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Dilated cardiomyopathy (DCM) and myocarditis: Classification, clinical and autoimmune features

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Abstract

Dilated cardiomyopathy (DCM), a leading cause of heart failure and heart transplantation in younger adults, is characterized by dilatation and impaired contraction of the left or both ventricles; it may be idiopathic, familial/genetic (20-30%), viral, and/or immune. On endomyocardial biopsy there is chronic inflammation in 30-40% of cases. Mutations in genes encoding myocyte structural proteins, cardiotoxic noxae and infectious agents are known causes; due to high aetiologic and genetic heterogeneity, the gene defects identified so far account for a tiny proportion of the familial cases. In at least two thirds of patients, DCM remains idiopathic. Myocarditis may be idiopathic, infectious or autoimmune and may heal or lead to DCM. Circulating heart-reactive autoantibodies are found in myocarditis/DCM patients and symptom-free relatives at higher frequency than in normal or noninflammatory heart disease control groups. These autoantibodies are directed against multiple antigens, some of which are expressed only in the heart (organ-specific); some autoantibodies have functional effects on cardiac myocytes in vitro as well as in animal models. Depletion of nonantigen-specific antibodies by extracorporeal immunoadsorption is associated with improved ventricular function and reduced cardiac symptoms in some DCM patients, suggesting that autoantibodies may also have a functional role in humans. Immunosuppression seems beneficial in patients who are virus-negative and cardiac autoantibody positive. Prospective family studies have shown that cardiac-specific autoantibodies are present in at least 60% of both familial and non familial pedigrees and predict DCM development among asymptomatic relatives, years before clinical and echocardiographic evidence of disease. Animal models have shown the autoimmune myocarditis/DCM can be induced by virus as well as reproduced by immunization with a well-characterized autoantigen, cardiac myosin. Thus, in a substantial proportion of patients, myocarditis and DCM represent different stages of an organ-specific autoimmune disease, that represents the final common pathogenetic pathway of infectious and noninfectious myocardial injuries in genetically predisposed individuals.

Key words: myocarditis, inflammatory cardiomyopathies, dilated cardiomyopathy, cardiac autoantibodies, autoimmunity

List of abbreviations

AHA	anti-heart autoantibodies
ANT	adenine nucleotide translocator
BCKD-E2	branched chain α -ketoacid dehydrogenase dihydrolipoyl transacylase
CB3	Coxsackie B3
cTnl	cardiac Troponin I
DCM	dilated cardiomyopathy
ELISA	enzyme-linked immunosorbent assay
EMB	endomyocardial biopsy
HSP	heat shock proteins
MHC	myosin heavy chain
PKA	protein kinase A
PCR	polymerase chain reaction
s-I IFL	standard indirect immunofluorescence
SPRIA	indirect micro solid-phase radioimmunoassay

Introduction

According to the current WHO classification of cardiomyopathies, dilated cardiomyopathy (DCM) is characterized by dilatation and impaired contraction of the left or both ventricles; it may be idiopathic, familial/genetic, viral, and/or immune [1]. The diagnosis of DCM requires exclusion of known, specific causes of heart failure, including coronary artery disease. On endomyocardial biopsy (EMB) there is myocyte loss, compensatory hypertrophy, fibrous tissue and immunohistochemical findings consistent with chronic inflammation (myocarditis) in 30-40% of cases. DCM represents a leading cause of severe heart failure and heart transplantation in younger adults, with an annual incidence of up to 100 patients and a prevalence of 300 to 400 patients per million in the USA. DCM is familial in 20-30% of cases. Mutations in genes encoding myocyte structural proteins, cardiotoxic substances and drugs and infectious agents, especially viruses, are known causes; however, due to high aetiologic and genetic heterogeneity, the gene defects iden-

tified so far account for a tiny proportion of the familial cases [2]. In at least two-thirds of patients, aetiology of DCM remains idiopathic.

Myocarditis is an inflammatory disease of the myocardium, and is diagnosed by EMB using established histological, immunological and immunohistochemical criteria; it may be idiopathic, infectious or autoimmune and may heal or lead to DCM [1-4]. Etiopathogenic causes of myocarditis are detailed in Table 1.

