Inflammatory Dilated Cardiomyopathy (DCMI)

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Abstract

Cardiomyopathies are heart muscle diseases, which have been defined by their central hemodynamics and macropathology and divided in five major forms: dilated (DCM), hypertrophic (HCM), restrictive (RCM), right ventricular (RVCM), and nonclassifiable cardiomyopathies (NCCM). Furthermore, the most recent WHO/WHF definition also comprises, among the specific cardiomyopathies, inflammatory cardiomyopathy as a distinct entity, defined as myocarditis in association with cardiac dysfunction. Idiopathic, autoimmune, and infectious forms of inflammatory cardiomyopathy were recognized. Viral cardiomyopathy has been defined as viral persistence in a dilated heart. It may be accompanied by myocardial inflammation and then termed inflammatory viral cardiomyopathy (or viral myocarditis with cardiomegaly). If no inflammation is observed in the biopsy of a dilated heart (< 14 lymphocytes and macrophages/mm²), the term viral cardiomyopathy or viral persistence in DCM should be applied according to the WHF Task Force recommendations.

Within the German heart failure net it is the authors' working hypothesis, that DCM shares genetic risk factors with other diseases of presumed autoimmune etiology and, therefore, the same multiple genes in combination with environmental factors lead to numerous different autoimmune diseases including DCM. Therefore, the authors' primary goal is to acquire epidemiologic data of patients with DCM regarding an infectious and inflammatory etiology of the disease. Circumstantial evidence points to a major role of viral myocarditis in the etiology of DCM. The common presence of viral genetic material in the myocardium of patients with DCM provides the most compelling evidence, but proof of causality is still lacking. In addition, autoimmune reactions have been described in many studies, indicating them as an important etiologic factor. Nevertheless, data on the proportion of patients, in whom both mechanisms play a role are still missing.

A pivotal role for autoimmunity in a substantial proportion of patients with DCM is supported by the presence of organ-specific autoantibodies, inflammatory infiltrates and pro-inflammatory cytotoxic cytokines. Furthermore, familial occurrence of DCM has been described in about 20–30% of cases, with the presence of autoantibodies and abnormal cytokine profiles in first-degree relatives with asymptomatic left ventricular enlargement. This suggests the involvement of a disrupted humoral and cellular immunity early in the development of the disease. A similar pattern of humoral and cellular immune dysregulation has been described in other autoimmune diseases. There is considerable evidence that genetic factors play an important role in the pathogenesis of DCM, either as contributors to the susceptibility to environmental factors or as determinants of functional and structural changes that characterize the phenotypic expression of the disease.

Yet, it is not known whether the susceptibility to immunologically mediated myocardial damage reflects the presence of genetic risk factors shared by other autoimmune diseases. Preliminary investigations suggest, that this is the case, because the frequency of autoimmune disorders other than DCM was higher in first-degree relatives of the subjects with DCM including juvenile diabetes, rheumatoid arthritis, thyroiditis, psoriasis, and asthma.

The nature of the genetic risk is undetermined and probably involves genes in the major histocompatibility (MHC) locus as well as other susceptibility loci. Therefore, the authors started their investigation with the search for MHC class 2 DQ polymorphisms in the peripheral blood of patients with DCM in parallel to the search for new interesting susceptibility loci by the use of the microarray analysis regarding genes responsible for inflammatory and autoimmune diseases. By this approach a new insight in the familial clustering of other autoimmune diseases in patients with DCM and in genetic predisposition can be expected.

Key Words:

Inflammatory cardiomyopathy · Polymerase chain reaction · Viral infection · Immunohistochemistry

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Schlüsselwörter:
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Inflammatorische dilatative Kardiomyopathie (DCMI)

Zusammenfassung

Kardiomyopathien werden nach der letzten Klassifikation der WHO/ISFC-(WHF-)Task Force nach ihren makropathologischen und hämodynamischen Kriterien in fünf Formen eingeteilt: dilatative (DCM), hypertrophische (HCM), restriktive (RCM), rechtsventrikuläre (RVCM) und nicht klassifizierbare Herzmuskelerkrankungen (NCCM). Unter den spezifischen Kardiomyopathien wurde die inflammatorische Kardiomyopathie als Myokarditis mit hämodynamischer Dysfunktion eingereiht. Eine virale Kardiomyopathie wurde als Persistenz viralen Genoms bei einer DCM definiert, die mit einer histologisch validierten Entzündung einhergehen kann. Inflammation liegt nach den Empfehlungen der WHF-Task Force vor, wenn sich ≥ 14 Lymphozyten und Makrophagen/mm² in einer Myokardbiopsie finden. Dann liegt eine virale inflammatorische Kardiomyopathie oder Virusmyokarditis vor.

