or muscarinic M_2 acetylcholine receptor (M_2R) [6,7], as well as cardiac-specific peptides such as myosin [8] or troponin I [9] can directly lead to the generation of AAbs and myocarditis- or DCM-like phenotype in experimental animals. These findings support the development of AAbs upon exposure to •self-antigens•, thereby establishing the first step for specific AAbs as contributors in the development of DCM. Clinical and translational research studies regarding these specific AAbs are illustrated in Table 1.

Anti-myosin Autoantibody

The anti-myosin AAb has long been studied for its causal roles in the pathology of myocarditis or DCM. In 1987, immunization with cardiac myosin was found to induce anti-myosin AAbs and myocarditis in certain strains of mice [8]. However, since transfer of serum with high titer anti-myosin AAbs from C.B-17 mice to SCID (severe combined immune deficiency) mice failed to cause myocarditis [10], the pathogenic role of anti-myosin AAbs was questioned by some researchers. It has been proposed that myosin or a similar protein was present in the extracellular matrix of susceptible mouse strains [11]. And the pathogenic effects of anti-myosin AAbs was mediated at least partly by reacting with β-adrenergic receptor and activating downstream protein kinase A pathway [12]. In human, anti-myosin AAbs are detected in 20–30% of patients with DCM and 4–30% in those with ICM [13,14]. However, the clinical findings regarding the significance of anti-myosin AAbs are inconsistent. One study showed that persistence of anti-myosin AAb was associated with milder symptoms at presentation and stable disease [13]. Whereas anti-myosin AAbs were also shown to associate with deterioration of left ventricular function in patients with biopsy-proven chronic myocarditis [15].

Autoantibody against β_1-Adrenergic Receptor (β_1AR-AAb)

Detectable circulating AAbs against β_1AR have been observed in approximately 30–40% of patients with chronic heart failure due to DCM [4,16–19]. β_1AR-AAb shows agonist-like effects [20–23], inducing receptor uncoupling [4,24,25], myocyte apoptosis [26], sustained calcium influx resulting in electric instability of the heart [27], and persistent myocardial damage [5]. These effects were abolished by β-blockers *in vitro*[23,28] and *in vivo* [4]. There have also been prior reports demonstrating the association between detectable β_1AR-AAb and increased mortality [28] as well as the occurrence of fatal ventricular arrhythmias and sudden death [4,29] in patients with DCM. However, a majority of the subjects in these association studies were not receiving anti-adrenergic therapy at the time. Interestingly, more favorable recovery of cardiac performance in response to β-blocker therapy was observed in β_1AR-AAb-positive patients compared to β_1AR-AAb-negative patients [30]. These findings are consistent with the hypothesis that heightened adrenergic drive associated with β_1AR-AAb generation in DCM can lead to adverse cardiac consequences. Besides anti-adrenergic therapy, studies are currently ongoing to determine if an aptamer for neutralizing β_1AR-AAbs may curtail disease progression and perhaps even facilitate recovery [31].

It is important to note that not all of the detectable β_1AR-AAbs uniformly exerts their adverse physiological effects across the spectrum of clinical conditions. While β_1AR-AAbs were also detectable in patients with valvular or hypertensive heart disease (or even in some healthy subjects), AAbs in these non-DCM individuals were functionally inactive [32,33]. Furthermore, the presence of β_1AR-AAb was consistently associated with increased mortality risk largely in DCM and not in ischemic cardiomyopathy [28]. Evidence also suggests that some β_1AR-AAbs can be generated, at least partly, by cardiac loading or damage. In an analysis of weaned DCM patients who tested positive for β_1AR-AAb prior to implantation of left ventricular assist device (LVAD), β_1AR-AAb disappeared after 3 to 31 weeks following LV unloading by LVAD support in 33 of 34 patients [34]. The potential mechanism of these inconsistencies has not yet been elucidated, and further studies are needed.