Pathology of myocarditis

Based only on histopathologic criteria, several distinct types of myocarditis have been identified: lymphocytic, eosinophilic, polymorphous, giant cell, and granulomatous myocarditis. Lymphocytic myocarditis is the most common type of myocarditis in Western countries and most cases are documented or presumed to have viral origin. In order to develop uniform, and reproducible morphologic criteria for the pathologic diagnosis of myocarditis, a panel of cardiac pathologists developed a classification of the disease based on histologic features of EMB specimens [3]. The Dallas classification adopts two different types of terminology for the first EMB and for the second one, which should be scheduled after at least 6 weeks (Tab 2). The first EMB may recognise active myocarditis in the presence of inflammatory cell infiltrates associated with necrosis or degeneration of cardiomyocytes, borderline myocarditis when only the inflammatory cells are seen, or absence of myocarditis in the absence of inflammation. On the second or follow-up EMB, the pathologist, comparing the morphological findings with those observed in the preceding biopsy sample, may identify persistent, resolving or healed myocarditis. Myocarditis can be labelled as "persistent" or "resolving" only if a diagnosis of myocarditis is unequivocally achieved on a previous EMB.

Table 1: Etiopathogenetic agents associated with myocarditis/inflammatory cardiomyopathy

<p>1. Infective Myocarditis</p> <p>Bacterial</p> <p>Spirochetal</p> <p>Fungal</p> <p>Protozoal</p> <p>Parasitic</p> <p>Rickettsial</p> <p>Viral</p>	<p>Staphylococcus, Streptococcus, Pneumococcus, Meningococcus, Gonococcus, Salmonella, Corynebacterium diphtheriae, Haemophilus influenzae, Mycobacterium (tuberculosis), Mycoplasma pneumoniae, Brucella</p> <p>Borrelia (Lyme disease), Leptospira (Weil disease)</p> <p>Aspergillus, Actinomyces, Blastomyces, Candida, Coccidioides, Cryptococcus, Histoplasma, Mucormycoses, Nocardia, Sporothrix</p> <p>Trypanosoma cruzi, Toxoplasma gondii, Entamoeba, Leishmania</p> <p>Trichinella spiralis, Echinococcus granulosus, Tenia solium</p> <p>Coxiella burnetii (Q fever), R. rickettsii (Rocky Mountain spotted fever), R. tsutsugamuschi</p> <p>coxsackievirus A and B, echovirus, poliovirus, hepatitis viruses, influenza A and B viruses, adenovirus, respiratory syncytial virus, mumps virus, measles virus, rubella virus, dengue virus, Chikungunya virus, yellow fever virus, Junin virus, Lassa fever virus, lymphocytic choriomeningitis virus, herpes simplex virus, varicella-zoster, human herpes virus-6, cytomegalovirus, Epstein-Barr virus, variola virus, vaccinia virus, parvovirus B19, rabies virus, human immunodeficiency virus-1</p>
<p>2. Immune-mediated Myocarditis</p> <p>Allergens</p> <p>Alloantigens</p> <p>Autoantigens</p>	<p>Tetanus toxoid, Vaccines, Serum sickness</p> <p>Drugs: penicillin, cefaclor, colchicine, furosemide, isoniazid, lidocaine, tetracycline, sulfonamides, phenytoin, phenylbutazone, methyl dopa, thiazide diuretics, amitriptyline</p> <p>Heart transplant rejection</p> <p>Idiopathic: Virus-negative lymphocytic, virus-negative giant cell</p> <p>Associated with autoimmune or immune-oriented disorders: systemic lupus erythematosus, rheumatoid arthritis, Churg-Strauss syndrome, Kawasaki's disease, inflammatory bowel disease, scleroderma, polymyositis, myasthenia gravis, insulin-dependent diabetes mellitus, thyrotoxicosis, sarcoidosis, Wegener's granulomatosis</p>
<p>3. Toxic Myocarditis</p> <p>Drugs</p> <p>Heavy Metals</p> <p>Miscellaneous</p> <p>Hormones</p> <p>Physical agents</p>	<p>amphetamines, anthracyclines, cocaine, cyclophosphamide, ethanol, fluorouracil, lithium, catecholamines, hemetine, interleukin-2, trastuzumab, clozapine</p> <p>copper, iron, lead</p> <p>scorpion sting, snake, and spider bites, bee and wasp stings, carbon monoxide, inhalants, phosphorus, arsenic, sodium azide</p> <p>Pheochromocytoma, Vitamins: beri-beri</p> <p>Radiation, electric shock</p>