Im Herzinsuffizienznetz geht das Projekt der Autoren von der Hypothese aus, dass die DCM ein genetisches Risikoprofil mit anderen Autoimmunkrankheiten teilt und deshalb verschiedene Gene (Polymorphismen) zusammen mit Umweltfaktoren zu autoimmunen Krankheitsbildern führen, zu denen auch die DCM zählt. Mit dieser Hypothese gut vereinbar sind der Nachweis von viraler DNA oder RNA im Myokard oder autoimmune Phänomene bei Patienten, z.T. auch ihren (noch) nicht betroffenen Angehörigen. Ungeklärt sind bisher allerdings die kausalen ätiopathogenetischen Verknüpfungen oder Trennlinien zwischen den beiden Befunden: So lassen sich bei einem Großteil der DCM-Patienten zirkulierende und biopsiegebundene organspezifische Autoantikörper, entzündliche Infiltrate und die Freisetzung proinflammatorischer Zytokine nachweisen. Andererseits ist anzunehmen, dass mindestens 20–30% der DCMs familiär gehäuft auftreten, allerdings ohne dass dabei in allen Fällen eine monogenetische Erkrankung vorliegen muss. Es ist anzunehmen, dass genetische Faktoren deshalb entweder als Suszeptibilitätsfaktoren für Umwelteinflüsse (wie eine Virusinfektion, Stress usw.) oder als direkte Determinanten des funktionellen und strukturellen Phänotyps Kardiomyopathie verantwortlich sind. Dabei ist es weiteren Untersuchungen vorbehalten zu klären, ob die Prädisposition für eine autoreaktive kardiale Schädigung sich derselben genetischen Mechanismen wie bei anderen Autoimmunerkrankungen bedient. Bemerkenswert ist die Prävalenz anderer autoimmuner Erkrankungen bei einem substantiellen Anteil von Indexpatienten mit DCM und Verwandten ersten Grades, wie z.B. juveniler Diabetes, Rheuma, Thyreoiditis, Psoriasis, Asthma usw., in eigenen bisherigen Untersuchungen.

Die Autoren leiten daraus ab, dass für die Abschätzung des genetisch prädisponierenden Risikos auch Gene des MHC („major histocompatibility complex") eine Rolle spielen, und haben sich in einem ersten Ansatz deren Untersuchung vorgenommen.

Introduction

The cardiomyopathies constitute a group of diseases in which the dominant feature is direct involvement of the heart muscle itself. Five different forms are recognized: dilated (DCM), hypertrophic (HCM), restrictive (RCM), right ventricular (RVCM), and nonclassifiable cardiomyopathies (NCCM) with distinct hemodynamic properties. In 1995, the WHO/ISFC (WHF) Task Force on the Definition and Classification of Cardiomyopathies established several changes in the terminology [1]. The term cardiomyopathy is no longer reserved for the idiopathic forms but can be used interchangeably with the term heart muscle disease including specific, secondary forms. RVCM, valvular, hypertensive, ischemic, and inflammatory cardiomyopathy have been introduced. Idiopathic, autoimmune, and infectious forms of inflammatory cardiomyopathy were also recognized. Viral cardiomyopathy is defined as viral persistence in a dilated heart. It may be accompanied by myocardial inflammation and then termed inflammatory viral cardiomyopathy. A task force of the World Heart Federation's (WHF) Council on Cardiomyopathies specified inflammation in the endomyocardial biopsy quantatively either as foci of lymphocytes and/or ≥ 14 lymphocytes and macrophages/mm² [2, 14]. The term viral cardiomyopathy or viral persistence in DCM should be applied, if only viral RNA or DNA but no inflammation is present. If both virus and inflammation are found, viral inflammatory cardiomyopathy is the correct term [2].

If progressive, cardiomyopathies lead to either systolic pump failure, which is often accompanied or preceded by an impairment of the diastolic compliance.

Primary and secondary forms of cardiomyopathies have a different etiopathogenesis. The

following factors have been postulated to lead to left ventricular compromise in DCMs:

- myocardial inflammation (autoimmune, viral, or postviral) mediated by the effector cells of the immune system (cytotoxic T-lymphocytes, natural killer [NK] cells, macrophages) [3–7];
- locoregional effect of inflammatory mediators (iNOS activation, cytokines), released by the infiltrating lymphocytes, macrophages or endothelial cells;
- direct interaction of the antibodies against the β-receptor, myolemma, membrane receptors or proteins, mitochondrial or microsomal membrane or enzymes [4, 6, 8–10];
- toxins impairing the membrane transport mechanisms or biochemical processes (alcohol, anthracyclines, cocaine, etc.);
- loss or dysfunction of the matrix proteins (i.e., dystrophin, laminins, etc.).

