Definition of Inflammatory Cardiomyopathy (Myocarditis): On the Way to Consensus

A Status Report

Bernhard Maisch, Irene Portig, Arsen Ristic, Günther Hufnagel, Sabine Pankuweit[1]

Abstract

This article reviews the current state of consensus reached for the diagnosis of myocarditis and dilated cardiomyopathy on the basis of conventional histopathological and immunohistochemical methods for inflammatory infiltrates in addition to molecular biological methods for persistence of viral genome in endomyocardial biopsies.

Additionally, a brief overview is presented stating the current knowledge on effector mechanisms of the immune system in myocarditis and dilated cardiomyopathy.

Key Words: Myocarditis · Dilated cardiomyopathy with and without inflammation · Effector systems of the immune system · Viral heart disease

Definition der entzündlichen Kardiomyopathie (Myokarditis): Auf dem Weg zu einem Konsens. Ein Statusreport

Zusammenfassung

Die Definition der Kardiomyopathie mit und ohne Entzündungsreaktion sowie die mögliche Beteiligung von Effektormechanismen des humoralen und zellulären Immunsystems an ihrer Pathogenese war in den letzten Jahren Gegenstand der Forschung.

Um einen Konsens für die Diagnose dilatative Kardiomyopathie mit und ohne Entzündung zu erreichen, kamen in Marburg Experten auf den Gebieten Histopathologie und Virologie zusammen, um Kriterien zu erarbeiten, die die 1987 publizierten Dallas-Kriterien zur Diagnostik der Myokarditis erweitern und mit Hilfe neuer Technologien präzisieren.

Histopathologie: Für die Diagnose „Myokarditis“ wird ein Minimum von 14 Leukozyten/mm^2, bestehend aus T-Lymphozyten (CD3) oder aktivierten T-Lymphozyten (zum Beispiel CD45RO), gefordert (allerdings dürfen bis zu vier Makrophagen eingeschlossen werden). Ein fokaler entzündlicher Prozess wird beschrieben, wenn sich mindestens drei Lymphozyten in einem Nest außerhalb von Gefäßen befinden. Wenn solche Foci vorhanden sind, kann eine Myokarditis diagnostiziert werden, auch wenn nicht die kritische Zahl von 14 Leukozyten/mm^2 erreicht werden. Wenn Leukozyten, fokal oder diffus, in fibrotischen Arealen auftreten, kann man von einem reparativen Prozess sprechen.

In Anlehnung an die Dallas-Kriterien (1987 von Aretz et al. publiziert, siehe Tabelle 1) unterscheidet man bei der ersten Biopsie die akute (mit Myozytolyse) von der „Borderline“ (ohne Myozytolyse) bzw. keiner Myokarditis. Werden konsekutive Biopsien entnommen, kann eine persistierende (= akute) von einer abheilenden und abgeheilten Myokarditis unterschieden werden. Die neue modifizierte Klassifikation ist quantitativ:

1. Akute Myokarditis: entzündliches Infiltrat (diffus, fokal oder konfluierend) mit ≥ 14 Leukozyten/mm^2. Zur Unterstützung werden immunhistochemische Verfahren zur Subklassifizierung der Leukozyten herangezogen. Nekrose ist obligat, Fibrose kann vorhanden sein;
2. chronische Myokarditis: entzündliches Infiltrat (diffus, fokal oder konfluierend) mit ≥ 14 Leukozyten/mm^2. Keine Nekrose erforderlich, Fibrose ist möglich;
3. keine Myokarditis: keine oder weniger als 14 Leukozyten/mm^2;
4. persistierende Myokarditis (zweite Biopsie): Kriterien wie in 1 oder 2;
5. abheilende Myokarditis (zweite Biopsie): Kriterien wie in 1 oder 2, der immunologische Prozess ist aber spärlicher;
6. abgeheilte Myokarditis (zweite Biopsie): entsprechend den Dallas-Kriterien.

Virologie: Da beim Erwachsenen die Anzucht von Viren aus Biopsien meist erfolglos verläuft, werden sensitivere molekularbiologische Methoden, wie die PCR und/oder die In-situ-Hybridisierung eingesetzt. Im Falle von HIV, Hepatitis C und Zytomegalie und Borreliose wird die serologische Diagnostik zusätzlich verwendet. In Marburg wurde Konsens darüber erreicht, welche Methodik im Einzelnen im Hin-

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blick auf Sensitivität und Reproduzierbarkeit zu bevorzugen ist.

Im zweiten Abschnitt der Arbeit werden Effektormechanismen des Immunsystems vorgestellt, für die in tierexperimentellen Studien bzw. Untersuchungen an Serum oder Endomyokardbiopsien vom Menschen ein möglicher Zusammenhang mit der Pathogenese der dilatativen Kardiomyopathie mit oder ohne Entzündungsreaktion gefunden wurde. In Tabelle 2 sind Autoantikörper sowie deren möglicher Pathomechanismus dargestellt. Im Anschluss werden neuere Untersuchungen zur möglichen Rolle von Zytokinen, Adhäsionsmolekülen und Immunkomplexen kurz zusammengefasst.

