Ernst Schering Research Foundation Workshop 55
Chronic Viral and Inflammatory Cardiomyopathy

Ernst Schering Research Foundation
Workshop 55

Chronic Viral and Inflammatory Cardiomyopathy

H.-P. Schultheiss, J.-F. Kapp, G. Grötzbach
Editors

With 68 Figures

Springer

Series Editors: G. Stock and M. Lessl

ISSN 0947-6075

ISBN-10 3-540-23971-5 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-23971-0 Springer Berlin Heidelberg New York

Library of Congress Control Number: 2005930272

Springer is a part of Springer Science+Business Media
springeronline.com

Printed in Germany

Editor: Dr. Ute Heilmann, Heidelberg
Desk Editor: Wilma McHugh, Heidelberg
Production Editor: Michael Hübert, Leipzig
Cover design: design & production, Heidelberg
Typesetting and production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig
21/3152/YL – 5 4 3 2 1 0 Printed on acid-free paper

Preface

Following its tradition of promoting novel areas of scientific discourse, the Ernst Schering Research Foundation (ESRF) hosted this workshop on chronic viral and inflammatory cardiomyopathy. In late October 2004, scientists from Canada, Germany, the Georgian Republic, Great Britain, Italy, Japan, the Netherlands, Israel, Sweden, and the United States gathered in Berlin to discuss their concepts, hypotheses, and latest findings on myocarditis and cardiomyopathy.

This expert meeting was held in cooperation with the German Research Foundation, which in the same year had supported transregional collaborative research activities entitled "Inflammatory Cardiomyopathy – Molecular Pathogenesis and Therapy."

Organizing the workshop, our efforts strove to render tighter the network between the distinct disciplines involved in cardiomyopathy research, building bridges between its molecular, pathogenetic, diagnostic, and therapeutic determinants.

It all began as a story of a neighborhood, and we would like to express our hope that this long-term project, which will require much and intensive cooperative work among the participants, will evolve to become a story of a good neighborhood.

Schering and the Charité, with their four campuses – the Mitte, Benjamin Franklin, Berlin-Buch, and Virchow clinics – are good neighbors and cooperate in many research and development tasks. Equally, on a personal level, two of us (H.P. Schultheiss and J-F Kapp) are neigh-

bors ourselves, and the proximity of our houses makes it possible that we sometimes meet when we are out for a walk. On one of these occasions, Schering's expert meeting on interferon-beta in multiple sclerosis

1. Karin Klingel, Tübingen, Germany
2. Kirk U. Knowlton, San Diego, USA
3. Jeffrey Towbin, Houston, USA
4. Akira Matsumori, Kyoto, Japan
5. Thomas-C. Bock, Tübingen, Germany
6. Stephane Heymans, Maastricht, Netherlands
7. Hugo A. Katus, Heidelberg, Germany
8. Finn Waagstein, Gothenburg, Sweden
9. Mathias Pauschinger, Berlin, Germany
10. Vincenz Hombach, Ulm, Germany
11. Nodar N. Kipshidze, Tbilissi, Georgia
12. Ruprecht Zierz, Schering AG, Berlin, Germany
13. Susanne Modrow, Regensburg, Germany
14. Udo Sechtem, Stuttgart, Germany
15. Andre Keren, Jerusalem, Israel
16. Wolfgang Poller, Berlin, Germany
17. Uwe Kühl, Berlin, Germany
18. Peter Liu, Toronto, Canada
19. Bernhard Maisch, Marburg, Germany
20. Andrea Frustaci, Milan, Italy
21. William McKenna, London, UK
22. Noel Rose, Baltimore, USA
23. Heinz-Peter Schultheiss, Berlin, Germany
24. Stefan Felix, Greifswald, Germany
25. Hans-Dieter Volk, Berlin, Germany
26. Claus-Steffen Stürzebecher, Schering AG, Berlin, Germany
27. Georg Grötzbach, Schering AG, Berlin, Germany
28. Christian Kraus, Schering AG, Berlin, Germany

came up, and on the next walk, we pondered the concept of treating virus-induced cardiomyopathy. Becoming quite enthusiastic at the end of the walk, the budget for a pilot study using interferon-beta to verify this concept had been set. The outcome of the pilot study encouraged us to proceed to the currently ongoing formal phase II protocol.

Cardiac inflammation is difficult to diagnose, and even if diagnosed, can we treat it effectively? This question was raised in 1772 by Jean-Baptiste de Sénac, physician to Louis XV. At that time, he could have chosen almost any disease and proper diagnosis would not have made any difference as far as effective treatment was concerned – independent of whether or not the diagnosis itself was rather straightforward or difficult.

Now, 230 years after de Sénac's doubts, there is some hope – maybe even some confidence – that we might have indeed finally identified a potential treatment for cardiac inflammation, and we have also managed to make its diagnosis an almost routine procedure.