Symptoms usually develop gradually, and some patients are asymptomatic despite left ventricular dilatation for months or even years. The most striking symptoms of DCM are those of left ventricular systolic failure, but right-sided heart failure may also occur and is associated with a particularly poor prognosis [11]).

About 30–40% of individuals with DCM have a familial form of the disease [12]. If monogenetic, the mode of inheritance is mostly autosomal dominant, rarely X-chromosomal or autosomal recessive. Mitochondrial inheritance is most often seen in childhood forms [13]. Since the penetrance and expression of the genes are highly variable, several other predisposing and environmental factors are responsible for the development of the cardiomyopathy. To establish prevalence and prognostic value of single mutations, large multicenter registries and international studies are ongoing.

According to the WHO/ISFC (WHF) criteria inflammatory cardiomyopathy is defined as myocarditis in association with cardiac dysfunction [1, 2, 14]. The diagnosis of inflammatory dilated cardiomyopathy cannot be established without endomyocardial biopsy. In acute (active) myocarditis, necrosis and/or degeneration of adjacent myocytes are present, whereas in chronic myocarditis, necrosis is not an obligatory feature. When referring to the Dallas criteria [15], the term acute myocarditis corresponds to active myocarditis, chronic myocarditis may also comprise borderline or healing myocarditis in H&E (hematoxylin-eosin) staining (Table 1). The inflammatory infiltrate should be subclassified as lymphocytic, eosinophilic, neutrophilic, giant cell, granulomatous, or mixed. The distribution should be classified as focal, confluent, or diffuse (Figure 1).

For the definition of myocarditis a minimum of 14 infiltrating leukocytes/mm^2, preferably T-lymphocytes or activated T-cells (including up to four macrophages), are necessary [2]. In case of nests of leukocytes (three or more lymphocytes, preferably T-cells) located outside the lumen of a vessel, a focal inflammatory process (myocarditis) is diagnosed. If foci of T-lymphocytes are present, myocarditis can be diagnosed due to the nature of the infiltrate even when the critical number of 14 leukocytes/mm^2 is not reached. If the focal or diffuse leukocytes are localized in fibrotic areas, the process may be termed reparative.

The amount of fibrosis should be described as: no fibrosis (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3). Distribution should be outlined as endocardial, replacement, or interstitial. The following terminology is to be used:

First biopsy:

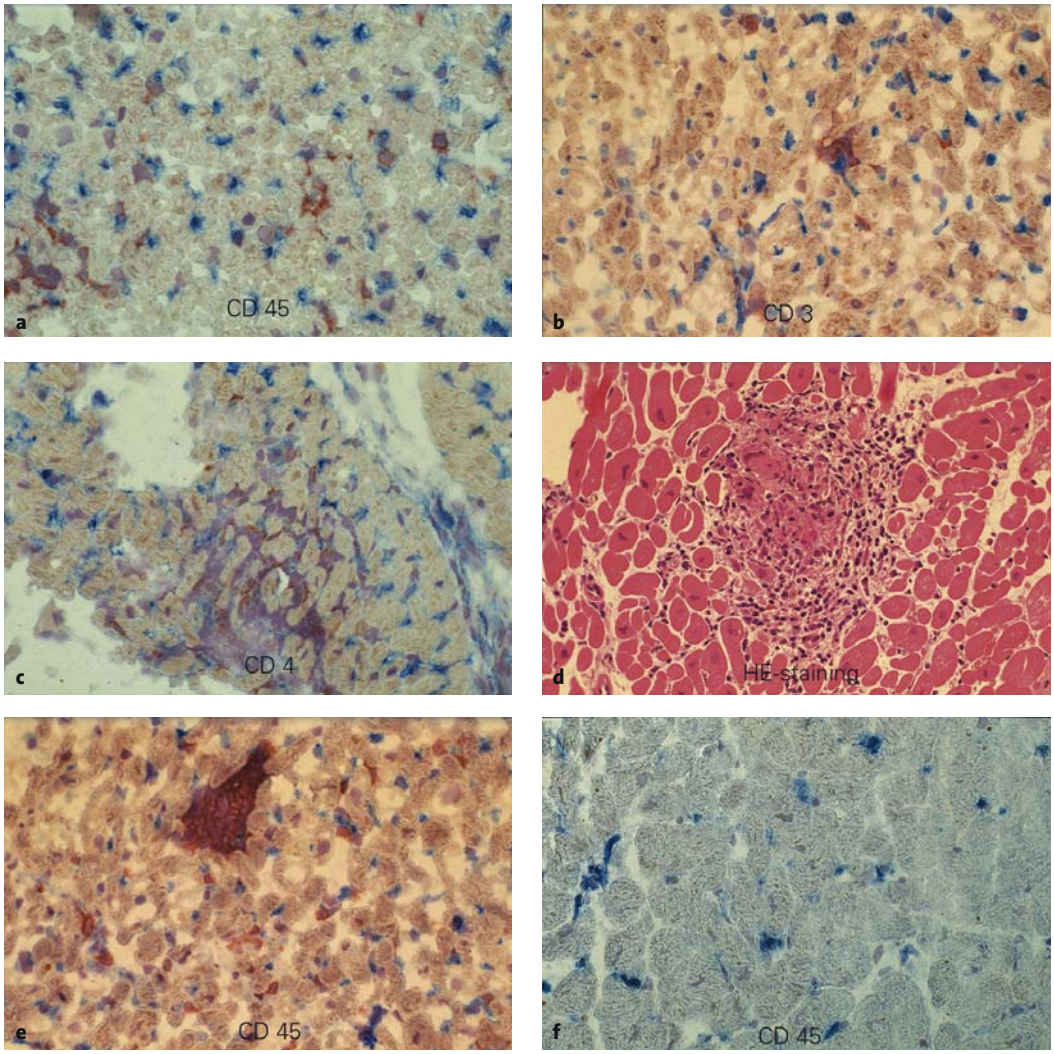
1. Acute (active) myocarditis: a clear-cut infiltrate (diffuse, focal, or confluent), quantitated by immunohistochemistry (≥ 14 lymphocytes and macrophages/mm^2). Necrosis or degeneration are compulsory, fibrosis should be graded.