Table 2: Myocarditis: Histopathological criteria from Dallas Classification (Aretz, Am J Cardiovasc Pathol 1987;1:3-14)

I EMB	II EMB
Active (Inflammatory cells + necrosis/degeneration)	Persistent (with or without Fibrosis)
Borderline (Inflammatory cells)	Resolving (with or without Fibrosis)
Negative	Healed (with or without Fibrosis)

Although the Dallas criteria include the advantage of using a simple, universally accepted and standardized terminology, they have some important limitations. In the original Dallas classification, other histologic types of inflammatory infiltrate (e.g. lymphocytic, eosinophilic, neutrophilic or giant cell) are just mentioned in the view of a possible differentiation between a primary (or idiopathic) form of myocarditis from a process secondary to a known cause [3]. It is not specified if the terminology (e.g. active, borderline, healing with or without fibrosis) that is currently used in the most common form (lymphocytic myocarditis) can be equally applied to the other forms. Myocyte changes including frank necrosis and degeneration have been described in the original report from Aretz [3], however specification of the type and extension of myocyte damage was not included in the classification. It is very difficult to foresee the natural history of the disease and the specific timing of its evolution, however, when EMB is performed and a thorough morphological evaluation carried out, it is mandatory to add temporal information of the lesion, and highlight the clinical history in order to provide indications on the disease course. This approach may avoid the unrestricted use of the term borderline myocarditis. This term should not be inappropriately used for chronic forms, which instead are defined "inflammatory cardiomyopathy" [1].

The intensity and distribution of the inflammatory infiltrate are highly variable, ranging from a solitary small focus to multifocal aggregates to diffuse myocardial involve-

ment. On routine haematoxylin eosin stained sections it may be difficult to characterize interstitial cells, since normal myocardial components such as mast cells, fibroblast nuclei cut in cross section, pericytes, histiocytes, and endothelial cells may resemble lymphocytes [5,6]. Recently, a cut off of <14 leucocytes/mm² with the presence of T lymphocytes <7 cells/mm² has been considered a more realistic value [7]. Most experts in the field agree that an actual increase of sensitivity of EMB has been reached combining immunohistochemistry and routine histology. A large panel of monoclonal and polyclonal antibodies is now mandatory to identify and characterize the inflammatory cell population as well as the activated immunological processes [5,6]. Angelini et al showed that in patients who did not fulfil the Dallas criteria, immunohistochemistry allowed to detect inflammatory infiltrates in the myocardium, characterized by T-lymphocytes and macrophages, thus excluding the possibility of acute ischemia, that is characterized by a neutrophilic infiltrate [5,6]. The presence of an inflammatory infiltrate on immunohistochemical analysis together with molecular detection of genomic sequences for cardiotropic viruses by polymerase chain reaction (PCR) increases the diagnostic accuracy of EMB in those cases of myocarditis in which the application only of the Dallas criteria would otherwise have failed to detect and characterize the inflammatory disease [5,6,8-9].

Clinical features in myocarditis

The clinical features of myocarditis are heterogeneous [10-15]. Cardiac involvement may be preceded (1-2 weeks) by a systemic flu-like illness. Myocarditis may be subclinical, causing minor symptoms (palpitation, atypical chest pain), ECG abnormalities (atrioventricular conduction disturbance, bundle branch block, ST and T-wave changes), or arrhythmias (paroxysmal atrial fibrillation or ventricular arrhythmias) with or without global or regional left and/or right ventricular dysfunction. Pericardial involvement commonly coexists with myocarditis. Other presentations of myocarditis include syncope, sudden death, acute right or left ventricular failure, cardiogenic shock, or DCM. A syndrome mimicking acute myocardial infarction, but with normal coronary arteries, may also occur. Prognosis of myocarditis is thought to be good, with complete recovery in the majority of patients. However, myocarditis can cause fulminant and fatal heart failure. Relapses may occur, and about one third of patients will develop residual mild left ventricular dysfunction or DCM. Thus, in a patient subset, myocarditis and DCM represent the acute and chronic stages of an inflammatory disease of the myocardium, which can be viral, post infectious immune or primarily organ-specific autoimmune [1-4].