Schlüsselwörter: Myokarditis · Dilatative Kardiomyopathie mit und ohne Entzündung · Effektorsysteme des Immunsystems · Virale Herzerkrankung

The definition of cardiomyopathy with and without inflammation and the contribution of different effector and modulator organs of the immune system to the clinical manifestations have been a continuous and sometimes controversial effort of scientists working in this field over the past decades.

Historically the investigation of the immunological effector and modulator mechanisms began with the demonstration of infiltrating lymphocytes in endomyocardial biopsies or at necropsy (reviewed in [1]), continued with demonstration of antibodies against different cardiac tissue components, whose debated relevance was partly defined by in-vitro or in-vivo experiments (reviewed in [4]). Organ-specificity, cross-reactivity to the microbial and antigenic mimicri are issues still at stake, particularly when taking into account that by PCR we are able today to demonstrate the viral or bacterial pathogen in the myocardial tissue specimen (reviewed in [23]). The demonstration of mediators, cytokines and adhesion molecules and their effect on myocytes, endothelial cells and fibroblasts boostered the discussion on humoral factors involved in the pathogenesis of cardiomyopathy again. At present a complex concept of the pathogenesis has evolved and reached consensus "in principle".

It is the purpose of the following overview to systematically and briefly describe the present state of consensus reached for the diagnosis of myocarditis and dilated cardiomyopathy from the point of view of immunology and to outline areas of further research or ongoing controversy by taking stock of our present knowledge in cellular, antibody and immune complex and cytokine-mediated immunoreactivity.

The Cellular Effector Mechanisms – Role of Lymphocytes and Macrophages

The World Health Organisation/International Society and Federation of Cardiology Task Force (ISFC, presently World Heart Federation, WHF) on the Definition and Classification of Cardiomyopathies from 1995 introduced several changes in the definition and classification of heart muscle diseases [22]. Since then, the term cardiomyopathy is no longer reserved for the idiopathic forms but can be used interchangeably with the term heart muscle diseases. Right ventricular cardiomyopathy, valvular, hypertensive, ischemic or inflammatory cardiomyopathy have been introduced for the first time as cardiomyopathies.

This new definition of the hemodynamically identified group of dilated cardiomyopathies also comprises inflammatory cardiomyopathy. It has been defined as "myocarditis in association with cardiac dysfunction". Idiopathic, autoimmune, and infectious forms of inflammatory cardiomyopathy were recognized. The definition of inflammation was left open with reference to the Dallas classification, however, which distinguished by conventional HE staining active myocarditis in the first biopsy, ongoing (persistent) myocarditis, healing or healed (resolved) in a subsequent biopsy.

Within the context of inflammatory cardiomyopathy terms such as active/acute or chronic myocarditis, the association between pericardial disease and myocarditis (perimyocarditis), autoreactive or virally induced myocarditis needed further explanation and diagnostic consensus. Therefore, the WHF Council on Cardiomyopathies formed 2 expert committees, one with international experts on histopathology and immunohistochemistry, and another one with international experts on the molecular diagnoses of infective or viral cardiomyopathies, which convened in separate sessions in Marburg, Germany[2]. Both committees formulated new definitions on chronic myocarditis and inflammatory dilated cardiomyopathy and on viral cardiomyopathies which are reported here in brief and will be published in extenso soon.

Expert Committee on the Histology of Dilated Inflammatory Cardiomyopathy

The committee defined myocarditis as a process characterized by an inflammatory infiltrate of the myocardium. In acute (active) myocarditis necrosis and/or degeneration of adjacent myocytes is required, whereas in chronic myocarditis necrosis is not an obligatory feature by definition (Figures 1a and 1b). When referring to the Dallas criteria the term acute myocarditis corresponds to active myocarditis, chronic myocarditis may be defined as comprising borderline or healing myocarditis.

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, respectively.

The panel has chosen for the definition of myocarditis a minimum of 14 infiltrating leukocytes/mm^2 preferably T lymphocytes (CD45RO) or activated T cells (e. g. CD45RO) + (up to 4 macrophages may be included in this total amount). The total number is more than 2 standard deviations above the number of leukocytes found in control tissue [4, 22, 23]. In case of nests of leukocytes (≥ 3 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 and distribution of fibrosis should be described similarly as no (Grade 0), mild (Grade 1), moderate (Grade 2), or severe (Grade 3). Localization or formation of fibrosis should be outlined as endocardial, replacement, or interstitial. Thus the following terminology was adopted (Table 1):

First Biopsy

1. Acute (active) myocarditis: a clear-cut infiltrate (diffuse, focal or confluent) of ≥ 14 leukocytes/mm^2 (preferably activated T cells). The amount of the infiltrate should be quantitated by immunohistochemistry. Necrosis or degeneration are compulsory, fibrosis may be absent or present and should be graded;
2. chronic myocarditis: an infiltrate of ≥ 14 leukocytes/mm^2 (diffuse, focal or confluent, preferably ac-