Table 1. Histological criteria for the diagnosis of myocarditis according to the Dallas classification [15] and World Heart Federation (WHF) criteria [2, 14]. DCMI: inflammatory dilated cardiomyopathy.

Tabelle 1. Histologische Kriterien der Diagnose Myokarditis nach den Dallas-Kriterien [15] und den Kriterien der World Heart Federation (WHF) [2, 14]. DCMI: inflammatorische dilatative Kardiomyopathie.

		Histopathology				
	Dallas terminology	**Infiltrate**	**Myocytolysis**	**Edema**	**WHF terminology**	**Immunohistochemistry**
First biopsy	Active myocarditis	+	+	+	Active myocarditis	Corresponds to Dallas criteria[a]
	Borderline myocarditis	+	–	–	Chronic myocarditis/DCMI	≥ 14 lymphocytes/mm^2
Second biopsy	Ongoing myocarditis	+	+	+	Ongoing myocarditis	Corresponds to Dallas criteria[a]
	Resolving/healing myocarditis	+	–	–	Resolving or chronic myocarditis/DCMI	≥ 14 lymphocytes/mm^2
	Resolved myocarditis	–	–	–	Resolved myocarditis	< 14 lymphocytes/mm^2

[a] Immunohistochemistry aids in subclassification of infiltrating cells



Figures 1a to 1f. Immunohistochemical and histological staining of endomyocardial biopsies.
a, b) Chronic myocarditis with scattered lymphocytic infiltrates.
c–e) Acute myocarditis with a large infiltrate of T-cells.
f) Biopsy without inflammatory infiltrate.

Abbildungen 1a bis 1f. Immunhistochemie und Histologie von Endomyokardbiopsien.
a, b) Chronische Myokarditis mit eingestreuten lymphozytären Infiltraten.
c–e) Akute Myokarditis mit großen T-Lymphozyten-Infiltraten.
f) Kontrollbiopsie (ohne Infiltrate).

2. Chronic myocarditis: an infiltrate of ≥ 14 lymphocytes and macrophages/mm^2 (diffuse, focal, or confluent) quantitated by immunohistochemistry. Necrosis or degeneration are usually not evident, fibrosis should be graded.
3. No myocarditis: no infiltrating cells or < 14 leukocytes/mm^2.

Subsequent biopsies:

1. Ongoing (persistent) myocarditis. Criteria as in 1 or 2 (features of an acute or chronic myocarditis).
2. Resolving (healing) myocarditis. Criteria as in 1 or 2 but the immunologic processes are sparser than in the first biopsy.
3. Resolved (healed) myocarditis. Criteria correspond to the Dallas classification.

The presence of chronic inflammatory cells (e.g., lymphocytes, monocytes or macrophages) defined by histology and/or immunohistochemistry in association with the cardiomyopathic changes define dilated cardiomyopathy with inflammation (DCMI).

By the use of these quantitative criteria of 14 infiltrating cells (lymphocytes and macrophages/mm^2) consensus could be reached in the large majority of cases on DCMI (inflammatory cardiomyopathy).