Autoimmune features in myocarditis/DCM

Autoimmune diseases fulfil at least two of the major criteria proposed by Rose [16-17]. Fulfilled Rose-Witebski autoimmune criteria in human myocarditis/DCM include familial aggregation and clustering of autoimmune diseases in patients and relatives, a weak association with HLA-DR4, DR5, lymph mononuclear cell infiltrates and/or abnormal expression of HLA class II and adhesion molecules on cardiac endothelium on EMB, in the affected patients and family members, increased levels of circulating cytokines and

cardiac autoantibodies in patients and family members, experimental models of both antibody-mediated and cell-mediated autoimmune myocarditis/DCM following immunization with relevant autoantigen(s), the best characterized of which are cardiac myosin and the beta-1 adrenergic receptor [18-33] (Table 3).

Anti-heart autoantibodies (AHA) by standard indirect immunofluorescence (s-I IFL)

Several researchers reported antibodies to distinct cardiac antigens in myocarditis and DCM by s-I IFL, but the organ- and disease-specificity of these antibody types were not always evaluated [16]. These antibodies were either cross-reactive or untested on skeletal muscle. In addition, it remained unclear whether these antibodies were disease-specific for myocarditis/DCM, because disease controls were not always tested [16].

Using indirect s-I IFL on 4 µm-thick unfixed fresh frozen cryostat sections of blood group O normal human atrium, ventricle and skeletal muscle, and absorption with human heart and skeletal muscle and rat liver, organ-specific IgG anti-heart autoantibodies (AHA) giving a diffuse cytoplasmic staining pattern of myocytes, and a negative pattern on skeletal muscle, were found in about one third of myocarditis/DCM patients and their symptom-free family members, in 1% of patients with other cardiac disease, in 3% of normal subjects, and in 17% of patients without cardiac disease, but with autoimmune polyendocrinopathy [16,19,23-24,34]. AHA of the cross-reactive 1 type, which exhibited partial organ-specificity for heart antigens by absorption and gave a fine striational staining pattern on myocytes, but were negative or only weakly stained skeletal muscle, were also more frequently detected in DCM/myocarditis than in controls. Conversely, AHA of the cross-reactive 2 type, which were entirely skeletal muscle cross-reactive by absorption and gave a broad striational "myas-

Table 3: Fullfilled Rose-Witebsky autoimmune features in myocarditis/DCM

<p>Major</p> <ul style="list-style-type: none"> • Mononuclear cell infiltration and abnormal HLA expression in the target organ (organ-specific disease) in the absence of infectious agents or known inflammatory causes: yes • Circulating autoantibodies and/or autoreactive lymphocytes in patients and in unaffected family members: yes • Autoantibody and/or autoreactive lymphocytes in situ within the affected tissue: yes • Identification and isolation of autoantigen (s) involved: yes • Disease induced in animals by immunisation with relevant autoantigen, and/or passive transfer of serum, purified autoantibody and/or lymphocytes: yes • Efficacy of immunosuppressive therapy: controversial <p>Minor</p> <p>a) Common to all autoimmune disorders</p> <ul style="list-style-type: none"> • Middle-aged women most frequently affected: no • Familial aggregation: yes • HLA association: controversial • Hypergammaglobulinemia: no • Clinical course characterized by exacerbations and remissions: yes • Autoimmune diseases associated in the same patient or in family members: yes <p>a) Typical of organ-specific autoimmune disorders</p> <ul style="list-style-type: none"> • Autoantigens at low concentration: not known • Autoantibodies directly against organ-specific autoantigens: yes • Immunopathology mediated by Type II, IV, V, VI reactions: yes • Induction of antibodies induces an organ-specific disease/phenotype: yes • Transfer of autoantibodies also transfers the disease/phenotype: yes

thenic" pattern on heart and skeletal muscle, were found in similar proportions among groups [19,23-24,34]. Prospective family studies have shown that AHA are present in at least 60% of both familial and non familial pedigrees, and are independent predictors of DCM development in symptom-free relatives at 5 year follow-up, in keeping with the view that autoimmunity is clinically relevant in the majority of myocarditis/DCM cases [19-20]. More recently AHA and anti-intercalated disk autoantibodies (AIDA), also detected by s-I IFL, have been found at increased frequency in idiopathic recurrent acute pericarditis, providing evidence for autoimmunity in this rare idiopathic heart disease [35].