When talking about dilated cardiomyopathy, precise diagnosis is important, because differentiation between autoimmune inflammatory car-

diomyopathy on the one hand, and cardiomyopathy with viral persistence on the other, allows therapeutic benefit from immunosuppressive treatment for one disease and from antiviral interferon-beta treatment for the other.

Constituting an invasive tool, endomyocardial biopsy provides valuable diagnostic information including virus identification, and morphological and immunohistochemical patterns. For the long-term, it would be desirable to develop serological markers for gaining prognostic and etiologic data. Experience from predicting transplant rejection was presented, hoping to be adopted for the identification of biomarkers. In view of the limitations of the current classification, a new histomorphological staging of myocarditis and inflammatory cardiomyopathy was proposed. As a new non-invasive approach, magnetic resonance imaging may gain importance in the diagnosis of cardiomyopathy, yet for the time being, this technique is still in too early a stage.

The interconnectedness of genetics and susceptibility to disease, of viral and non-viral inflammation, and of the role of immunity and the development of autoimmunity is a fascinating and much-discussed labyrinth. Cardiotropism needs to be verified for parvovirus B19, HHV6, and HVC.

Pilot experience in treating virus-positive patients with interferon-beta was presented. The audience appreciated these results hinting at a first causal treatment of viral cardiomyopathy. Other approaches dealt with receptor binding and protease inhibitors. Immunosuppression was found to be beneficial to virus-negative myocarditis patients.

In a special session it was controversially and spiritedly discussed whether or not (1) virus persistence was a determinant for progression, (2) autoimmunity was of significance, (3) parvovirus was relevant, and (4) there was a specific matrix destruction in the course of cardiomyopathy. The pertaining statements have also been included in this volume.

We are convinced that the unique composition of and the broad spectrum covered by this two-day workshop will be of exceptional value to the readers of volume 55.

Heinz-Peter Schultheiss
Joachim-Friedrich Kapp
Georg Grötzbach

Contents

V Diagnosis and Treatment

List of Editors and Contributors

Editors

Schultheiss, H.-P.
Department of Cardiology and Pneumology, Campus Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, Germany
(e-mail: heinz-peter.Schultheiss@charite.de)

Kapp, J.-F.
Berlex Inc. Specialized Therapeutics,
M1/3-9, Montville NJ 07045, USA
(e-mail: joachim-friedrich.kapp@schering.de)

Grötzbach, G.
Schering AG, Medical Development, Specialized Therapeutics,
Cardiovascular Europe,
13342 Berlin, Germany
(e-mail: georg.groetzbach@schering.de)

Contributors

Angelini, A.
University of Padua Medical School,
Via A. Gabelli, 61, 35121 Padova, Italy

Anker, S.D.
Department of Clinical Cardiology, Imperial College School of Medicine,
National Heart & Lung Institute,
Dovehouse Street, London, SW3 6LY, UK

and
Division of Applied Cachexia Research, Department of Cardiology,
Charité Medical School, Campus Virchow-Klinikum, Berlin, Germany

Bock, C.-T.
Department of Molecular pathology, Institute of Pathology,
University Hospital of Tübingen,
Liebermeisterstr. 8, 72076 Tübingen, Germany
(e-mail: thomas.bock@med.uni-tuebingen.de)

Caforio, A.L.P.
Division of Cardiology, Department of Cardiological,
Thoracic and Vascular Sciences
Centro "V. Gallucci", Policlinico University of Padua,
Via Giustiniani, 2, 35128 Padova, Italy
(e-mail: alida.caforio@unipd.it)

Calabrese, F.
University of Padua Medical School,
Via A. Gabelli, 61, 35121 Padova, Italy

Carturan, E.
University of Padua Medical School,
Via A. Gabelli, 61, 35121 Padova, Italy

Chimenti, C.
Cardiology Department, Catholic University Largo A. Gemelli 8,
00168 Rome, Italy

Chu, G.
Heart and Stroke, Richard Lewar Centre of Excellence,
NCSB 11-1266, Toronto General Hospital,
200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada

Doehner, W.
Division of Applied Cachexia Research, Department of Cardiology,
Charité Medical School, Campus Virchow-Klinikum, Berlin, Germany

Fechner, H.
Department of Cardiology and Pneumology, Campus Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, Germany

Felix, S.B.
Department of Internal Medicine B,
Ernst-Moritz-Arndt University Greifswald,
Friedrich-Loeffler Str. 23a, 17487 Greifswald, Germany
(e-mail: felix@uni-greifswald.de)

Frustaci, A.
Cardiology Department, Catholic University Largo A. Gemelli 8,
00168 Rome, Italy
(e-mail: biocard@rm.unicatt.it)

Fuse, K.
Heart and Stroke, Richard Lewar Centre of Excellence,
NCSB 11-1266, Toronto General Hospital,
200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada

von Haehling, S.
Department of Clinical Cardiology, Imperial College School of Medicine,
National Heart & Lung Institute,
Dovehouse Street, London, SW3 6LY, UK
(e-mail: stephan.von.haehling@web.de)