Autoreactive Inflammatory Cardiomyopathy

If no viral or bacterial RNA or DNA is detected but inflammation is present, the diagnosis is autoreactive myocarditis or inflammatory cardiomyopathy. Infection is causing an antiviral humoral and cellular immune response. Both direct viral cytotoxicity and lytic effect and the antiviral immune response can initiate inflammation and

Table 2. Antibodies to cardiac antigen and their possible cross-reactivity and pathomechanism. Ach: acetylcholine; AH: aconitate hydratase; AMA: antimitochondrial antibody; AMLA: antimyolemmal antibody; ANA: antinuclear antigen; ANCA: anti-neutrophil cytoplasmic antigen; ANT: adenine nucleotide translocator; ASA: antisarcolemmal antibody; CK: creatine kinase; DLD: dihydrolipoamide dehydrogenase; hsp: heat-shock protein; NADD: nicotinamide-adenine dinucleotide dehydrogenase; PK: pyruvate kinase; SR-Ca-ATPase: sarcoplasmic reticulum calcium ATPase; UCR: ubiquinol-cytochrome-c reductase.

Tabelle 2. Antikörper gegen Herzantigen und ihre möglichen Kreuzreaktionen und Pathomechanismen. Ach: Acetylcholin; AH: Akonitat-Hydratase; AMA: antimitochondriale Antikörper; AMLA: antimyolemmale Antikörper; ANA: antinukleäre Antikörper; ANCA: antineutrophile zytoplasmatische Antikörper; ANT: Adeninnukleotid-Translokator; ASA: antisarkolemmale Antikörper; CK: Kreatinkinase; DLD: Dihydrolipoamid-Dehydrogenase; hsp: Hitzeschockprotein; NADD: Nikotinamid-Adenin-Dinukleotid-Dehydrogenase; PK: Pyruvatkinase; SR-Ca-ATPase: sarkoplasmatische Retikulum-Calcium-ATPase; UCR: Ubichinol-Cytochrom-c-Reductase.

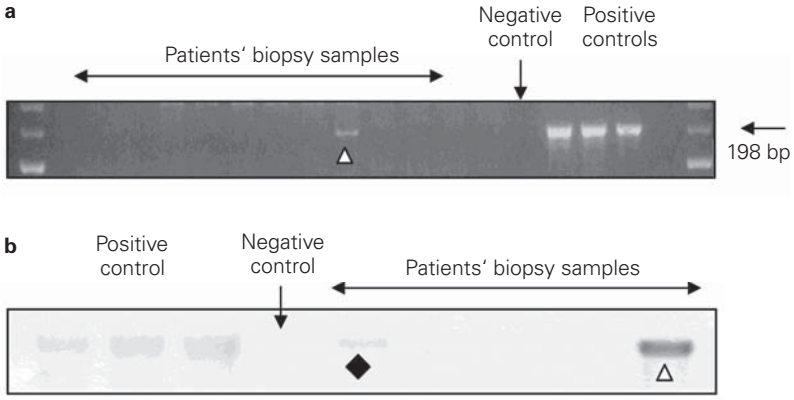
Antigen	Antibody	Cross-reactivity[a]	Pathomechanism[a]	Author (for references see [16])
Actin	Anti-actin	Unknown	Unknown	Maisch 1993
Ach-receptor	Anti-Ach	Unknown	Bradycardia?	Goin 1999
AH	Anti-AH	Unknown	Impairment of energy metabolism?	Pankuweit 1997
PK	Anti-PK			
DLD	Anti-DLD			
CK	Anti-CK			
ANT	Anti-ANT	Enterovirus?	Impairment of energy metabolism	Schulze 1995
Beta1 receptor	Anti-β1	Enterovirus?	Positive chronotropic*	Wallukat 1995
Beta1 receptor	Anti-β1		Negative inotropic?	Limas 1990
Ca^{2+} channel	Anti-Ca^{2+}	ANT? Enterovirus?	Unknown	Schulze 1999
Carnitine	Anti-carnitine		Unknown	Otto 1999
Conduction system	Anti-sinus	Unknown	Conduction defect?	Maisch 1986
	Anti-AV node			
	Anti-Purkinje			
Desmin	Anti-desmin		Unknown	Obermayer, Maisch 1987
Hsp60	Anti-hsp60	Multiple	Unknown	Portig 1998
Hsp70	Anti-hsp70			
Vimentin	Anti-vimentin			
Laminin	Anti-laminin		Unknown	Maisch 1987
Mitochondria/microsomes	AMA	Multiple*	Inhibition of sarcosine dehydrogenase	Klein 1990, Pohlner 1997
Myolemma	AMLA	Enterovirus*	Lytic*	Maisch 1993
Myosin	Anti-myosin		Negative inotropic?	Wittner, Maisch 1983
Myosin	Anti-myosin	Enterovirus?	Negative inotropic?	Caforio 1996
NADD	Anti-NADD	Unknown	Impaired energy metabolism?	Pohlner 1997
UCR	Anti-UCR			
Nuclear antigen	ANA	Unknown	Immune complex-mediated	Naparstek 1993
ENA	ENA		Degranulation of neutrophils?	
	ANCA		AV block	
	Anti-SSA			
	Anti-SSB			
Sarcolemma	ASA	Enterovirus*	Lytic*	Maisch 1987
SR-Ca-ATPase			Metabolic interactions	Khaw 1995