Autoantibody against Muscarinic M2 Acetylcholine Receptor

Circulating AAbs against the second extracellular loop of M_2R (M_2R-AAb) have been detected in a large number of cardiovascular diseases such as DCM and chronic Chagas' disease [35–39]. M_2R-AAbs extracted from DCM patients induced a significant decrease in Ca^{2+} currents [39], negative chronotropic effect *in vitro* [37] and supraventricular arrhythmia *ex vivo* [38] which were blocked by M_2 antagonist [38,39] or a synthetic peptide derived from the second extracellular loop of M_2R [38]. M_2R-AAbs were also detected along with the induction of DCM-like morphology in the heart of mice receiving adoptive transfer of splenocytes from M_2R null mice immunized with synthetic M_2R peptide [40]. In addition, AAbs from these mice induced a significant decrease in Ca^{2+} currents in ventricular cardiomyocytes, which was blocked by M_2R antagonists or synthetic M_2R peptides [40]. Clinically the presence of M_2R-AAbs was strongly and independently associated with the comorbidity of atrial fibrillation [38], and may serve as an independent predictor for the recurrence of atrial fibrillation after catheter ablation [41].

Autoantibody against Troponin I

Monoclonal antibodies against cardiac troponin I (cTnI) induce chamber dilatation and contractile dysfunction of hearts of wild-type mice [9]. Interestingly, the immunization of cTnI, but not cardiac troponin T, induced severe autoimmune inflammation in the mouse myocardium [42]. Monoclonal cTnI-AAbs extracted from mice following immunization with recombinant cTnI are able to increase the voltage-dependent L-type calcium current in cardiomyocytes of wild-type mice [9].

Despite promising data regarding the effects of cTnI-AAbs in animal models, the pathogenic role of cTnI-AAbs in humans and their possible involvement in the pathogenesis of DCM remains controversial. Although the cTnI-AAbs were reported to be detected in DCM and ICM patients [43,44], IgG preparations reactive towards cTnI did not exhibit measurable effects on Ca^{2+} transients in cultured neonatal rat ventricular cardiomyocytes, nor did they bind the respective cells by direct immunofluorescence [43]. When examining cellular autoimmune responses from peripheral blood mononuclear cells, there were no significant differences between DCM patients with versus without detectable cTnI-AAb levels [45]. Furthermore, in the analysis of 95 patients with DCM, circulating cTnI-AAbs was not associated with patients' clinical status or outcome, while the presence of cTnI was associated with worse cardiac function, morphology, and worse clinical outcome [46]. In contrast, another study showed that cTnI-AAb positive patients had significantly higher left ventricular ejection fraction and more favorable outcomes than negative patients with DCM, but not ICM [44].

Data from Immunoadsorption Studies

Like other autoimmune disorders, the removal of pathogenic AAbs by immunoadsorption (IA) therapy can be considered as a therapeutic option for DCM patients. Clinical studies have consistently demonstrated that non-specific IA can lead to improved hemodynamics and cardiac function, better survival and quality of life in DCM patients [47–54] (Table 2). Specifically, IA using the column specific for β_1AR-AAbs was effective in alleviating the cardiac dysfunction in an observational series of patients with DCM [55,56]. However, many of these studies are small in sample size and few have blinding or control group comparisons. Moreover, it is also important to point out that non-specific IA was effective in both patients with β_1AR-AAbs and those without [57]. These findings suggest the possibility that the causal mechanism in the pathology of DCM cannot be explained solely by β_1AR-AAbs, and that other immune or non-immune factors may influence the effectiveness of IA in this population.

The potential contribution of cTnI-AAbs is less certain. In a small study, the presence of cTnI-AAbs was not associated with the effect of IA therapy which removes such AAbs. Despite being statistically insignificant, the study even showed a weaker effect of IA on the absolute change of LVEF after 6 months in cTnI-AAb positive patients [58].