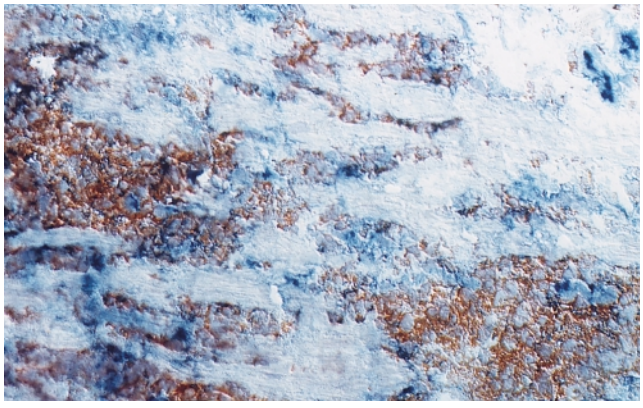


Figure 1a – Abbildung 1a

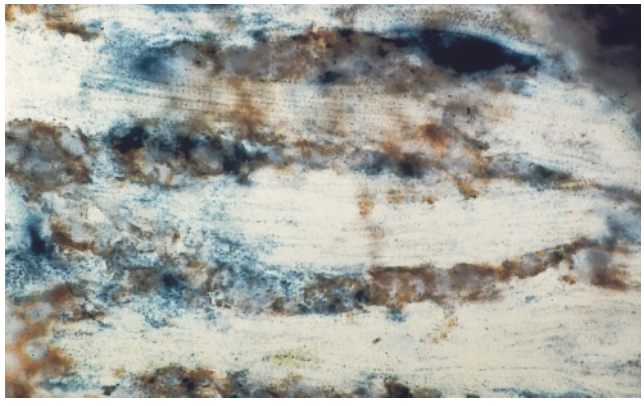


Figure 1b – Abbildung 1b

Figures 1a and 1b
Cryostat sections demonstrating CD4 positive lymphocytes in chronic myocarditis. a) At low magnification (80×); b) at high magnification (400×) using mabs to CD4 (Becton).

Abbildungen 1a und 1b
Kryostatschnitt der CD4-positiven Lymphozyteninfiltrate bei chronischer Myokarditis. a) Als Übersicht (Vergrößerung × 80); b) als Ausschnitt (Vergrößerung × 400). Markierung mit Anti-CD4-monoklonalem Antikörper (Becton).

[2] The expert committee on histopathology and histochemistry included B. Bültman, S. Factor, H.-J. Gröne, G. Hufnagel, K. Kawamura, U. Kühl, B. Maisch, E. J. Olsen, S. Pankuweit and R. Virmani, invited consultants were W. McKenna, P. J. Richardson, G. Thiene, H.-P. Schultheiß and M. Sekiguchi. The expert committee on viral heart disease included C. Aepinus, K. Aitken, E. Arbustini, L. Archard, C. Baboonian, N. Bowles, S. Broor, G. Hufnagel, R. Kandolf, P. Liu, B. Maisch, A. Matsumori, W. McKenna, S. Pankuweit, M. Pauschinger, H.-P. Schultheiß, W. Slenczka, M. Sole, K. K. Talwar, J. Towbin and S. Tracy. Invited consultants were A. Bayes de Luna, J. F. Goodwin, P. J. Richardson.

	Diagnosis	Dallas Classification (Histopathology) Infiltrate	Myocytolysis	Edema	WHF-Classification
1st biopsy	Active myocarditis	+	+	+	≥ 14 cells/mm²
	Borderline myocarditis	+	–	–	≥ 14 cells/mm²
2nd biopsy	Ongoing myocarditis	+	+	+	≥ 14 cells/mm²
	Resolving/healing myocarditis	+	–	–	≥ 14 cells/mm²
	Resolved myocarditis	–	–	–	< 14 cells/mm²

Table 1
Conventional histological criteria for the diagnosis of myocarditis according to the Dallas classification [1] and the new WHF-classification.

Tabelle 1
Konventionelle lichtmikroskopische Kriterien der Myokarditis (Dallas-Klassifikation und die neue WHF-Klassifikation).

tivated T cells). Quantification should be made by immunohistochemistry. Necrosis or degeneration are usually not evident, fibrosis may be absent or present and should be graded;
3. no myocarditis: no infiltrating cells or < 14 leukocytes/mm².

Subsequent Biopsies
4. Ongoing (persistent) myocarditis: criteria as in 1 or 2 (features of an acute or chronic myocarditis);
5. resolving (healing) myocarditis: criteria as in 1 or 2 but the immunological process is sparser than in the first biopsy;
6. resolved (healed) myocarditis: corresponds to the Dallas classification.

The panel tested the new definition on the histology and immunohistology sections available from standard hematoxylin-eosin (HE) and van Gieson staining and on cryostat sections from the 2 centers evaluating the biopsies of the multicenter randomized double blind treatment trial on myocarditis (ESETCID, European Study on the Epidemiology and Treatment of Cardiac Inflammatory Disease [11]) with conventional HE staining and immunostainings for CD45RO, CD2, CD3, CD4, CD 8, CD79a, CD68 from the core lab in 56 patients, who entered ESETCID out of 1,615 patients screened for chronic myocarditis. By the use of the quantitative criteria of 14 infiltrating cells (lymphocytes and macrophages/mm²) consensus could be reached in the large majority of cases on inflammatory cardiomyopathy.