a*: experimentally proven; ?: hypothetical

myocytolysis. Following infection, autoreactivity against myocardium can develop based on the cross-reactivity or activation of the autoreactive clone. Anticardiac autoreactivity is regulated by the T-suppressor and T-helper cells and the humoral and cellular effector mechanisms resulting in the clinical forms of acute, subacute, and chronic forms of myocarditis or perimyocarditis. Cytotoxic effect of the cellular immunity, various cytokines (e.g., tumor necrosis factor-[TNF-]α, interleukin-[IL-]6), and cardiac autoantibodies (Table 2) can cause or contribute to the inflammation and myocytolysis. If the myocardial infection cannot be proven by PCR or in situ hybridization and at the same time there is an impairment of myocardial contractility, the diagnosis of autoreactive inflammatory cardiomyopathy can be established.

Viral cardiomyopathy

Viral RNA or DNA can be present in inflammatory cardiomyopathy or in dilated cardiac dysfunction without inflammation. The spectrum of the infectious agents that could be involved in the viral cardiomyopathy varies with the geographic



Figures 2a and 2b. Molecular biological investigation of endomyocardial biopsies.
a) Detection of enterovirus-positive endomyocardial biopsies by polymerase chain reaction (PCR). Negative and positive controls and base-pair marker (bp) are indicated.
b) Southern blot hybridization analyses made from the above indicated agarose gel (for this reason the wrong way round). An additional positive sample (◆) was detected because of the increased sensitivity using Southern blot hybridization.

Abbildungen 2a und 2b. Molekularbiologische Untersuchungen von Endomyokardbiopsien.
a) Nachweis von enteroviraler RNA mittels Polymerase-Kettenreaktion (PCR). Negative und positive Kontrollen sowie Basenpaare (bp) sind angegeben.
b) Southern-Blot-Hybridisierung der obigen PCR im Agarosegel (deshalb ist die Reihenfolge umgedreht). Eine zusätzliche positive Probe (◆) lässt sich infolge der erhöhten Sensitivität bei Southern-Blot-Hybridisierung zeigen.

region, the patient's age, application of different therapeutic procedures, and additional diseases. Numerous viruses may be associated with clinical evidence of myocarditis [17–19]. The parvovirus B19, enteroviruses, and adenoviruses represent the most commonly identified etiologic agents of human viral cardiomyopathy [20, 21].

Both *coxsackieviruses* A and B may produce myocarditis, although infection with coxsackie B is more common [22, 23]. At least 50% of healthy active adults have detectable serum antibodies to coxsackie B, indicating prior infection. Depending on the investigated population the frequency in the Marburg PCR registry was 8% (Figures 2a and 2b). Active replication could be detected by demonstration of minus strands by polymerase chain reaction (PCR) [22]. The myocardium appears to be particularly susceptible to the effects of coxsackie infection because of the affinity of myocardium for the viral particles. Noutsias et al. [24] have found human coxsackie-adenovirus receptor (hCAR) co-localized with integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ on the cardiomyocyte sarcolemma and upregulated in DCM. This study suggested that low hCAR abundance may render normal human myocardium resistant to CAR-dependent viruses, whereas reexpression of hCAR, as observed in DCM, may determine cardiac susceptibility to viral infections.

Adenoviruses account for 3–5% of acute respiratory infections in children but for < 2% of respiratory illnesses in civilian adults. Nearly 100% of adults have serum antibody to multiple serotypes. The frequency of this virus in PCR-positive DCM patients is 6% in the Marburg registry. Similar findings were reported by Pauschinger et al. [25] revealing 12/94 positive patients (12.8%) by nested PCR. The detection of adenovirus was associated with considerably reduced graft survival after cardiac transplantation in a pediatric population [26].

Parvovirus B19 infects most humans early in life without any major sequelae. It was recently recognized that parvovirus B19 can cause myocarditis and either latent or active viral cardiomyopathy with high virus copy numbers in endomyocardial biopsies [27]. Mean number of viral copies detected in patients with DCMI was 2,013 in comparison to 57 copies detected in DCM and 44 copies detected in HCM. In the recent PCR series parvovirus B19 has been observed in 30% up to 67% of investigated endomyocardial biopsy samples of patients with DCM and myocarditis [20, 21].

Although clinically apparent myocarditis is rare in *influenza*, the presence of preexisting cardiovascular disease greatly increases the risk of morbidity and mortality [28]. During epidemics, 5–10% of infected patients may experience cardiac symptoms [29]. Postmortem findings in fatal cases include biventricular dilatation, with evidence of a mononuclear infiltrate, especially in perivascular areas. The frequency of this virus in PCR-positive DCM patients is 0.5% in the Marburg registry.