"Cardio-depressant Autoantibody"

Some groups have shown that IgG antibodies purified from patients with DCM may induce a negative inotropic effect or reduction of calcium transient in adult rat cardiomyocytes [59,60] or chick embryo *ex vivo* systems [61,62]. Often referred to as the "cardio-depressant" AAb, the epitope(s) of such AAbs have not been identified. Interestingly, patients with cardio-depressant AAbs demonstrated an acute increase in cardiac index and improvement in left ventricular ejection fraction following IA therapy, while those without such AAbs did not show significant changes [60,62]. Nevertheless, cardio-depressant effects of these AAbs are unlikely to be induced by either the $F(ab')^2$ or Fc fragments alone. Reconstitution of the Fc part by incubation of cardiomyocytes with DCM-$F(ab')^2$ fragments followed by goat-anti-human-F(ab')-IgG again induced reduction of cell shortening and of calcium transients [63]. Taken together, these findings suggest that AAbs require Fc fragment binding to Fc receptor as well as $F(ab')^2$ fragments binding to the epitope peptide in order to exert its pathological effect, and hence the effects of the AAb vary depending on the structure of the Fc fragment.

The Significance of IgG3 Subclass AAbs

IgG has 4 subclasses and they differ from one another both immunologically and functionally. Antibodies capable of triggering effector functions and the most likely to be involved in immunoregulatory activities are IgG3 and IgG1. Complement activation through binding of C1q is most effective with IgG3 [64]. Affinity-purified IgG fractions positive for IgG3 anti-myosin AAb significantly inhibited the contraction of cardiomyocytes to a greater extent than those with non-IgG3 AAb *in vitro* [65]. In 1999, Warraich et al reported anti-myosin AAbs belonging to IgG3 subclass were selectively raised in patients with DCM compared with HF patients of an ischemic etiology [66]. Indices of hemodynamic dysfunction also significantly correlated with anti-myosin AAb titer belonging to IgG3, but not total IgG in patients with DCM [67]. AAbs against some other cardiac components belonging to the IgG3 subclass were also detected in patients with DCM and their presence were an independent predictor of the presence of cardio-depressant AAb [68]. The importance of IgG3 AAbs was supported when cardiac function was improved with IA via anti-human IgG columns (high affinity for all IgG subclasses) compared to using protein A (high affinity for IgG1, 2, and 4, but low affinity for IgG3) [69]. Column eluent from an anti-IgG column, but not from a protein A column exerted cardio-depressant effects on rat cardiomyocytes, whereas such cardio-depressant effects were abolished after removal of IgG3 subclass from eluent of an anti-IgG column [70]. Furthermore, IA using a protein A column with a modified protocol to effectively eliminate IgG3 subclass demonstrated better hemodynamic improvement in patients with DCM [71]. Previously, we performed an IA study for patients with refractory DCM by utilizing a tryptophan column. IA therapy removed IgG3 efficiently and to a greater extent than the other subclasses [54]. Left ventricular ejection fraction significantly increased after IA and such improvement in cardiac function correlated with the AAb titer belonging to IgG3 subclass more than total IgG, which also suggested that the removal of IgG3-AAb is important to maximize the effect of IA for the patients with DCM [62]. Therefore, AAb belonging to the IgG3 subclass may play a pivotal role in the onset and progression of cardiac dysfunction in new-onset

DCM and the therapeutic efficacy of IA. From these findings, anti-cardiac AAbs belonging to IgG3 subclass might be involved in the pathogenesis of DCM, but further research is needed to elucidate this issue.

Autoimmune Dysregulation as a Possible Cause of Pregnancy-related Cardiovascular Diseases

While most research has been focused on deciphering the cause versus consequence theories of AAb generation in the setting of DCM, the potential role of autoimmune dysregulation has also been extended to other at-risk clinical states. Recently, detection of AAbs in pregnancy-related cardiovascular diseases point to extension of this concept beyond dilated cardiomyopathy.