Hager, S.
Division of Cardiology
Robert-Bosch-Krankenhaus
Auerbachstraße 110, 70876 Stuttgart, Germany
(e-mail: stefan.hager@rbk.de)

Heymans, S.
Experimental and Molecular Cardiology Laboratory/CARIM,
Department of Cardiology, University Hospital Maastricht,
PO Box 5800, 6202 AZ Maastricht, The Netherlands
(e-mail: s.heymans@cardio.unimaas.nl)

Kallwellis-Opara, A.
Department of Cardiology and Pneumology, Campus-Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
(e-mail: angela.kallwellis-opara@charite.de)

Keren, A.
Heiden Department of Cardiology, Bikur Cholim Hospital,
Jerusalem, Israel
(e-mail: andrek@cc.huji.ac.il)

Knowlton, K.U.
Department of Medicine, University of California,
San Diego School of Medicine,
9500 Gilman Drive, 0613K, La Jolla, CA 92093, USA
(e-mail: kknowlton@ucsd.edu)

Kühl, U.
Department of Cardiology and Pneumology, Campus Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, Germany
(e-mail: uwe.kuehl@charite.de)

Liu, P.
Heart and Stroke, Richard Lewar Centre of Excellence,
NCSB 11-1266, Toronto General Hospital,
200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada
(e-mail: peter.liu@utoronto.ca)

Liu, Y.
Heart and Stroke, Richard Lewar Centre of Excellence,
NCSB 11-1266, Toronto General Hospital,
200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada

Mahon, N.G.
Cardiovascular Department, Mater Misericordiae Hospital,
Dublin, Ireland

Mahrholdt, H.
Division of Cardiology
Robert-Bosch-Krankenhaus
Auerbachstraße 110, 70876 Stuttgart, Germany
(e-mail: heike.mahrholdt@rbk.de)

Matsumori, A.
Department of Cardiovascular Medicine,
Kyoto University Graduate School of Medicine,
54 Kawahara-cho Shogoing, Sakyo-ku, Kyoto 606-8507, Japan
(e-mail: amat@kuhp.kyoto-u.ac.jp)

McKenna, W.J.
The Heart Hospital,
University College London Hospitals NHS Foundation Trust,
London, United Kingdom

Modrow, S.
Institut für Medizinische Mikrobiologie und Hygiene,
Universität Regensburg,
Franz-Josef-Strauß Allee 11, 93053 Regensburg, Germany
(e-mail: susanne.modrow@klinik.uni-regensburg.de)

Opavsky, A.
Heart and Stroke, Richard Lewar Centre of Excellence,
NCSB 11-1266, Toronto General Hospital,
200 Elizabeth Street, Toronto, Ontario, M5G 2C4, Canada

Pauschinger, M.
Department of Cardiology and Pneumology, Campus-Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
(e-mail: matthias.pauschinger@charite.de)

Pieroni, M.
Cardiology Department, Catholic University Largo A. Gemelli 8,
00168 Rome, Italy

Poller, W.
Department of Cardiology and Pneumology, Campus-Benjamin Franklin,
Charité University Medicine Berlin,
Hindenburgdamm 30, 12200 Berlin, Germany
(e-mail: wolfgang.poller@charite.de)

Rose, N.R.
Departments of Pathology and of Molecular Microbiology and Immunology,
Bloomberg School of Public Health – E5014,
615 North Wolfe Street, Baltimore, MD 21205, USA
(e-mail: nrrose@jhsph.edu)

Sechtem, U.
Cardiology and Pulmology, Robert-Bosch-Krankenhaus,
Auerbachstrasse 110, 70376 Stuttgart, Germany,
(e-mail: udo.sechtem@rbk.de)

Thiene, G.
University of Padua Medical School,
Via A. Gabelli, 61, 35121 Padova, Italy
(e-mail: gaetano.thiene@unipd.it)

Towbin, J.A.
Baylor College of Medicine, Texas Children's Hospital,
6621 Fannin Street, MC 19345-C, Houston, TX 77030, USA
(e-mail: jtowbin@bcm.tmc.edu)

Vogelsberg, H.
Division of Cardiology
Robert-Bosch-Krankenhaus
Auerbachstraße 110, 70876 Stuttgart, Germany
(e-mail: holger.vogelsberg@rbk.de)

Waagstein, F.
Wallenberg Laboratory, Sahlgrenska Academy,
Göteborg University,
Göteborg, Sweden
(e-mail: finn.waagstein@wlab.gu.se)