Definition of Chronic Myocarditis (DCMi)

The WHF Expert Committee on the Histology of Inflammatory Cardiomyopathy introduced chronic myocarditis explicitly as a histologically defined independent category (presence of a diffuse or focal leukocytic infiltrate or foci of lymphocytes associated with the presence of myocellular hypertrophy, focal or diffuse interstitial, replacement and/or perivascular fibrosis and non-obligatory microvascular changes) for dilated cardiomyopathies. 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 chronic myocarditis or dilated cardiomyopathy with inflammation (DCMi).

Thus histologically-wise chronic myocarditis was defined interchangeably with dilated cardiomyopathy with inflammation or inflammatory cardiomyopathy (DCMi). Considerable variability in the histological diagnosis of chronic myocarditis can often be resolved by immunostaining, which could be helpful in providing more uniform and quantitative criteria for the diagnosis of myocarditis and dilated cardiomyopathy and for the present and future treatment trials.

Expert Committee on the Definition of Viral Cardiomyopathy

Since isolation of the virus from swabs or tissue is possible only in the acute phase of infection it is unlikely to succeed with this method in patients with longer lasting diseases or chronic infections. Enteroviruses have therefore been effectively isolated only in pediatric patients. The isolation of the active virus remains a standard procedure but has the disadvantage of being time consuming. It was therefore deliberately excluded in this analysis.

A higher sensitivity was achieved with molecular techniques. It is well documented that molecular techniques e. g., gene amplification are significantly more sensitive than standard histochemical techniques for the detection of viral proteins.

Except for HIV, hepatitis C, cytomegalovirus and of the bacterium borrelia Burgdorferi infections serological assessment of antiviral antibodies appeared to be of

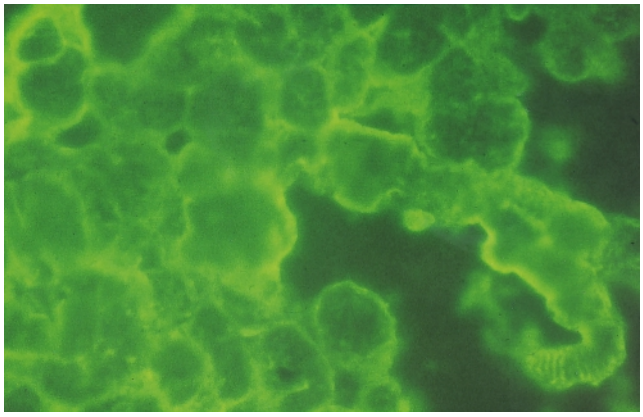


Figure 2a
Demonstration of IgG binding (FITC-labeled F(ab)2 antihuman IgG) in dilated cardiomyopathy with inflammation.

Abbildung 2a
Nachweis einer IgG-Bindung (FITC-markiertes antihumanes IgG, F(ab)2-Spaltprodukt) bei dilatativer Kardiomyopathie mit Inflammation. C3-IgM und IgA-Fixation sprechen für einen akuten immunologischen Prozess.

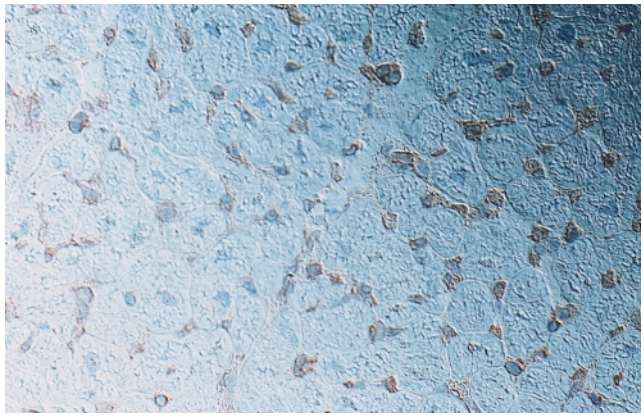


Figure 2b
Demonstration of major histocompatibility class II expression on capillaries and interstitial tissue, a common finding in heart failure patients.

Abbildung 2b
Nachweis einer vermehrten Expression von MHC-Klasse-II-Molekülen auf Kapillaren und im Interstitium, ein häufiger, oft wenig spezifischer Befund bei Herzinsuffizienz.

limited diagnostic value with respect to the actual disease status of the patients and for the critical issue if the viral genome is present in the myocardium.

The WHF expert panel reached a consensus on current diagnostic approaches to viral heart disease by means of an international, multicenter and blinded interlaboratory study. Detection of viral nucleic acid in the myocardium was regarded as indicative of virus infection of the heart. The PCR technique was selected for this study because of its rapidity, wide availability, high sensitivity, and specificity. In-situ hybridization, not carried out in this trial, offers near equivalent sensitivity to PCR combined with localization of the virus on the cellular level, with the draw back of the lack of rapidity. PCR primers can be designed to be specific to amplify any member of a particular virus group. The individual agent group can then be identified by direct sequencing of the PCR product.

The highest sensitivity and reproducibility for the detection of enteroviral genomes were achieved with frozen tissue (100%) in 5 out of 9 centers. Reverse transcription (RT) PCR of enterovirus RNA from fixed embedded tissue was less reliable, probably with false negatives and the frequent failure to amplify sequences from the processed mRNA of control house keeping genes. Detection of enterovirus sequences in formalin-fixed samples was less convincing. The incidence of hepatitis C virus in formalin-fixed tissue (15%) was remarkable. PCR for the genomic sequences of DNA viruses in formalin-fixed tissue is less critical and adeno- (12.5 to 22.5%), cytomegalo- (5%) and Epstein-Barr virus (2.5%) could also be detected in formalin-fixed tissue.

