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Identification of novel antigens contributing to autoimmunity in cardiovascular diseases☆

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ABSTRACT

In myocarditis and dilated cardiomyopathy (DCM) patients the immune system may play an important role in disease progression. In this study, we aimed to identify new antigens as a target for autoimmune response that might play a crucial role in these diseases. Therefore, a peptide-array was used to investigate antibody binding profiles in patients with autoimmune myocarditis or DCM compared to healthy controls and thus to identify disease relevant antigens. To analyze the pathogenicity of the identified antigens, an experimental autoimmune myocarditis (EAM) model was used. Hereby, 3 peptide sequences, derived from myosin-binding-protein-C (MYBPC) fast-type, RNA-binding-protein 20 (RBM20), and dystrophin, showed pathogenic effects on the myocardium of mice. In summary, 3 potentially cardiopathogenic peptides (MYBPC fast-type, RBM20, dystrophin) were identified. Thus, this study could serve as a basis for future investigations aimed at determining further antigens leading to pathogenic effects on the myocardium of DCM as well as myocarditis patients.

1. Introduction

Cardiovascular diseases remain a major worldwide health concern due to their high prevalence, and considerable morbidity and mortality [1]. Although autoimmune mechanisms are a well-established factor underlying a wide range of systemic and organ-specific disorders, their involvement in heart diseases has remained, until recently, largely understudied. Advances in the understanding of the pathogenesis of various cardiovascular disorders, however, have revealed the important role of autoimmunity in cardiac damage. Autoimmune processes are believed to result from local or diffuse inflammatory processes related to microbial infection or ischemic cardiomyocyte injury [2,3], explaining the frequently observed abnormalities in the immune system in myocarditis and dilated cardiomyopathy (DCM) patients [4–6]. In addition, the patient's genetic predisposition has been shown to determine the susceptibility to autoimmune reactions and the phenotypic disease expression [7,8].

Emerging evidence demonstrates that autoimmune damage in the context of many cardiovascular disorders is associated with the generation of functional antibodies against various proteins [9]. The constantly growing list of these antigens includes proteins associated with the sarcolemma (*e.g.* cardiac myosin, troponin I (TnI), laminins) [10–13], mitochondria [14], and membrane [15,16].

The contribution of distinct antigens to cardiovascular diseases and their progression has recently been reported [6,17]. The pathogenic role of these antigens is currently intensively investigated. In fact, studies in experimental animal models and clinical data have indicated the ability of a few antigens to induce an immune reaction, which may trigger cardiac dysfunction and heart failure. For instance, immune reactions directed against β1-adrenergic receptor have been assigned a pivotal role in the pathomechanism of DCM [18].

These antigens can play an important role in disease progression as well. Thus, the detection of circulating antibodies directed against these antigens has already been used for predicting disease outcome among relatives of DCM patients before symptom onset [8].

To date, a large-scale systematic study of the antigens targeted by circulating antibodies in sera of patients with heart diseases has been lacking. Therefore, in this study antigen profiles of groups of patients with distinct cardiovascular disorders — autoimmune myocarditis

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(tested virus-negative) and DCM — were investigated compared to healthy individuals (normal) on a peptide-array spotted with short overlapping peptides representing cardiovascular antigens. In-depth analysis of the data served to identify the respective antigenic epitopes. To validate the pathogenic effect of these epitopes, mice were immunized with the corresponding peptide sequences. Subsequently, their cardiac functional parameter was determined and histological analyses of the heart were performed. Furthermore, the chemokine/chemokine receptor and the cytokine mRNA expression levels in the myocardium were investigated. In this context, various proteins that induced a myocardial inflammation could be identified. Thus, this work could serve as a basis for future clinical investigations aimed at determining additional pathogenic antigens of DCM as well as myocarditis patients and establishing new specific therapeutic approaches.

2. Materials and methods

2.1. Study population

All patients were treated with optimal medical therapy and received a cardiac catheterisation. Every medical procedure was performed according to the guidelines for medical treatment of heart failure.

Myocarditis patients showed decreased left ventricular ejection fraction (LVEF) and no coronary heart disease excluded by coronary angiography. Endomyocardial biopsies were assessed for inflammation with hematoxylin and eosin (HE) and immunohistological staining (CD3+ and CD11a+/LFA-1+ lymphocytes, CD11b+/Mac-1+ macrophages, CD8+ T lymphocytes, granzyme B) [19]. All biopsies showed positive myocardial inflammation score according to the Dallas criteria and criteria of the World Heart Federation (WHF) [20] and were virus negative for enterovirus, adenovirus, Epstein–Barr virus, erythrovirus and human herpes virus 6 [19].

DCM was defined as a clinical diagnosis of heart failure, associated with LV dilatation and systolic dysfunction (LVEF < 45%) in the absence of secondary causes of heart failure such as coronary artery disease (CAD) and valvular heart disease [21].