I Chronic Viral and Inflammatory Cardiomyopathy

Overview and Outlook

1 Overview on Chronic Viral Cardiomyopathy/Chronic Myocarditis

H.-P. Schultheiss, U. Kühl

Abstract. Myocarditis is most often induced by cardiotropic viruses and often resolves with minimal cardiac remodelling and without discernable prognostic impact. Acute myocarditis has a highly diverse clinical presentation (asymptomatic, infarct-like presentation, atrioventricular (AV)-block, atrial fibrillation, sudden death due to ventricular tachycardia, fulminant myocarditis with severely depressed contractility). Progression of myocarditis to its sequela, dilated cardiomyopathy (DCM), has been documented in 20% of cases and is pathogenically linked to chronic inflammation and viral persistence. Persistence of cardiotropic viruses (enterovirus, adenovirus) constitutes one of the predominant aetiological factors in DCM. Additionally, circulating autoantibodies to distinct cardiac autoantigens have been described in patients with DCM, providing evidence for autoimmune involvement. Since clinical com-

plaints of myocarditis and DCM are unspecific, a positive effect of any specific therapy depends on an accurate biopsy-based diagnosis and characterization of the patients with histological, immunohistological and molecular biological methods (PCR), which have developed into sensitive tools for the detection of different viruses, active viral replication, and myocardial inflammation. The immunohistochemical characterization of infiltrates has supported a new era in the diagnosis of myocardial inflammation compared with the Dallas criteria, which has led to a new entity of secondary cardiomyopathies acknowledged by the WHO, the inflammatory cardiomyopathies (DCMi). Immunohistochemically quantified lymphocytes significantly better reflect troponin levels and correlate with findings by anti-myosin scintigraphy compared with the histological analysis. Furthermore, the orchestrated induction of endothelial cell adhesion molecules (CAMs) in 65% of DCM patients has confirmed that CAM induction is a prerequisite for lymphocytic infiltration in DCMi. The combination of these immunohistological with molecular biological diagnostic techniques of virus analysis allows a further classification of dilated cardiomyopathy by differentiating the disease entity in subgroups of virus-positive and virus-negative patients with or without cardiac inflammation. Further analysis of the predominant Th1-/Th2-immune response may provide additional prognostic information on the natural course of the disease. This differential analysis improves the clinical management of patients and is an indispensable prerequisite for the development of specific antiviral or immunomodulatory treatment strategies.

1.1 Introduction

Cardiomyopathies are diseases of the heart characterized by ventricular dysfunction that is not caused by primary heart diseases, e.g. hypertension or congenital, valvular, coronary, arterial or pericardial abnormalities. They are classified as primary cardiomyopathies if the origin of contractile dysfunction is unknown (dilated cardiomyopathy, hypertrophic cardiomyopathy) and as secondary or specific cardiomyopathies if the heart is affected in association with specific infectious, immunological, metabolic, neuromuscular or toxic diseases.

Dilated cardiomyopathy (DCM) is one of the most common cardiomyopathy entities. Its yearly incidence is 5–8 cases per 100,000, the age-corrected prevalence is 36 cases with 17 hospitalizations, and 3.8 deaths per 100,000 per year are due to DCM. DCM affects males most

frequently, with a sex ratio of 3:1, manifesting predominantly between the third and fifth decades (Olbrich 2001). In a homogeneous population of young military servicemen, the incidence of myocarditis was reported 0.17 per 1,000 man-years (Karjalainen and Heikkila 1999), but the real numbers are expected to be substantially higher due to the often subclinical presentation of acute myocarditis and misinterpretation of unspecific symptoms (Kühl et al. 1997a).

During the past decade, the basis of left ventricle (LV) dysfunction has begun to unravel. In approximately 30%–40% of cases, the disorder is inherited; autosomal- dominant inheritance is most common (although X-linked, autosomal recessive and mitochondrial inheritance occurs) (Towbin and Bowles 2000). In the remaining patients, the disorder is presumed to be acquired, with inflammatory heart disease playing an important role (Towbin and Bowles 2002; Towbin and Bowles 2001). Evolution of acute myocarditis to dilated cardiomyopathy (DCM), which occurs in 21% of the patients within a mean follow-up of 33 months (D'Ambrosio et al. 2001), gave rise to the hypothesis that certain DCM cases might be due to sequela of a "chronic myocarditis" (Fig. 1). Acknowledging the unequivocal evidence on the chronic inflammatory process involved in DCM, the 1995 report of the WHO/ISFC Task Force

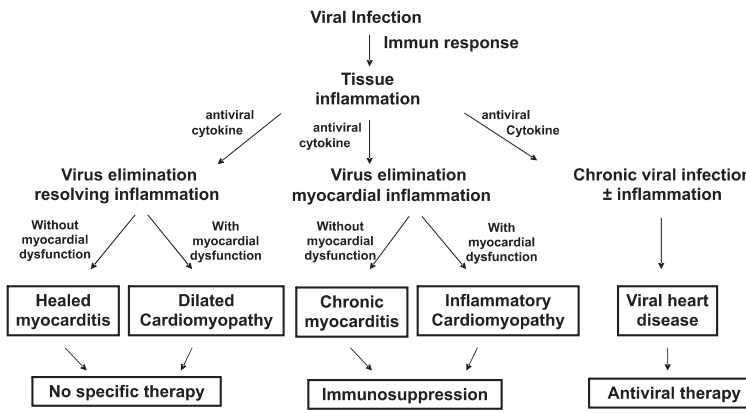


Fig. 1. Natural course of virus induced myocarditis developing DCM/ inflammatory cardiomyopathy

on the Definition and Classification of Cardiomyopathies introduced the new entity "inflammatory cardiomyopathy" among other specific cardiomyopathies (DCMi) (Richardson et al. 1996), which is characterized by idiopathic heart failure with evidence for intramyocardial inflammation.