New data from 2 laboratories indicate that also Parvo B19 is a new, not yet well established cardiotropic virus with a prevalence well in the range of enterovirus (data from Marburg and by R. Kandolf, Tübingen (presented in Mannheim 2000).

As entero- and adenoviruses are probably the most common agents of viral heart muscle disease, RT-PCR is required to amplify these viral genomic RNA sequences. The centers' experience was that fresh frozen tissue (biopsy) is the material of choice, giving high sensitivity and specificity of detection. Nested PCR seems desirable to detect a low copy number of enteroviral RNA in chronic disease, but single-step PCR with Southern blot gave equivalent positive results in the tissue samples and dilution experiments in this study.

An advantage of PCR over slot-blot or in-situ hybridization techniques is that group-specific primers can be used to amplify viral sequences and the particular agent can be identified subsequently by direct nucleotide sequencing of the PCR product.

On the basis of the interlaboratory analysis the second WHF Expert Panel on Viral Cardiomyopathies has given for the first time a reliable comparative analysis of cardiac tissue samples infected in part with cardiotropic viruses. The high reproducibility of results for enterovirus positive samples in frozen material by the methods outlined here is an important step for the standardization of diagnostic criteria on viral or inflammatory cardiomyopathy. It has also clearly demonstrated that hepatitis C is an important RNA virus to be considered, as are DNA viruses e. g., adeno- and cytomegalovirus. These findings have to be taken into account on future diagnostic and therapeutic studies in the field of dilated cardiomyopathies and will be published in extenso shortly.

Antibodies in Dilated Cardiomyopathy with and without Inflammation

Autoantibodies belong to effector mechanisms of the immune system responsible for tissue damage in various autoimmune diseases. These autoantibodies are produced as a result of failure or breakdown of the mechanisms normally responsible for maintaining self-tolerance. Polyclonal activation of lymphocytes and cross-reactions between foreign and (neo-)self antigens (molecular mimicry) are important factors contributing to autoimmunity.

It is now widely accepted that the majority of antibodies directed against cardiac antigens, found in sera of patients with dilated cardiomyopathy, do not play a major pathogenetic role but are indicative of damage to cardiac tissue and show a tendency towards autoimmune disease [2, 10, 13].

However, experiments in animals have shown that autoantibodies may well have a share in compromising myocardial function. For example, antimyolemmal antibodies, detected by direct immunofluorescence, exhibit lytic properties towards isolated rat cardiomyocytes in the presence of complement or lymphocytes [9]. Antibodies to receptors of the sympathetic and parasympathetic nervous system have been shown to modulate myocardial performance in vitro [6, 8, 27]. Antibodies to the Ca^{2+} channel cross-reacting with the adenin-nucleotide translocator depressing myocardial function are an attractive but yet unproven hypothesis]24, 25]. Antibodies binding to mitochondrial proteins are believed to impair the energy metabolism of myocardial cells [19]. Antibodies binding to nuclear antigens (ANA, ENA) have been shown to form immune complexes and anti-neutrophil cytoplasmic antibodies (ANCA) are thought to degranulate activated neutrophils thereby leading to vasculitis in small vessels. Thereby, autoantibodies found in lupus erythematosus and panarteriitis nodosa can explain systemic complications including those of the heart [16].

Moreover, recent data on the improvement of myocardial function of patients with dilated cardiomyopathy following plasmapheresis to eliminate circulating antibodies show that we may still underestimate the role of autoantibodies in dilated cardiomyopathy with and without inflammation [15].

Methodology and Results

Autoantibodies directed against cardiac antigens have been detected in serum of patients with dilated cardiomyopathy for diagnostic purposes using indirect immunofluoresence on isolated rat cardiomyocytes and direct immunofluorescence on endomyocardial biopsies. The fluorescence patterns helps to distinguish antibodies directed against sarcolemmal, myolemmal and cytoplasmic proteins.

IgG bound to the autologous biopsy specimen has been detected in more than 80% of patients with myocarditis and inflammatory dilated cardiomyopathy, IgM and IgA in conjunction with complement C3 (a possible marker for the discrimination of acute and chronic disease) was found less frequently.

One- and two-dimensional immunoblot followed by n-terminal amino acid sequencing have been used to identify corresponding antigens of autoantibodies in serum of patients with dilated cardiomyopathy [18–20] (Table 2).

Since for most antibodies only in vitro pathophysiological relevance was demonstrated, they can be at present considered best to be diagnostic markers of cardiac myocytolysis and consecutive autoreactivity. In the hands of individual laboratories they serve an appropriate role as an indicator system of humoral activation.