Peripartum Cardiomyopathy

Peripartum cardiomyopathy (PPCM) is a serious disease during pregnancy with unknown etiology. Because of its rarity the cause or pathology of the disease has not yet been fully investigated. A literature search only revealed a few published investigations which examined the correlation between autoimmunity and PPCM. However, the hypothesis of autoimmunity as a possible cause of PPCM is supported by the findings that AAbs against adenine nucleotide translocator, branched chain α-keto acid dehydrogenase and cardiac myosin were found to be detected in patients with PPCM [72]. Furthermore, the serum titer of these three antibodies was observed to be higher in patients with versus without PPCM [72]. Whereas the IgG3 subclass anti-myosin AAbs was predominant in DCM patients, AAbs detected in PPCM patients were subclass nonspecific. However, among these PPCM patients, the presence of AAbs belonging to IgG3 was still associated with higher NYHA functional class and heart rate [73]. Although some potential correlation between IgG3-antimyosin AAbs and the pathology of PPCM would be suspected, this study was cross-sectional in design and more longitudinal data will be needed to elucidate this issue. On the other hand, functionally active β_1AR-AAb was detected in PPCM patients as well [74]. Recently it was reported that β_1AR-AAb and M_2R-AAbs were detected in 60% and 46% of PPCM patients, respectively. The frequency and titer of both β_1AR-AAb and M_2R-AAbs had a positive correlation with left ventricular dimension, plasma NT-proBNP level, and NYHA functional class and a negative correlation with LVEF. After 12 months the titer and frequency of both AAbs significantly decreased [75]. Taken together, we can consider that the onset and course of PPCM accompanies some autoimmune activation, and it subsides in accordance with the amelioration of disease activity. However, further examination will be needed to elucidate the causal relationship between anti-cardiac AAbs and the pathology of PPCM.

Preeclampsia

Preeclampsia is characterized by proteinuria and hypertension during pregnancy. It is characterized by the presence of AAbs that bind to and activate the angiotensin II type1 receptor (AT_1R) [76]. AAbs against AT_1R (AT_1R-AAb) isolated from sera of preeclamptic women increased reactive oxygen species (ROS) production, the NADPH oxidase components [77], and intracellular Ca2+ concentration *in vitro* [78]. Interestingly, the

procedure of clipping ovarian arteries in pregnant rats to induce placental ischemia produced hypertension and proteinuria, representing the clinical features of preeclampsia [79]. A follow-up study found that AT_1R-AAb was produced in this rat preeclampsia model [80]. AT_1R-AAbs also were found to be detected in TNF-α infused pregnant rats, while non-pregnant rats did not produce AT_1R-AAbs [80]. Passive transfer of AT_1R-AAb extracted from preeclampsia patients induced hypertension and proteinuria in pregnant mice, but not in non-pregnant ones [81]. Collectively, AT_1R-AAb works as one of the pathogenic factors in preeclampsia. However, the detailed mechanism by which AT_1R-AAb is produced is still unknown.

Challenges in Clinical Translation

Despite these promising experimental findings, the applicability of AAbs testing and translation into clinical practice remains challenging. The first challenge is the issue of generalizability of findings, and the lack of broad clinical availability of testing. AAbs are largely measured in single-center, cross-sectional research studies (and often at a single-time point), in which autoimmune responses may differ among individuals or among geographic locations. In other words, their biological variability is largely unknown. The second challenge is the issue of availability and harmonization of laboratory assays. None of the AAb assays are clinically available; even measurements of AAbs against the same protein may vary widely, as they were developed by individual research laboratories without standardization. Finally, it has become increasingly clear that not all affected individuals have detectable AAbs. In addition, those with detectable AAbs may already have other established etiologies of heart failure, such as genetic/inherited or ischemic cardiomyopathies. It is therefore conceivable that pathogenic contributions of AAbs may only be applicable to a subset of susceptible individuals [32,33,82]. Whether targeting these subgroups may alter the natural history of the disease warrants further investigations.