Myocarditis and DCMi in the Western world are most often caused by cardiotropic viruses (Mason 2002). A number of similar viruses have been identified in biopsies of patients with myocarditis and DCM (Kühl et al. 2003a, 2005). Despite the increasing role of the systemic inflammatory response involved secondarily in the progression of congestive heart failure irrespective of the underlying pathogenesis (Anker and von Haehling 2004) DCMi refers exclusively to DCM with primary anti-cardiac inflammatory and/or viral aetiology. This differentiation between systemic and organ-specific inflammation is pivotal for the consideration of diagnostic procedures on endomyocardial biopsies (EMBs) and treatment modalities targeting intramyocardial inflammation and/or cardiotropic viral infection. The use of EMBs is indispensable for the diagnosis of DCMi, which mainly comprises the detection and characterization of intramyocardial inflammation by immunohistological evaluation, and the proof of viral infection by molecular biological techniques (Table 1). This interdisciplinary approach has expanded our understanding of the pathogenesis of DCMi, and these insights were ultimately decisive for the design of immunomodulatory trials with beneficial outcomes in DCMi patients (Noutsias et al. 2004; Mason et al. 1995; Frustaci et al. 2003; Dec and Fuster 1994a; Kühl et al. 2003b; Wojnicz et al. 2001).

1.2 Diagnosis of Myocarditis and DCMi

The prognosis, as well as the initial clinical presentation of patients with myocarditis and DCM, is highly variable and ranges from spontaneous improvement to rapidly progressive heart failure and sudden cardiac death (D'Ambrosio et al. 2001). To date, no clinical parameter has proved to be a relevant discriminator of prognosis or pathogenesis. In contrast, both of the major pathogenic hallmarks of DCMi, namely viral persistence and intramyocardial inflammation diagnosed in EMBs, have

Table 1. Analysis of endomyocardial biopsies

	Histology	Immunohistology	PCR
Morphological evaluation of myocardial tissue	+	–	–
Acute myocarditis/necrosis	(+)*	–	–
Detection and quantification of inflammation	–	+	–
Detection of adhesion molecules (ICAM, VCAM)	–	+	–
Subtyping of infiltrating cells (cytotoxic cells)	–	+	–
Differentiation of immune response (Th1/2)	–	+	–
Detection of different viruses (subtypes)	–	–	+
Detection of viral replication/load	–	–	+

*Low sensitivity, high sampling error

been associated with adverse prognosis in DCM (Why et al. 1994a; Fujioka et al. 2000; 21 Angelini et al. 2002; Kanzaki et al. 2001).

- The clinical symptoms of myocarditis and DCMi are unspecific (Table 2).
- A diagnosis of inflammation based only on clinical history/presentation and non-invasive examinations is not possible.
- The unequivocal diagnosis of chronic myocarditis/inflammatory cardiomyopathy can be only achieved by analysis of endomyocardial biopsies including :
    1. Histology (in the acute phase)
    2. Immunohistology (in the chronic phase)
    3. Molecular biology/virology

Table 2. Clinical symptoms of virus-negative and virus-positive patients

	Negative	EV	PVB19	HHV6	PVB/HHV
Total (n)	260	54	210	89	56
Infection	48%	45%	49%	35%	42%
Tiredness	69%	82%	72%	59%	82%
Angina pectoris	40%	10%	47%	36%	40%
Dyspnea on exertion	55%	50%	60%	64%	77%
Pericardial effusion	6%	2%	7%	5%	13%
Impaired contractility (global)	68%	71%	68%	69%	69%
Impaired contractility (region.)	43%	24%	31%	44%	25%
Rhythm disturbances	46%	50%	51%	38%	62%
SVES	7%	9%	11%	4%	6%
VES	23%	9%	24%	19%	18%
Atrial fibrillation	19%	25%	16%	18%	23%
Ventricular tachycardia	9%	0%	13%	4%	7%