Autoantibodies to myosin heavy chain (MHC), other sarcolemmal autoantigens and heat shock proteins (HSP)

Two of the autoantigens recognized by the AHA in DCM, were identified as α and β myosin heavy chain (MHC) isoforms, by Western blotting; several bands due to yet unknown antigens were also detected [25]. The α isoform is expressed solely within the atrial myocardium. Antibodies to this molecule represent organ-specific AHA. The identification of α and β MHC as autoantigens in human DCM parallels what is seen in the experimental model [17,36,37] and in human myocarditis [24,26]. These findings have been confirmed by various groups [26,27]; a study suggested that the disease-specific anti-myosin antibodies in DCM sera are mainly of IgG3 subclass [27]. In some studies the anti-myosin antibodies were associated with dete-

rioration of cardiac function [16,26]. Others found antibodies to heat shock proteins (HSP) -60 and HSP-70 at higher frequency in DCM than in control subjects [16].

One study reported the effects of anti-cardiac myosin autoantibodies from human DCM patients, affinity-purified by immunoaffinity column chromatography, on contractility, L-type Ca^{2+} current and Ca^{2+} transients in continuously perfused rat ventricular myocytes [38]. The antibodies reduced the capacity of electrical field-stimulated myocytes to contract in a dose-dependent manner, but inhibition of contraction, expressed as a percentage of untreated cells, was independent of antibody titre. Myocytes treated with DCM antibodies raised peak systolic and diastolic levels of $[\text{Ca}^{2+}]_i$ in response to an increase in beating frequency (0.2 to 3.0 Hz) [38]. However, the antibodies were not internalized by myocytes and had no effect on L-type Ca^{2+} current, thus it was speculated that altered sensitivity of the myofilaments to $[\text{Ca}^{2+}]_i$ might be involved as a potential mechanism of anti-cardiac myosin antibody mediated impairment in DCM [38]. These *in vitro* data suggest that some of the anti-myosin antibodies, similar to other antibody specificities, may have a functional role in human DCM, influencing contractility of myocytes. Myosin is an intracellular protein, thus there are two major hypotheses, which may be not mutually exclusive, to explain interruption of tolerance to this autoantigen. These include molecular mimicry, since cross-reactive epitopes between cardiac myosin and infectious agents have been found, and myocyte necrosis due to viral infection or other tissue insults [37, 39-40]. Both mechanisms would explain the association of viral infection with autoimmune myocarditis/DCM. Infection with Coxsackie B3 (CB3) virus triggers antimyosin reactivity and autoimmune myocarditis in many mouse strains, and immunization with cardiac myosin induces organ-specific myocardial disease in the same susceptible strains [41-42]. In some strains, such as Balb/c mice, CB3 virus-induced or myosin-induced myo-

carditis is T cell-mediated [42], whereas in other strains, such as DBA/2 mice, it is an antibody-mediated disease [43]. The same may apply to humans, so that the antimyosin antibodies may be directly pathogenic in some, but not all patients with myocarditis/DCM according to different immunogenetic backgrounds, isotype [43] and/or subclass specificity of these antibodies [27]. In keeping with this, an experimental model of myosin-induced autoimmune myocarditis has revealed an additional mechanism by which anti-cardiac myosin autoantibodies may lead to heart dysfunction [44]. Li et al showed that anti-cardiac myosin antibodies induced by immunization with cardiac myosin or its pathogenic peptide S2-16 target the β -adrenergic receptor on the heart cell surface and specifically induce camp-dependent protein kinase A (PKA) activity in heart cells. Antibody-mediated cell signalling of PKA was inhibited by cardiac myosin, anti-IgG, or specific inhibitors of the β -adrenergic receptor pathway [44]. Monoclonal antibodies specific for cardiac myosin confirmed the cross-reactive mimicry between cardiac myosin and the β -adrenergic receptor. In addition, passive transfer of purified IgG from cardiac-myosin immunized rats produced IgG deposition and apoptosis and DCM-like changes in the heart of normal recipients [44].