Unrecognized infection with *cytomegalovirus* (CMV) is common in childhood, and the majority of the adult population has antibodies to CMV [30]. Primary infection after the age of 35 years is uncommon, and generalized infection usually occurs only in immunosuppressed patients [31]. The current frequency of this virus in PCR-positive DCM patients is 3% in the Marburg registry.

Clinical cardiac involvement in *hepatitis* is rare. There are contested data implicating hepatitis virus C (HCV) infection as an etiologic factor in at least some cases of human viral cardiomyopathy [32]. Fulminant myocarditis with congestive heart failure, hypotension, and death may occur in rare cases.

Cardiac involvement occurs in about one quarter to one half of patients infected with *human immunodeficiency virus* (HIV) [33]. However, it leads to clinically apparent heart disease in

only approximately 10%. Congestive heart failure due to left ventricular dilatation and dysfunction is the most common finding [34].

Aims of the BMBF Project IKARIUS: Inflammatory/Familial Cardiomyopathy: Is there a Link to Autoimmune Diseases?

In a yet not defined part of patients with inflammatory/familial cardiomyopathy, the phenotype DCM is assumed to be the end stage of a multifactorial etiopathogenetic pathophysiology. Precipitating factors include enhanced autoimmunity, predisposition for viral infections, environmental factors in addition to a specific "genetic background" of the individual patient [35, 36]. It is unresolved, whether the susceptibility to immunologically mediated myocardial damage reflects the presence of genetic risk factors shared by other autoimmune diseases, such as psoriasis, arthritis, thyroiditis, and asthma [37] or is cardiospecific with individual realization factors. Epidemiologic investigations in patients with autoimmune diseases have shown, that in addition to a specific genetic alteration secondary inducing factors are responsible for the onset of the disease, which may lead to different phenotypes of autoimmune diseases in a single family. From this the working hypothesis has been derived that inflammatory DCM is the end stage of an autoimmune cardiac disease that goes along with the activation of susceptibility genes, which are common to other autoimmune diseases.

The original aims of the project are

1. the inclusion of patients within the cohort of the network with DCM (ejection fraction [EF] < 45%, left ventricular end-diastolic diameter > 56 mm) and, in addition,
2. the inclusion of all relevant data regarding a possible familial, infectious or autoimmune etiology of the disease. This should help us get for the first time data on the proportion of patients with sporadic or familial inflammatory or non-inflammatory DCM within the cohort of patients with DCM.

To reach this goal, a questionnaire was added to the CRFs, to ascertain data regarding a possible familial history for each patient not only for cardiac diseases, but also for autoimmune disorders. A pedigree of all patients is available. Data regarding a possible infectious or inflammatory etiology of the disease are available by investigation of the endomyocardial biopsy and peripheral blood.

Derived from the database, the biopsy and serum bank, further aims of the project are

1. the search for a genetic link to autoimmune diseases in patients with familial/inflammatory DCM and the
2. the search for a genetic predisposition for autoimmune diseases in patients with DCM, especially inflammatory diseases.

To reach these aims, peripheral blood of all included patients was sent first to the biomaterial bank in Berlin, Germany. DNA extracted from peripheral blood was distributed to the laboratories working in a second project for the detection of genetic abnormalities in the genes for structural proteins, which are known to be associated with DCM. In addition, we started screening by a candidate gene approach in endomyocardial biopsies of patients with DCM using microchip technology and the investigation for polymorphisms in the HLA class II DQ locus in the patient cohort.

So far, we have included 53 index patients (45 male, eight female) with an age of 49 ± 13 years, an EF of 29% ± 7%, and a left ventricular end-diastolic diameter of 70 ± 10 mm. To identify the patients within the hospital (ambulances, wards), we have screened a total of 895 patients (by May 23), who fulfilled at least one entrance criterion (Henry Index > 117%), using our echocardiography database. All patients, who were screened, were included in our original database, from which inclusion in the IKARIUS study was organized. So far, only twelve patients were not able or denied to be enrolled in the trial. Blood samples and endomyocardial biopsies from all patients were taken for further investigations, a pedigree of all patients including data for a familial history for heart diseases or autoimmune diseases is available.

So far, we identified 13 families (25%) with a positive familial history for DCM, three of them with a DCMI and three of them with an infectious etiology of DCM. In six families we found, in addition, a history of autoimmune diseases, including juvenile diabetes, asthma, and psoriasis. In the remaining 40 patients with sporadic DCM, we found 25 patients without inflammation, six of them with a viral etiology. In 15 patients with sporadic DCMI, we identified five with a viral etiology. In 15 of the 40 patients with sporadic DCM we detected a positive familial history for autoimmune diseases. As the recruitment of the patients is in progress, patient numbers change weekly. Outcome measurements regarding clinical data are available in 1 year from now the earliest, when the follow-up data for the first 50 patients will be evaluated.