SVES, supraventricular extra beats; VES, ventricular extra beats

1.3 Histology

Histological analysis has been considered the "gold standard" for diagnosing cardiomyopathies. This is true for acute myocarditis; but the histological diagnosis of chronic myocarditis and DCMi causes difficulties and the results are contradictory. According to the Dallas criteria, myocarditis in its acute stage is histologically defined by lymphocytic infiltrates in association with myocyte necrosis. The histological definition of chronic myocarditis according to the Dallas classification (histologically "borderline and ongoing myocarditis") demands the presence of infiltrating lymphocytes without further histomorphological signs of myocyte injury or immunohistological features of a persisting, activated inflammatory process in the first and the control biopsy. These cellular infiltrates in chronic heart failure, however, are often sparse or focal and therefore might be missed by sampling error (Shanes et al. 1987; Hauck et al. 1989). Moreover, it is difficult to differentiate non-

inflammatory interstitial cells from infiltrating lymphocytes by light microscopy. This leads to a misinterpretation of interstitial cells as inflammatory lymphocytes and thus to an over- or underestimation of the degree of inflammation. Ultimately, one has to keep in mind that infiltrating lymphocytes, especially if not activated, are not necessarily representative for an ongoing immune process affecting the entire myocardium. Those two mechanisms (misinterpretation of interstitial cells and sampling error) are mainly responsible for the low diagnostic yield in the histological analysis and explain the often-reported high "interobserver variability".

1.4 Immunohistology

Meanwhile, immunohistological methods have been successfully introduced into the diagnosis of an inflammatory myocardial process. In contrast to routine histology with the above-explained difficulties in detecting lymphocytes, cardiac inflammation is, in addition to the quantification of lymphocytes, immunohistologically characterized by different markers of cell activation and the enhanced expression of histocompatibility antigens and adhesion molecules (Noutsias et al. 2002a,b). The sensitivity and specificity of monoclonal antibodies, directed against specific epitopes of immunocompetent cells, allow the unambiguous identification, characterization and quantification of inflammatory cells infiltrating myocardial tissues (Noutsias et al. 2003a). If the number of immunoreactive T lymphocytes is determined by the use of anti-CD2, -CD3, -CD4 or -CD8-antibodies, tissues with a mean number of lymphocytes exceeding 2.0 per high-power field (7.0 cells/mm^2) can be considered to have pathologically increased lymphocytic infiltrations (Fig. 2a, b) because additional markers of immune activation such as an expression of adhesion molecules are found to be enhanced in more than 90% of these cardiac tissues (Fig. 2d). Control tissues with mean lymphocyte counts of 0.7 ± 0.4 cells per high-power field (0.0–5.0/mm^2) express these markers in less than 30% of the tissues (Fig. 2c; see comments of Kühl et al. 1997b; also Kühl et al. 1997a; Noutsias et al. 1999; Bowles et al. 1986).

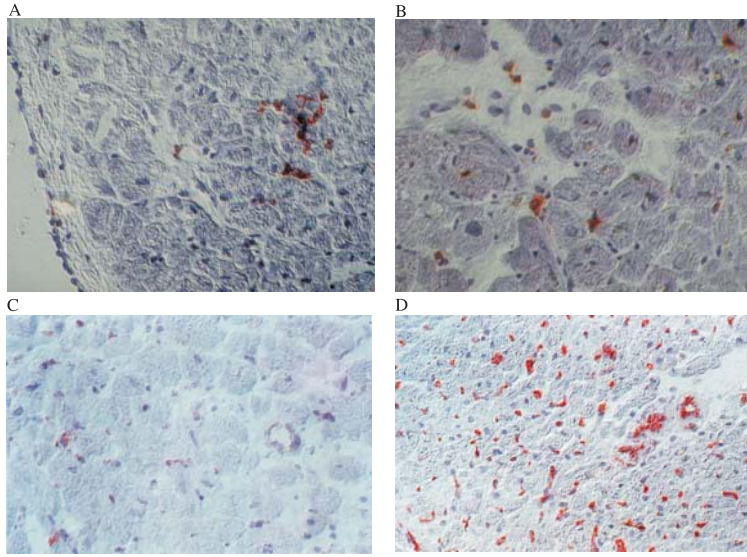
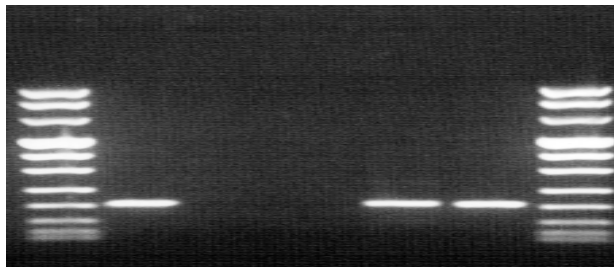


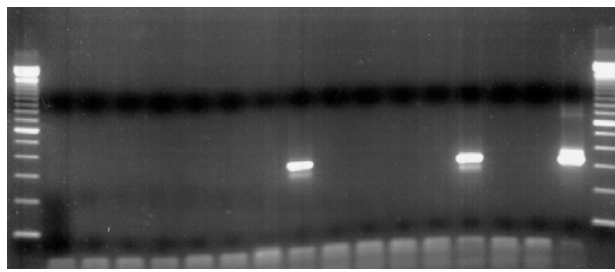
Fig. 2a–d. Immunohistological analysis of lymphocytes and adhesion molecules (ICAM-1) in endomyocardial biopsies. **a** Focal $CD3^+$-lymphocytic infiltration. **b** Diffuse $CD3^+$-lymphocytic infiltration. **c** Normal expression of ICAM-1 in a CD3-negative tissue. **d** Enhanced expression of ICAM-1 in a CD3-positive tissue