Cytokines

Myocarditis induced by viral infection is believed to be one of the major pathogenetic mechanisms of inflammatory dilated cardiomyopathy. Although the etiology of progression to myocardial failure is not

Antigen	Antibody	Cross-reactivity with * experimentally proven ? hypothetical	Pathomechanism * experimentally proven ? hypothetical	Reference
Actin	α-Actin	Unknown	Unknown	Maisch et al. 1993 [9]
ACh-receptor	α-ACh	Unknown	Bradycardia?	Goin et al.1999 [6]
AH PK DLD CK	α-AH α-PK α-DLD α-CK	Unknown	Impairment of energy metabolism?	Pankuweit et al.1997 [18]
ANT	α-ANT	Enterovirus?	Impairment of energy metabolism	Schulze et al.1995 [25]
β1-receptor	α-b1	Enterovirus?	Pos. chronotropic*	Wallukat et al.1996 [27]
β1-receptor	α-b1		Neg. inotropic?	Limas 1990
Ca^{2+} channel	α-Ca^{2+}	ANT? Enterovirus?	Unknown	Schulze et al.1999 [24]
Carnitin	α-carnitin		Unknown	Otto 1998
Conduction	α-sinus α-AV node α-Purkinje	Unknown	Conduction defect?	Maisch et al.1986 [12]
Desmin	α-desmin		Unknown	Maisch 1994
hsp60 hsp70 Vimentin	α-hsp60 α-hsp70 α-vimentin	Multiple	Unknown	Portig et al. 1998 [20]
Laminin	α-laminin		Unknown	Maisch 1994
Myolemma	AMLA	Enterovirus*	Lytic*	Maisch et al. 1993 [9]
Myosin	α-myosin		Neg. inotropic?	Maisch 1987 [13]
Myosin	α-myosin	Enterovirus?	Neg. inotropic?	Carforio et al. 1996 [2]
NADD UCR	α-NADD α-UCR	Unknown	Impairment of energy metabolism?	Pohlner et al. 1997 [19]
Nuclear antigen ENA	ANA ENA ANCA α-SSA α-SSB	Unknown	Immune complex-mediated Degranulation of neutrophils? AV-Block	Naparstek et al. 1993 [16]
Sarcolemma	ASA	Enterovirus*	Lytic*	Maisch et al. 1993 [9]

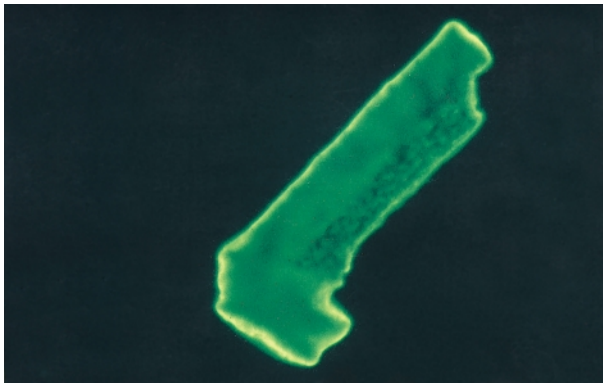
Table 2
Compilation of antibodies to cardiac antigen and their possible crossreactivity and pathomechanism (in alphabetical order) (ASA = antisarcolemmal antibody; AMLA = antimyolemmal antibody; ACh = acetylecholine; ANT = adenine nucleotide translocator; AH = aconitate hydratase; PK = pyruvate kinase; DLD = dihydrolipoamide dehydrogenase; CK = creatinine kinase; NADD = nicotinamideadenine dinucleotide dehydrogenase; UCR = ubiquinol-cytochrome-c reductase; hsp = heat shock protein; ANA = antinuclear antigen; ANCA = anti-neutrophil cytoplasmic antigen).

Tabelle 2
Übersicht antikardialer Antikörper, ihre diskutierte Kreuzreaktivität sowie Pathomechanismen (in alphabetischer Reihenfolge) (ASA = antisarkolemmale Antikörper, AMLA = antimyolemmale Antikörper; ACh = Azetylcholin; ANT = Adeninnukleotidtranslokator; AH = Akonitathydratase; PK = Pyruvatkinase; DLD = Dihydrolipoamiddehydrogenase; CK = Kreatinkinase; NADD = Nikotinamidadenindinukleotiddehydrogenase; UCR = Ubiquinol-cytochrome-c Reduktase; hsp = Heatshock- (Hitzeschock-) Protein; ANA = antinukläres Antigen; ANCA = antineutrophiles zytoplasmatisches Antigen).

Figures 3a to 3h
Demonstration of circulating antibodies to a) sarcolemma (ASA)/myolemma (AMLA) in isolated rat cardiocytes (magnification x 400). b) myosin in isolated rat cardio-cytes (magnification x 400). c) laminin in isolated rat cardiocytes (magnification x 400). d) actin in isolated rat cardiocytes(magnification x 400). e) Purkinje in bovine false tendon (magnification x 400). f) Antiinterfibrillary staining (e. g., antimitochondrial or anticytoplasmatic antibodies against isolated rat cardiomyocites (magnification x 400). g) Intracellular staining of Purkinje fiber in bovine false tendon (OFT) (magnification x 400). h) Antiendothelial staining (AEA) on Hep2 cells (magnification x 400).