Interestingly, mice deficient in the T cell costimulatory molecule PD1 spontaneously developed autoimmune DCM in the absence of inflammatory infiltrates, but accompanied by deposition of immune complexes on the surface of myocytes [45]. Sera from these mice contained high titer autoantibodies to cardiac Troponin I (cTnI); injected monoclonal antibodies to cTnI induced dilatation and heart dysfunction and were therefore considered responsible for the DCM in PD1 deficient mice [46]. The anti-cTnI antibodies possibly recognized cTnI expressed on the cardiomyocyte surface, did not cross-react with the Ca-channel, but enhanced the Ca^{2+} current [46], similar to the Ca^{2+} enhancement described for the antibodies against the adenine nucleotide translocator (ANT) [47-48],

the β_1 -adrenoceptor [28-31, 49-50], and cardiac myosin [38]. Thus it may be that distinct anti-cardiac autoantibody specificities contribute to the induction of humoral-mediated DCM, in the absence of myocardial inflammation, by affecting regulation of the Ca^{2+} current [18,46]. cTnI would be an organ-specific cardiac autoantigen; since one study failed to show a higher frequency of anti-cTnI antibodies in human DCM compared to ischemic heart disease [25], further clinical confirmatory work is needed.

Autoantibodies to sarcolemmal Na-K-ATPase

A study, using porcine cerebral cortex Na-K-ATPase as antigen by enzyme-linked immunosorbent assay (ELISA), found anti-Na-K-ATPase autoantibodies in 26% of DCM and in 2% of normal subjects, and suggested that they might possess biologic activity [51]. Cardiac sudden death was independently predicted by the presence of antibodies. The authors speculated that these antibodies might lead to electrical instability, because of abnormal Ca^{2+} handling by reduced Na-K-ATPase activity. It remains to be seen whether these antibodies are disease-specific for DCM. Sarcolemmal Na-K-ATPase does not seem to fulfil strict criteria of organ-specific cardiac autoantigen [16].

Autoantibodies to mitochondrial antigens

Antibodies against mitochondrial antigens, the M7 [52], the adenine nucleotide translocator (ANT) [47-48] and the branched chain a-ketoacid dehydrogenase dihydrolipoyl transacylase (BCKD-E2) [53] have also been detected. The M7 antibodies, detected by ELISA on beef heart mitochondria, were of IgG class and were found in 31% of DCM patients, 13% of those with myocarditis, 33% of controls with hypertrophic cardiomyopathy, but not in controls with other cardiac dis-

ease, immune-mediated disorders, or in normal subjects [52]. Using an indirect micro solid-phase radioimmunoassay (SPRIA) and ANT, a protein of the internal mitochondrial membrane, purified from beef heart, liver and kidney as antigen, anti-ANT antibodies were found in 57-91% of myocarditis/DCM sera, and in no controls with ischemic heart disease, or in normal subjects [47]. Mitochondrial antigens have generally been classified as nonorgan-specific. The heart-specificity of the M7 antibodies was shown by absorption studies, whereas these were not performed with the ANT and the BCKD-E2 antibodies. Experimentally induced affinity-purified anti-ANT antibodies cross-reacted with calcium channel complex proteins of rat cardiac myocytes, induced enhancement of transmembrane calcium current, and produced calcium-dependent cell lysis in the absence of complement [47-48]. Antibody-dependent cell lysis has not been shown using the antibodies present in patients' sera.

Autoantibodies to β -adrenergic and β_2 -muscarinic receptors

Using a binding inhibition assay on rat cardiac membranes, a significant inhibitory activity, attributed to anti- β_1 -adrenoceptor IgG antibodies, was found in 30-75% of DCM sera, 37% of disease controls and 18% of sera from normal subjects [28-29]. Magnusson et al., using as antigens synthetic peptides analogous to the sequences of the second extra cellular loop of β_1 - and β_2 adrenergic receptors by ELISA, found antibodies in 31% of DCM patients, 12% of normal subjects and in none of the disease controls [30].

When analysed in a functional test system of spontaneously beating neonatal rat myocytes, antibody positive DCM sera [28] or the affinity purified β_1 -receptor antibodies [30] increased the beating frequency of isolated myocytes in vitro. β_1 -blocking drugs inhibited the effect of the antibodies. Stimulating anti β_1 -receptor antibodies were present in 96% of myocarditis and 26-95% of DCM

sera, in 8-10% of controls with ischemic heart disease and 0-19% of normal subjects [16]. It has been suggested that this antibody-mediated stimulation of the β_1 -receptor, observed *in vitro*, could occur *in vivo*.