1.5 Molecular Biology/Virology

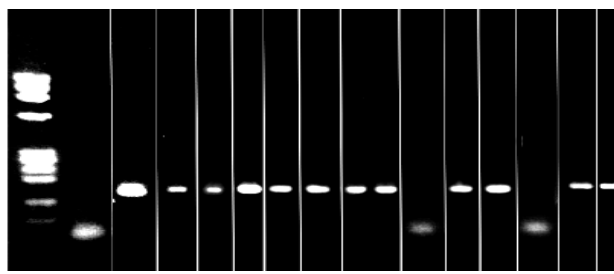
Positive serological tests cannot prove virus infection of the myocardium. The proof of a viral affliction of the myocardium warrants analysis of endomyocardial biopsies with more sensitive molecular biological methods, such as in situ hybridization or polymerase chain reaction (PCR) (Bowles et al. 1986; Why et al. 1994b). These methods enable the detection of viral RNA and DNA in tissues with even low numbers of viral copies (Fig. 3). Enteroviral genome was confirmed by PCR amplification in ca. 15% of EMBs from myocarditis and DCM patients (Baboonian and Treasure 1997). The adverse prognostic impact of enteroviral persistence in DCM was identified early (Dec and Fuster 1994a), and recent results indicate a special importance of the replicative infection



Pauschinger et al. Circ. 1999



Pauschinger et al. Circ. 1999



Kühl/Kandolf et al. Circ. 2003

Fig. 3. Detection of different viral genomes by nested polymerase chain reaction (nPCR). *EV*, enterovirus (from Pauschinger et al. 1999a); *ADV*, adenovirus (from Pauschinger et al. 1999b); *PVB19*, Parvivirus B19 (from Kühl et al. 2003b)

mode in a significant proportion of DCM patients (Fujioka et al. 2000; Pauschinger et al. 1999a). Adenovirus has been elucidated as a further cardiotropic virus in DCM (Pauschinger et al. 1999b). There is an evolving relevance of further cardiotropic viruses, especially parvovirus B19, human herpes-virus 6, hepatitis C and Epstein-Barr virus (Kühl et al. 2003a; Frustaci et al. 2003; Matsumori 2001).

Of note, investigations on the tissue distribution of these viruses revealed profound differences: Whereas the enteroviral genomes have been detected within cardiomyocytes by in situ hybridization, endothelial cells are the primary targets of parvovirus B19 infection (Kandolf et al. 1987; Li et al. 2000; Bultmann et al. 2003). In this context, the pattern of viral receptors might be a decisively important issue (Noutsias et al. 2001; Poller et al. 2002). At present, we are lacking detailed data on the prognostic impact, pathways of viral entry and persistence of these recently identified cardiotropic viruses. However, the retrospective analysis by Frustaci et al. confirmed that patients with persistence of these viruses (except for hepatitis C) do not improve, or even deteriorate under immunosuppression, which infers that myocardial persistence of these viruses may have a similar pathogenic and prognostic relevance as coxsackievirus (Frustaci et al. 2003).

Employing group-specific primers, homology screening for various enteroviral and adenoviral strains can be conducted in addition to the differentiation of actively replicating virus from non- replicating, resting viral genomes (Pauschinger et al. 1999a; Calabrese and Thiene 2003). Quantification of virus loads by real-time PCR and sequence analysis of possibly pathogenic virus subtypes complete both the diagnostic accuracy and pathophysiological understanding of cardiotropic viral infections.

1.6 Biopsy-Based Classification of DCMi

The combined use of quantitative and qualitative histological and immunohistological analysis of myocardial inflammation with quantitative and qualitative molecular biological analytical methods allows a more detailed differential diagnosis in patients with clinically suspected myocarditis or DCMi (Noutsias et al. 2004; Pauschinger et al. 2004). As far as acute viral myocarditis is concerned, the Dallas classification has

enabled a more standardized assessment of the histological changes in the myocardium. The clinical benefit, however, is limited by the fact that histological recording of myocytolysis is only possible for a short period of time during the acute disease (10–14 days) and usually no longer present at time points when later biopsies are taken in patients with chronic DCMi, which is considered a late sequela of virus-induced myocarditis. In chronic disease, this use of a combined set of analytical methods allows the identification of viral persistence and chronic-immunological processes as a possible cause for heart muscle injury in a considerable number of these patients (Kühl et al. 1996; Noutsias et al. 2003b). Three different entities of viral-induced dilated cardiomyopathy have been recognized (Fig. 1; Kühl et al. 1997b).