Abbildungen 3a bis 3h
Nachweis zirkulierender Antikörper. a) Sarkolemm (ASA/Myolemm (AMLA) auf isolierten Kardiomyozyten (Vergrößerung x 400). b) Laminin auf isolierten Rattenkardiozyten (Vergrößerung x 400). c) Sarkolemm auf isolierten Purkinjezellen des Hundes (Vergrößerung x 400). d) Myosin auf isolierten Rattenkardiozyten (Vergrößerung x 400). e) Actin auf isolierten Rattenkardiozyten (Vergrößerung x 400). f) Antiintrafibrilläre Fluoreszenz (antimitochondriale/antizytoplasmatische Antikörper auf isolierten Rattenkardiozyten (Vergrößerung x 400). g) Plasmatische Färbung isolierter Purkinje-Zellen am „Os false tendon“ des Rindes. h) Antiendotheliale Antikörper auf Hep2-Zellen (Vergrößerung x 400).

3a



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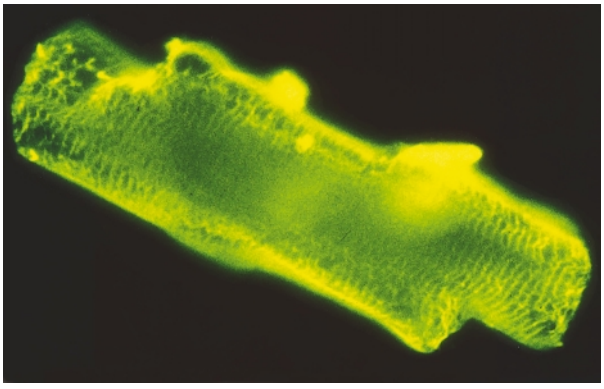

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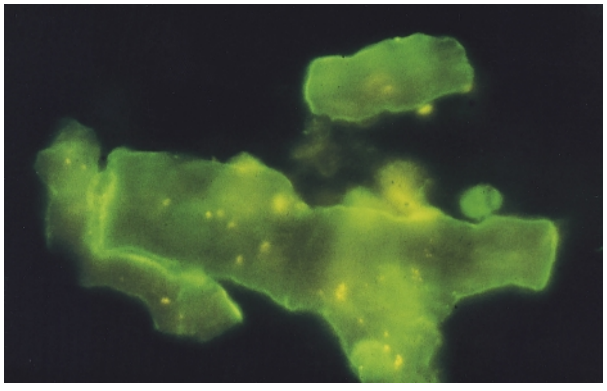

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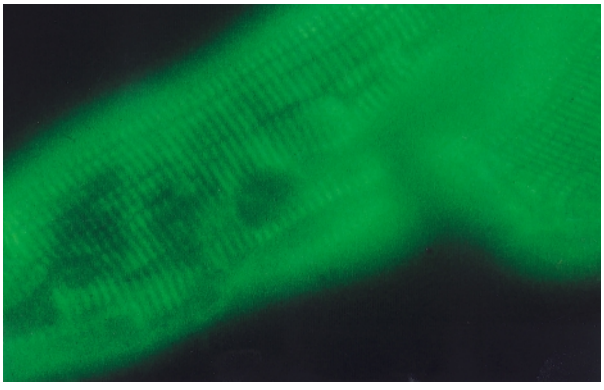

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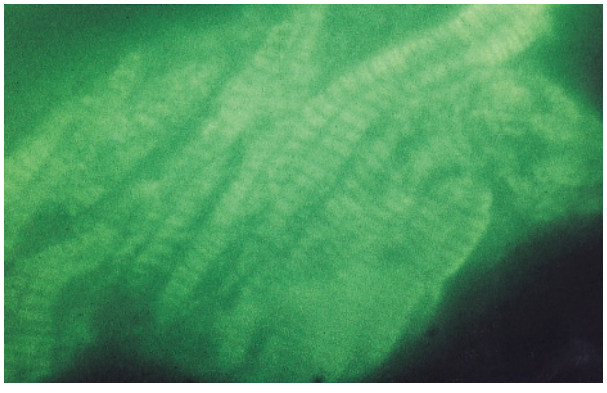

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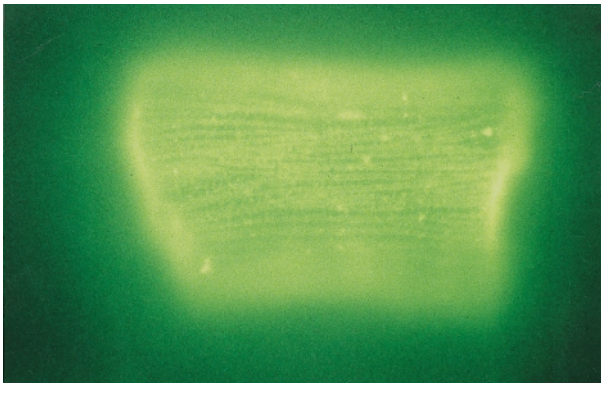
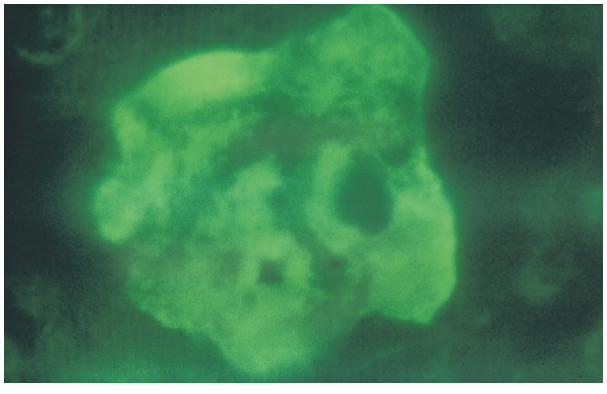
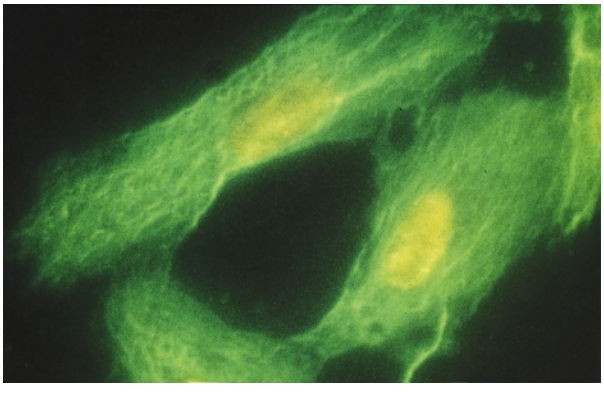