Healthy individuals served as controls (normal) and showed normal results after cardiac magnetic resonance stress imaging and echocardiography to rule out pulmonary hypertension, significant coronary artery disease, and left or right ventricular dysfunction due to any cause (MRI, labs, exercise echocardiography). The healthy individuals showed a normal physical exam and normal lab results, a NT-pro BNP below 125 ng/L, and a normal resting 12-lead electrocardiogram.

Table 1
List of cardiovascular antigens spotted on the array to identify disease-associated antigens.

2′,3′-cyclic-nucleotide 3′-phosphodiesterase	Gap junction alpha-1 protein	Plakophilin-2
5′-AMP-activated protein kinase catalytic subunit alpha-2	Glycogen synthase kinase-3 alpha	Plakophilin-3
5′-AMP-activated protein kinase subunit gamma-2	Glycogen synthase kinase-3 beta	Plakophilin-4
5-hydroxytryptamine receptor 4	Heart- and neural crest derivatives-expressed protein 1	Plasminogen activator inhibitor 1
60 kDa heat shock protein, mitochondrial	Heart- and neural crest derivatives-expressed protein 2	Platelet-derived growth factor C
Actin, alpha cardiac muscle 1	Heat shock protein beta-1	Platelet-derived growth factor D
Adrenomedullin (ADM)	Heat shock protein beta-3	Platelet-derived growth factor subunit A
Alpha-actinin-2	Heat shock protein HSP 90-alpha	Platelet-derived growth factor subunit B
Alpha-crystallin B chain	Heat shock protein HSP 90-beta	Potassium voltage-gated channel subfamily E member 2
Alpha-enolase	Heparin-binding growth factor 2	Potassium voltage-gated channel subfamily KQT member 1
Alpha-galactosidase A	High mobility group protein B1	POU domain, class 5 transcription factor 1
Ankyrin repeat domain-containing protein 1	Homeobox protein Nkx-2.5	Prelamin-A/C
Ankyrin-2	Insulin-like growth factor I	Presenilin-1
Annexin A2/A5/A6	Integrin-linked protein kinase	Presenilin-2
ATP-binding cassette sub-family C member 9	Intercellular adhesion molecule 1	Protein S100-A1
BAG family molecular chaperone regulator 3	Inward rectifier potassium channel 2	Protein Wnt-1, 2, 2b, 3, 3a, 4, 5a, 5b, 6, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11, 16
Beta-1 adrenergic receptor	Junction plakoglobin	Regulator of G-protein signaling 10
BTB/POZ domain-containing protein KCTD14	Junctophilin-2	Regulator of G-protein signaling 2
Cadherin-12	Laminin subunit alpha-1/2/3/4/5	Ryanodine receptor 2
Cadherin-2	Laminin subunit beta-1/2/3/4	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1
Calmodulin	Laminin subunit gamma-1/2/3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2
Calreticulin-3	Leptin	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3
Cardiac myosin light chain 1	LIM domain-binding protein 3	Serine/threonine-protein kinase TNNI3K
Cardiac myosin light chain 2	Lysosome-associated membrane glycoprotein 2	Serpin H1
Cardiac phospholamban	Metalloproteinase inhibitor 1	Sodium channel protein type 5 subunit alpha
Cathepsin B	Metalloproteinase inhibitor 2	Sodium channel subunit beta-4
Caveolin-2	Metalloproteinase inhibitor 3	Sodium/potassium-transporting ATPase subunit alpha-2
Caveolin-3	Metalloproteinase inhibitor 4	Sodium/potassium-transporting ATPase subunit alpha-3
Creatine kinase U-type, mitochondrial	Muscarinic acetylcholine receptor M2	Tafazzin
Cysteine and glycine-rich protein 3	Myocyte-specific enhancer factor 2C	Telethonin
Delta-sarcoglycan	Myopalladin	TIR domain-containing adapter molecule 1
Desmin	Myosin light chain 3	Titin
Desmoglein-3	Myosin light chain kinase, smooth muscle	Transcription factor GATA-4
Desmoplakin	Myosin regulatory light chain 2 atrial isoform	Transcription factor GATA-6
Dual specificity tyrosine-phosphorylation-regulated kinase 1A	Myosin regulatory light chain 2 ventricular/cardiac muscle isoform	Transforming growth factor beta-3
Dystrobrevin alpha	Myosin-6	Transmembrane protein 43
Dystrophin	Myosin-7	Tropomyosin alpha-1 chain
E3 ubiquitin-protein ligase TRIM21	Myosin-binding protein C, cardiac-type	Troponin C, slow skeletal and cardiac muscles
E3 ubiquitin-protein ligase TRIM63	Myozenin-2	Troponin I, cardiac muscle
Emerin	Natriuretic peptides A	Troponin T, cardiac muscle
Endothelin-1	Natriuretic peptides B	Vascular cell adhesion protein 1
E-selectin	Nebulette	Vinculin
Eyes absent homolog 4	Nexilin	Voltage-dependent anion-selective channel protein 1
Fatty acid-binding protein, heart	Obscurin	Voltage-dependent L-type calcium channel subunit alpha-1C
Four and a half LIM domains protein 2	Phenylethanolamine *N*-methyltransferase	
Fukutin	Plakophilin-1	

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