Conclusions

AAbs can be produced in various diseases and conditions. Initially it had been considered to act as a diagnostic marker for DCM of autoimmune origin. However, some of these AAbs are physiologically active and can function as causal factors in the pathogenesis of multiple cardiovascular diseases (including DCM, PPCM, and preeclampsia), particularly of the IgG3 subclass. Hence, detection of AAbs might identify those who have autoimmune origins that may warrant the early initiation of conventional antagonists against their pathogenic effects (e.g. β-blockers for β_1AR-AAb). Promising novel therapies such as IA which removes AAbs or AAb-neutralizing agents are also under investigation, albeit only providing temporary relief through a more invasive mechanism. We believe these therapeutic tools targeting pathogenic AAbs can potentially benefit selected individuals suffering from their intractable conditions. Still, further examination is necessary in order to elucidate the roles of AAbs as diagnostic tool in order to establish AAb-targeted personalized medicine for the population.

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Table 1

Summary of Studies of AAbs in DCM

Epitope of AAb	Physiological effect on pathology	Reproduction of disease by passive transfer	Induction of disease by immunization of epitope	Association with phenotype or outcomes in human	Improvement of pathology after removal	Comments
Myosin	+[12]	±[10,11]*	+[8]	+[13,15]	?	*Transfer of AAbs causes myocarditis only in certain strains of mice [10,11]
β_1AR	+++[4,20–27,29] −[32,33]*	+[5]	+[4]	++[28,83]† [30]‡	±[57] +[31,55]	*AAbs from some patients are functionally inactive [32,33]. †Associated with ventricular arrhythmia, sudden death [83] and cardiovascular death[28]. ‡Associated with more favorable response to β-blocker[30]
M_2R	++[35,37–39]	*(See comments)	+[6,7]	+[38,41]†	?	*Reproduced by adoptive transfer into Rag2 knockout mice of splenocytes from M_2R knockout mice immunized with M_2R protein. †Association with Af comorbid with DCM and recurrent after ablation[38,41]
Troponin I	+[9]	+[9]	+[42]	−?[46] +[44]*	−?[58]	*Associated with higher LVEF and more favorable outcomes in DCM[44]
Na-K-ATPase	+[84]	?	+[85]	+[84]*	?	*Associated with ventricular arrhythmia and sudden death [84]
“Cardio-depressant AAb”	+[59,60,61]	?	?	?	+[60,62]	Epitope unknown

AAb, autoantibody; DCM, dilated cardiomyopathy; β_1AR, β_1-adrenergic receptor; M_2R, muscarinic M_2 acetylcholine receptor; Af, atrial fibrillation; LVEF, left ventricular ejection fraction.

Table 2

Summary of IA Studies for Human DCM

IA Column	IV/IG	Number of Patients	Main Findings / Comments	References
Anti-human IgG	+	9	Induced acute hemodynamic benefit. Five alive patients showed the increase of LVEF at 3 years. Two deteriorated patients showed the re-increase in β_1AR-AAb titer.	[47,51]
	+	34 (control 17)	Improved LVEF at 12 months No significant reincrease in β_1AR-AAb was seen during the 1-year follow-up	[49]
	+	18 (control 9)	Improved cardiac function and hemodynamics at 3 months	[48]
	+	45	CI and LVEF improved significantly only in patients with “cardio-depressant AAb”	[60]
	+	22	The similar effect between patients with β_1AR-AAb and those without it	[57]
Anti-human IgG or Protein A	+	18 (anti-human IgG 9, protein A 9)	Anti-human IgG was superior to standard protein A column in terms of elimination IgG3 subclass and improvement of LVEF	[70]
Protein A	+	18	Improved treatment regimen for IgG3 subclass elimination induced hemodynamic benefit.	[71]
	+	22	Improved cardiac function and hemodynamics at 6 months	[52]
	−	4	Improved QOL Insignificant improvement of cardiac function at 6 months	[53]
Tryptophan	−	16	Improved cardiac function at 3 month. The increase in LVEF correlated with “cardio-depressant AAbs” and anti-cardiac AAbs belonging to IgG3 subclass. No control group	[54,62]
Column specific for β_1AR-AAb	−	8	Improved cardiac function at 1 year No control group	[55,56]

IA, immunoadsorption; IV/IG, intravenous immunoglobulin administration; LVEF, left ventricular ejection fraction; β_1AR-AAb, autoantibody against β_1-adrenergic receptor; CI, cardiac index; QOL, quality of life.