Fu et al., using as antigen a synthetic peptide analogous to the 169-193 sequence of the second extra cellular loop of human M2 muscarinic receptors by ELISA, showed anti-M2 antibodies in 39% of DCM sera and 7% of the normal subjects [54]. The immunization of rabbits with a synthetic peptide corresponding to the sequence of the second extracellular loop of either β_1 -adrenoceptors or M2-muscarinic receptors resulted in a moderate dilatation of the right ventricle with lymph mononuclear cell infiltrates within a year [55]. DCM was induced by immunizing inbred rats against the second extra cellular loop of β_1 - and β_2 adrenergic receptors and reproduced in healthy isogenic rats by passive transfer of the stimulatory autoantibodies from diseased rats [49]. Based on these results these authors suggested that anti- β_1 adrenergic receptor-induced DCM should be considered a disease entity of its own, similar to other receptor-directed autoimmune diseases, such as myasthenia gravis or Graves' disease [50]. It is puzzling that these receptors, established autoantigens in an organ-specific autoimmune disease such as myocarditis/DCM, are not strictly organ-specific cardiac components; in fact their distribution is not restricted to the heart, and there are no cardiac-specific isoforms [16].

The frequency of stimulating anti- β_1 -adrenergic receptor antibodies was rather low (about 10% in ischemic and 30% in DCM) using cell systems that have been advocated as gold standard tools for detection of functionally relevant antibodies in patients since they present the target receptor in its natural conformation [31], but new screening techniques have been developed such as functional fluorescence resonance energy transfer (FRET) assay [56]. A pilot study of immunoadsorption failed to show a correlation between hemodynamic benefit and anti- β_1 -receptor antibody positivity [57]. The patho-

genic contribution of anti- β_1 -receptor, anti-M2 receptor, anti-troponin, AHA and AIDA in human myocarditis/DCM and their relevance for prognosis and therapy is being actively investigated in a multicenter prospective trial, the ETICs trial [58].

Autoimmunity in myocarditis/DCM: clinical implications and conclusive remarks

The presence of organ- and disease-specific AHA of IgG class against myosin and other antigens supports the involvement of autoimmunity in at least one third of myocarditis/DCM patients and in 60% of both familial and non-familial pedigrees [10,16,19-20,23,24]. In overt DCM, AHA was associated with shorter duration and minor severity of symptoms [23]. In many patients who were AHA positive at diagnosis these markers were undetectable at follow-up [59]. In symptom-free relatives AHA preceded of 5 years other diagnostic abnormalities of heart dysfunction [20]. These findings suggest that AHA detected by s-I IFL are early markers. The absence of antibodies at diagnosis in some patients could indicate that cell-mediated mechanisms are predominant, and/or that autoimmunity is not involved; since the pre-clinical stage in DCM is prolonged [20], it might also relate to reduction of antibody levels with disease progression [59]. These findings have been obtained using standard autoimmune serology techniques, in particular s-I IFL, ELISA and immunoblotting, and have been confirmed by several groups [26,27,37,38]. The low frequency of AHA detected by s-I IFL in control patients with heart dysfunction not due to myocarditis/DCM [10,16,19-20,23,24], the decrease in antibody titres in advanced DCM and their detection in symptom-free relatives with a normal echocardiogram [19,20,59] suggest that these markers are not epiphenomena associated with tissue necrosis of various caus-

es, but represent specific markers of immune pathogenesis. AHA were found in similar proportions of patients with DCM and with biopsy-proven myocarditis according to the Dallas criteria, included in the Myocarditis Treatment Trial [24], suggesting that conventional histology does not distinguish between patients with and without an on-going immune-mediated process. The Myocarditis Treatment Trial failed to show an improvement in survival in biopsy-proven myocarditis with immunosuppressive therapy [11]; however, no immunohistochemical or serological markers (e.g. increased HLA expression on EMB and/or detection of serum AHA in the absence of viral genome in myocardial tissue) were used to identify those patients with immune-mediated pathogenesis in whom immunosuppression could have been beneficial [11]. Conversely, recent studies suggest its long-term benefit in immune-mediated myocarditis/DCM, identified by immunohistochemical markers on EMB or serum AHA in the absence of viral genome [12,13,60] and in giant cell myocarditis [15]. In a recent prospective study of biopsy-proven myocarditis, the disease was classified as autoimmune, (positive AHA, virus-negative PCR), in 48% of patients, viral (virus-positive PCR, negative AHA) in 9%, viral and immune (virus-positive PCR, positive AHA) in 12%, idiopathic and/or cell-mediated (virus-negative PCR, negative AHA) in 31% [10]. Thus, autoimmune myocarditis was the most common form, suggesting that a majority of patients may benefit of immunosuppression. The 31% of cases that were negative for both AHA and PCR [10] might be classified as "idiopathic myocarditis" and could reflect viral myocarditis, due to yet unknown pathogens, or, most likely, a cell-mediated autoimmune form, that might benefit of immunosuppression. AHA occurred in association with positive PCR for virus in 12% of patients [10]. These patients might be candidates for antiviral and, after virus clearance, immunosuppression or combined anti-viral and immunosuppressive therapy.

