Diagnosis and treatment of patients with virus induced inflammatory cardiomyopathy

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Chronic viral induced inflammatory cardiomyopathy causes progression of left ventricular dysfunction and is an important cause of dilated cardiomyopathy. Despite of the progress made in heart failure therapy, mortality of dilated cardiomyopathy is still 10% per year. Current heart failure therapy is symptomatic and does not impact on the specific underlying pathogenic mechanisms. Thus, specific treatment strategies that are directed against the underlying pathogenetic causes are required if myocarditis and its sequela, namely inflammatory cardiomyopathy, are to be successfully treated. Because histological and, especially, clinical diagnosis is fraught with numerous problems, an aetiological classification based on histology, immunohistochemistry and molecular biology is favourable, particularly in view of the improvements in methods made in recent years. The combination of these diagnostic techniques allows a new classification of dilated cardiomyopathy by differentiating the disease entity in subgroups of virus positive and virus negative patients with or without cardiac inflammation. This may not only contribute toward improving our understanding of underlying pathological mechanisms, but ultimately may also assist in developing specific immunomodulatory treatments. Preliminary results from ongoing treatment trials suggest that specific antiviral or anti-inflammatory treatment strategies are successful in patients who have been carefully selected and characterized according to biopsy-based diagnostic criteria.

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Introduction

Idiopathic dilated cardiomyopathy (DCM) is a serious disorder and is the most common cause of heart failure in young patients. Its prevalence in the U.S.A. is 36 cases per 1 million, being responsible for 25% of the more than 750,000 cases of heart failure reported and for approximately 250,000 deaths. The long-term outcome of approximately 50% of patients is terminal heart failure requiring cardiac transplantation[1,2]. A genetic origin for DCM has been reported in up to 25% of cases but the majority are sporadic, and a viral or immune pathogenesis is suspected in approximately 20–30% of idiopathic DCM[3–6]. Virus-induced myocarditis may progress to an autoimmune phase after resolution or amelioration of the initial infection, and than finally to progressive dilatation after resolution or amelioration of the immune injury[7]. Chronic viral infection of the myocardium has been detected in patients clinically presenting with myocarditis and DCM. Thus, although viruses are classically regarded as agents of self-limiting infections, a considerable body of evidence implicates them in the aetiology of chronic diseases, including DCM[8–10].

The diagnosis or exclusion of virus-induced inflammatory cardiomyopathy depends on accurate analysis of endomyocardial biopsies, and confusion in diagnosis and subsequent errors in therapy are particularly likely if inadequate diagnostic tools are employed. Even with the use of adequate diagnostic methods, it is difficult to make decisions regarding specific therapeutic strategies because of overlapping phases of the disease. Nevertheless, and in spite of some as yet unresolved shortcomings of the diagnostic procedures (e.g. sampling error), a rational decision regarding specific therapy requires the use of all currently available diagnostic tools.

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Current heart failure therapy is symptomatic and does not impact on the specific underlying pathogenic mechanisms. Progression of left ventricular dysfunction therefore cannot be halted by conventional heart failure therapies. Thus, specific treatment strategies that are directed against the underlying pathogenetic causes are required if myocarditis and its sequela, namely inflammatory cardiomyopathy, are to be successfully treated.

Pathogenetic mechanisms in inflammatory cardiomyopathy

Myocardial damage may occur during several stages of virus-induced cardiomyopathy (Fig. 1). The acute phase begins with myocardial cell infection and viral replication, culminating in cell lysis caused by virus. Early defence mechanisms include a cellular immune response that prevents further virus spreading and results in virus clearance. This is achieved by production of antiviral cytokines and elimination of virus-infected myocardial cells through cytokine-activated macrophages and natural killer cells, perforin mediated cell cytolysis (cytotoxic T lymphocytes) and induction of apoptosis\([11,12]\). Thus, both the viral pathogen itself and the protective antiviral immune response may produce irreversible loss of infected myocardial cells, thereby contributing to irreversible damage of the myocardium, long-term remodelling and progression of heart failure. Successful virus clearance and resolution of the inflammatory process therefore results in persistent sequelae.

If there is no history of virus-induced disease, then the observed clinical outcome is referred to as DCM. Persistent T-cell activation, induced by intrinsic myocardial antigens that cross-react with viral peptides (molecular mimicry), may induce an inflammatory process that is not terminated and escapes regular control mechanisms\([12]\) (see the review by Afanasyeva and Rose, in the present supplement). Resulting immune-mediated injury may further increase ventricular dysfunction. The pathogenetic mechanisms that are involved are poorly understood, but the first encounter with antigens may set the stage for later harmful autoimmune processes, when antigen-specific and antigen non-specific signals are presented to the immune system. For example, these signalling molecules include costimulatory cell surface markers such as CD28 or CD154, and soluble mediators such as interleukin (IL)-1, tumour necrosis factor-alpha, IL-12, interferon (IFN)-gamma, IL-4 and IL-13, all of which may play important roles in orchestrating the later adaptive immune response.

Diagnosis of inflammatory cardiomyopathy

Histological analysis

Myocardial inflammation is defined by the presence of infiltrating lymphocytes, either with (active myocarditis) or without (borderline myocarditis) myocyte necrosis.

Figure 1  Pathogenesis of virus-induced inflammatory cardiomyopathy.
Histologically, the diagnosis of a chronic inflammatory process is difficult to establish by light microscopy because of the often sparse or focally distributed cellular infiltrates, which may easily be missed by sampling error. Moreover, in haematoxylin and eosin stained sections, it is difficult if not impossible to differentiate unequivocally non-inflammatory cells (e.g. fibroblasts or pericytes) from infiltrating immune effector cells. Thus, misinterpretation of interstitial cells may lead to an overestimation or underestimation of the degree of the inflammatory process within the myocardium, in the absence of knowledge of the activity and cellular type of infiltration. As a result of low histological sensitivity, no myocardial inflammatory process is seen in the majority of biopsies from patients with DCM (Fig. 2). Thus, it is generally accepted that histological analysis does not contribute important information to the diagnosis of inflammatory cardiomyopathy.