Based on data from the literature, we assumed a proportion of 30% of all patients to suf-

fer from familial DCM; at present, the proportion is 25%. The number of patients with an infectious/inflammatory etiology of the disease is higher than expected (about 50% of patients with familial DCM, > 50% in the group of patients with sporadic DCM). In addition, the proportion of a positive familial history for autoimmune diseases is higher than expected (about 50% in both groups).

In parallel to the recruitment of patients we started the search for genes which might be disregulated in the endomyocardial biopsies from the 54 patients with DCM versus DCMI and myocarditis. Each biopsy was compared to control tissue by microarray analysis. A microarray chip with 560 genes involved in inflammatory diseases (Lab Arraytor human 500-1 cDNA) designed by the SIRS-Lab company (Jena, Germany) was used for these examinations. Because of the minimal starting material after RNA extraction from biopsies, we used the BD Atlas™ SMART™ Fluorescent Probe Amplification Kit (BD Biosciences) to amplify the extracted RNA to produce results that were comparable to those from pure RNA.

cDNA synthesis was performed by a PCR-based method (BD Super SMART™ cDNA synthesis) to produce high-quality cDNA, monofunctional N-hydroxysuccinimide-activated fluorescent dyes (Cy3, Cy5) were coupled to the cDNA. Then, a long-distance PCR (BD Advantage™ 2 PCR Kit) was carried out to amplify full-length cDNAs. After purification of the long-PCR products, probe synthesis was performed according to the manufacturer's instruction. Hybridization and analysis of the microarrays were performed at SIRS-Lab.

After scanning and analysis of the microarrays, differences in nine genes were detected. Significant differences could be detected between patients with DCM versus myocarditis and DCMI versus myocarditis. No differences in gene expression were detected in patients with DCM versus DCMI.

mRNA expression was different in genes encoding for complement components involved in the regulation of autoimmune diseases, as well as for genes involved in induction and regulation of apoptotic processes. Further on, differences were detected in genes involved in allergic or autoimmune diseases and in the regulation of the contraction/relaxation cycle. Further statistical analyses are in progress. These results are the starting point for investigations on the endomyocardial biopsies of the recruited patient cohort, as verification of the results from the microship analysis by the use of real-time RT-PCR in all patients' samples is in progress.

To identify genetic factors regarding the immune system probably controlling the susceptibility to myocarditis and DCM, HLA class II gene polymorphisms were analyzed by using the PCR/SSP (sequence-specific primer) technique. Except for a weak association with HLA-DR4, myocarditis or DCM predisposing effects of HLA genes have not been reported so far [38]. However, the HLA class II haplotype DQA1*0301/DQB1*0302 (DQ8) occurring in 80% of humans with juvenile diabetes confers strong susceptibility to myocarditis when expressed in murine class II-deficient ($mII^{-/-}$) transgenic mice [39, 40].

Thus, we typed HLA class II DQB1 alleles in 40 unrelated DCM and myocarditis patients whose diagnoses were established by clinical, echocardiographic, hemodynamic and immunohistochemical data. The control group consisted of 36 patients with cardiac diseases other than DCM (arterial hypertension and arrhythmias) and an EF > 60%. Only the DQB1 alleles were amplified by the DQ typing method (according to Olerup SSP AB, Sweden) and did not influence the DQB2 and DQB3 genes.

Conversely to the mouse model, we found a greater incidence of DQB1*0301 alleles in DCM and myocarditis patients when compared to the controls. The frequencies of the DQB1*0301 allele in the DCM, DCMI, and myocarditis patient groups were 38%, 50%, and 33%. Sequence comparison of the DQB1*0302 and DQB1*0301 alleles were overrepresented in patients with DCMI and myocarditis and revealed a single amino acid exchange (Leu > Tyr at position 26). Currently, we perform statistical analysis on DQB1*0301 expression to determine whether this allele has prognostic significance for deterioration of left ventricular function. High resolution of DQB1*0602 expression which is dominant protective in juvenile diabetes, is in progress.

The close cooperation between the centers contributing to IKARIUS will enable us for the first time to assess the role of autoimmune predisposition to the manifestations of myocardial (and perimyocardial) inflammation on a familial level. The interrelationship to viral infection and to other autoimmune disorders will be analyzed systematically and, in a selected patient cohort, also be related to the upregulation of proteins in biopsies by microchip technology.

The results of the trial should have impact on the better understanding of the etiology and mechanism of disease induction in different subgroups of the patients, possibly resulting in treat-

ment trials, for example, in patients with infectious etiology of the DCM.

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