Virus-negative myocarditis/DCM patients with cardiac-specific autoantibodies should also be included in future trials of immunosuppressive therapy.

Myosin fulfilled the expected criteria for organ-specific autoimmunity, in that immunization with cardiac but not skeletal myosin reproduced, in susceptible mouse strains, the human disease phenotype of myocarditis/DCM [17,36,37,41-44]. However, autoimmune diseases are often polyclonal, with production of autoantibodies to different autoantigens. Some of these autoantigens are involved earlier in disease and are more closely related to primary pathogenetic events compared to those, which play a role in secondary immunopathogenesis [17]. Both experimental and clinical evidence, in particular the multiplicity of autoantibody specificities identified so far, exists that this also applies to myocarditis/DCM.

Non antigen-specific IgG adsorption has been used in DCM patients with high titre antibodies to the β_1 -receptor, and it has been suggested that it has beneficial clinical effects, accompanied by undetectable antibody titres during follow-up [32]. This does not imply a direct pathogenic effect of the anti β_1 -receptor antibodies. The adsorption technique used was non-antigen specific; in addition, in antibody-mediated disorders the antibody titres rise again at the end of plasmapheresis. However, recently new evidence has been provided in favour of the possibility that the beneficial effect of immunoadsorption is related to removal of pathogenic cardio depressant autoantibodies of IgG3 subclass, although no conclusion is yet possible on the potential pathogenic role of a specific autoantibody [33,61]. It may be that this technique has a favourable immunomodulatory/immunosuppressive effect; in addition, IgG substitution performed after immunoadsorption to avoid infective complications of unselective IgG depletion, may have contributed to the observed hemodynamic improvement; randomized studies are underway. This does not undermine the possible role of any of the described antibodies

as predictive markers. It is still unknown whether subjects classified as seronegative for one antibody are positive for another, which is the temporal sequence of appearance of the various antibodies (anti-myosin, c-TnI, β -adrenoceptor, mitochondrial and other antigens) and whether single or multiple antigen-specific antibody tests will be superior to a non-antigen specific technique, such as s-I IFL, as screening tools. Collaborative work among laboratories testing the individual antibodies is underway [58].

In conclusion, several groups have shown that a subset of patients with myocarditis/idiopathic DCM and of their symptom-free relatives has circulating heart-reactive autoantibodies. These autoantibodies are directed against multiple antigens, some of which are strictly expressed in the myocardium (e.g. organ-specific for the heart), others are expressed in heart and skeletal muscle (e.g. muscle-specific). Distinct autoantibodies have also different prevalence in disease and normal controls (e.g. by IFL the organ-specific and cross-reactive-1 type AHA are disease-specific for DCM, some of the muscle-specific antibodies are not). Different antibody techniques detect one or more antibody specificities, thus they cannot be used interchangeably as screening tools. Antibody frequency in DCM vs. controls is expected to be different using distinct techniques; at present it is unknown whether the same subset (30-40%) of patients produce more than one antibody or different patient groups develop autoimmunity to different antigens. Antibodies of IgG class, which are shown to be cardiac and disease-specific for myocarditis/DCM, can be used as reliable markers of autoimmune pathogenesis for identifying patients in whom immunosuppression and/or immunomodulation therapy may be beneficial and their relatives at risk. Some of these autoantibodies may also have a functional role in patients, as suggested by *in vitro* data as well as by preliminary clinical observations, though further work is in progress to clarify this important issue.

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