1. Postmyocarditic viral heart disease. These patients clinically present as dilated cardiomyopathy. Immunohistologically, they are characterized by an inconspicuous endomyocardial biopsy without chronic inflammation. Molecular biological methods do not indicate viral persistence.
2. Chronic (auto)immune-mediated myocarditis/inflammatory cardiomyopathy. Immunohistochemical analysis of endomyocardial biopsy reveals an active inflammatory process within the myocardium in the absence of viral persistence.
3. Chronic viral heart disease. Histologically, these patients cannot be differentiated from postmyocarditic viral heart disease (healed myocarditis/DCM). Viral persistence can be detected by in situ hybridization or PCR. Virus-induced myocardial inflammation may be present.

The rationale for this aetiological differentiation of "dilated cardiomyopathy" into different phases of the disease is to better understand the pathomechanism that might give rise to a more specific treatment strategy. If the developmental process of postmyocarditic heart failure is accelerated by chronic inflammation and/or viral persistence, prognosis of patients might be dependent on whether virus and inflammation persist in the myocardium (Fig. 2).

Recent data obtained by this combined mode of diagnostic procedures indicate a high percentage of single and multiple viral infections of the myocardium in patients with clinically suspected myocarditis of

recent onset, myocarditis in the past and DCMi. Follow- up analysis of the patients reveals that spontaneous virus clearance is often associated with a spontaneous recovery of myocardial function. On the other hand, progression of myocardial dysfunction occurs in those patients who develop virus persistence, independent of the virus subtype involved. The patients with chronic virus persistence, however, often get benefit from a specific anti-viral treatment if this has been initiated early, before an irreversible virus-induced myocardial dysfunction has developed (Kühl et al. 2003b; Deonarain et al. 2004).

1.7 Unresolved Issues

With respect to the prevalence of virus-induced myocarditis/cardiomyopathies, the natural course of DCMi and their treatment responses, a number of open questions still exist.

Unknown are:

- The prevalence and clinical significance of various cardiotropic viruses
- The significance of endogenous and exogenous factors for viral infection, virus persistence or virus clearance
- The relevance of multiple viral infections, human pathogenic virus subtypes, genomic mutations predisposing for the disease, virus replication rate, or virus load, for the clinical spontaneous course of the disease
- The cause of highly variable virus receptor expression and its significance for the primary virus infection and the further course of the disease and
- The influence of the local and systemic antiviral immune response for the natural course of the disease?

1.8 Future Goals

- Development of risk response prognosis parameters
- Standardization of diagnostic methods
- Establishment of a causal differential therapy
- Development of diagnostic and therapeutic guidelines

Acknowledgements. This publication has been supported by the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich/Transregio 19.

References

Angelini A, Crosato M, Boffa GM, Calabrese F, Calzolari V, Chioin R, Daliento L, Thiene G (2002) Active versus borderline myocarditis: clinicopathological correlates and prognostic implications. Heart 87:210–215

Anker SD, von Haehling S (2004) Inflammatory mediators in chronic heart failure: an overview. Heart 90:464–470

Baboonian C, Treasure T (1997) Meta-analysis of the association of enteroviruses with human heart disease. Heart 78:539–543

Bowles NE, Richardson PJ, Olsen EG, Archard LC (1986) Detection of coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. Lancet 1:1120–1123

Bultmann BD, Klingel K, Sotlar K, Bock CT, Baba HA, Sauter M, Kandolf R (2003) Fatal parvovirus B19-associated myocarditis clinically mimicking ischemic heart disease: an endothelial cell-mediated disease. Hum Pathol 34:92–95

Calabrese F, Thiene G (2003) Myocarditis and inflammatory cardiomyopathy: microbiological and molecular biological aspects. Cardiovasc Res 60:11–25

D'Ambrosio A, Patti G, Manzoli A, Sinagra G, Di Lenarda A, Silvestri F, Di Sciascio G (2001) The fate of acute myocarditis between spontaneous improvement and evolution to dilated cardiomyopathy: a review. Heart 85:499–504

Dec GW, Fuster V (1994a) Idiopathic dilated cardiomyopathy [see comments]. N Engl J Med 331:1564–1575

Deonarain R, Cerullo D, Fuse K, Liu PP, Fish EN (2004) Protective role for interferon-b in coxsackievirus B3 infection. Circulation 110:3540–3543

Frustaci A, Chimenti C, Calabrese F, Pieroni M, Thiene G, Maseri A (2003) Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. Circulation 107:857–863

Fujioka S, Kitaura Y, Ukimura A, Deguchi H, Kawamura K, Isomura T, Suma H, Shimizu A (2000) Evaluation of viral infection in the myocardium of patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 36:1920–1926

Hauck AJ, Kearney DL, Edwards WD (1989) Evaluation of postmortem endomyocardial biopsy specimens from 38 patients with lymphocytic myocarditis: implications for role of sampling error. Mayo Clin Proc 64:1235–1245