3g



3h



fully understood, postulated mechanisms include an autoimmune process or persistent viral infection.

In any case, cytokines mediate activation and effector phase of innate and specific immunity, which are both important in controlling viral infection. The innate immune response not only has an important protective function but also serves to initiate and regulate subsequent specific immune responses.

There are 2 principal mechanisms of innate immunity against viruses: 1. Viral infection directly stimulates the production of Type I IFN (IFN-α and -β) by infected cells. Type I IFN inhibits viral replication by initiating the synthesis of a number of enzymes, that collectively interfere with replication of viral RNA or DNA. 2. Natural killer cells lyse a wide variety of virally infected cells, and are probably one of the principal mechanisms of immunity against viruses early in the course of infection, before specific immune responses have developed [28].

Il-12 and Il-15 are important components of the innate immune system modulating the pattern of subsequent specific cell-mediated immune responses.

Cytokines of innate immunity have not been reported to impair cardiac function per se, but defects affecting this system may contribute to a delayed clearance of virus from the heart. For example, Il-12 has been reported to reduce mortality, myocardial damage and viral replication in mouse heart tissue when administered exogenously.

In specific immunity various cytokines, chemokines and adhesion molecules are involved in regulating migration and activation of T- and B-cell responses including migration and activity of macrophages. Interest has focussed on TNF-α, IL-1, and Il-6, since elevated levels have been reported in plasma of patients with myocarditis. Moreover, plasma levels of TNF-α and Il-6 correlate with clinical signs of heart insufficiency in patients with dilated cardiomyopathy [14].

TNF depresses myocardial function. The mechanism of this action appears to involve induction and/or increase in activity of an enzyme in cardiac myocytes, nitric oxide synthase (NOS) that converts arginine to citrulline and NO. NO made by this enzyme inhibits myocardial contractility. In mouse experiments a correlation between an increase in Il-6, TNF-α and iNOS mRNA and reduced contractile performance was established.

Adhesion Molecules

The integrin family of heterodimeric leukocyte proteins functions primarily as adhesion molecules, although they may serve signaling functions as well. In the context of dilated cardiomyopathy with and without inflammation, LFA-1, E-selectin ligand, or VLA-4 and ICAM-1, -2, and -3, E-selectin, or VCAM-1, which are responsible for leukocyte adhesion to resting or cytokine-activated endothelium thereby mediating inflammatory reactions in affected tissue, have been studied extensively. Inflammatory endothelial activation is present in a large percentage of patients with dilated cardiomyopathy. A correlation was demonstrated between the expression of cell adhesion molecules (CAMs) and the immunohistological diagnosis of inflammatory dilated cardiomyopathy and counterreceptor-bearing intramyocardial infiltrates [5, 17].

The mechanisms leading to the expression of cytokine-induced CAMs on endothelium in patients with dilated cardiomyopathy preceding the inflammatory response are still not fully understood: In myosin-induced myocarditis, a mouse model, the expression of Class II MHC Ag and/or endothelial ICAM-1, induced by the administration of lipopolysaccharide, is a prerequisite for emigration of myosin-reactive T cells when passively transferred [21]. Similar mechanisms could be involved in humans during the systemic phase of viral infection.

Immune Complexes

Immune complexes composed of a soluble antigen and specific antibody are formed anywhere in the circulation and may deposit in vessel walls anywhere in the body. This leads to local activation of leukocytes and the complement system with resultant tissue injury. In patients with inflammatory dilated cardiomyopathy immune complexes have been found more frequently than in normals and in patients with dilated cardiomyopathy without inflammation [13]. These circulating immune complexes consisted of IgG, IgM, C3 and C4, in myocarditis IgM predominated [3]. Up to now, the nature of the soluble antigen (foreign or self?) has not been determined. Although detectable, immune complex deposition does not seem to play a major role in the pathogenesis of dilated cardiomyopathy.

Conclusion

With the introduction of immunohistochemical and molecular biological methods, the WHF Expert Panel has achieved an important improvement in the diagnostic procedure of inflammatory heart diseases. Further work is needed to understand underlying pathomechanisms in order to enable the introduction of new therapeutic strategies.

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