**Immunohistochemical analysis of inflammatory cells**

In order to obtain more specific diagnostic and prognostic information, immunohistological techniques have been introduced to identify and characterize mononuclear cellular infiltrates in myocardial biopsies of patients with DCM. Although myocarditis is detected in fewer than 2% of endomyocardial biopsies from patients with DCM on histological analysis (4-5% of biopsies are consistent with the diagnosis of borderline myocarditis), a myocardial inflammatory process is detected in 43% of endomyocardial biopsies when immunohistochemical staining procedures are employed (Fig. 2). One of the key questions still under debate is whether the relative low cell number (which is often seen in the myocardium of DCM patients) can be considered a marker for ongoing immunological processes that cause progression of the disease. Because long-term clinical complaints and outcome of patients usually do not correlate with the number of infiltrating cells, both subtype of infiltrating cells and detection of an even low-grade inflammatory processes spread within the entire myocardium are of more importance than the number of infiltrating lymphocytes. Using subtype-specific antibodies, immunohistochemical analysis revealed the presence of cytotoxic lymphocytes and natural killer cells in chronic inflamed myocardium (see the review by Noutsias et al., in the present supplement.[11,13]

**Immunohistochemical analysis of adhesion molecules**

In order to gain additional information on the myocardial immune process, we introduced an expanded panel of diagnostic immune markers (i.e. cellular adhesion molecules) into the immunohistological diagnosis of myocarditis.[14-16]. The expression patterns of cellular adhesion molecules differ substantially both in terms of quantity and quality. CD29 expression is not confined to endothelial and interstitial cells, but is also expressed within the extracellular matrix, exhibiting the greatest immunoreactivity of the spectrum of investigated adhesion receptors. Furthermore, whereas HLA, intercellular adhesion molecule-1, CD58 and CD29 are distributed homogenously within DCM biopsies, vascular cell adhesion molecule-1 and the selectins are confined to single post-capillary venules (Fig. 3).
Myocardial expressions of most adhesion molecules correlate with the number of inflammatory infiltrates and are enhanced within the entire myocardium if the mean lymphocyte number exceeds 2·0 CD3⁺ cells per high-power microscopic field (at magnification ×400), even in cases with focal lymphocytic infiltration. Combining enhanced expression of adhesion molecules on interstitial cells and the vascular endothelium with identification, quantification and subtyping of cellular infiltration improves diagnostic yield and reduces sampling error when compared with histological analysis (Fig.2)\[5,17\]. Therefore, using immunohistochemical analysis, we defined myocardial inflammation as the presence of more than 2·0 infiltrating T lymphocytes per high-power field (corresponding to 7·0 cells/mm²) in addition to an enhanced expression of adhesion molecules on interstitial cells or the vascular endothelium (Table1)\[5,17,18\].

Using digital imaging analysis, an observer-independent, standardized quantification of immunohistochemically marked infiltrates and adhesion molecules in endomyocardial biopsies is possible\[19,20\]. Moreover, digital imaging analysis enables the objective and quantitative evaluation of parameters that are not accessible via visual assessment (e.g. heart area, and quantitative muscle evaluation of cellular adhesion molecule immunoreactivity). This may ultimately permit us to overcome the differences reported in evaluation of endomyocardial biopsies between different laboratories\[19,20\].

### Table 1  Immunohistological definition of inflammatory cardiomyopathy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
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</thead>
<tbody>
<tr>
<td>Infiltrating lymphocytes (CD2⁺, CD3⁺, CD4⁺,CD8⁺)</td>
<td>&gt;7·0 cells . mm⁻²</td>
</tr>
<tr>
<td>Adhesion molecules (HLA I/II, CD54, CD59, VCAM-1)</td>
<td>Enhanced expression on interstitial cells and vascular endothelium</td>
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HLA = human leucocyte antigen; VCAM = vascular cell adhesion molecule.

### Virus analysis: detection of viral genome by nested PCR

Even positive serological tests cannot prove virus infection of the myocardium. A positive diagnosis of virus infection of the myocardium is only confirmed by analysis of endomyocardial biopsies using molecular biology methods such as in situ hybridization and polymerase chain reaction (PCR). Among 900 consecutive patients who underwent endomyocardial biopsy in our department in order to elucidate the origin of their persistent left ventricular dysfunction in the absence of apparent causes, enteroviral and adenosiral genomes were detected in 15% and 7%, respectively. For the virus analysis, primer pairs were designed to amplify the genomic sequence of adenoviruses that encodes the hexon protein region (5'-untranslated region). The adenovirus-specific primers were designed to detect all adenovirus serotypes while the enterovirus primers detect most enterovirus types\[10,21\]. Total RNA and genomic/viral DNA were isolated simultaneously from biopsy specimens (Fig. 4). Beta-actin primers were used as internal controls for the isolation of intact DNA and RNA. Adenovirus type 5 DNA isolated from infected cells and a cloned cDNA of coxsackievirus B3 83, were used as positive viral controls for PCR analysis, as was recently reported\[10,21\].
Biopsy derived diagnosis and classification of viral heart disease

Because pathological/anatomical and, especially, clinical diagnosis is fraught with numerous problems, an aetiological classification based on histology, immunohistochemistry and molecular biology is favourable, particularly in view of the improvements in methods made in recent years. Such a classification may not only contribute toward improving our understanding of underlying pathological mechanisms, but ultimately may also assist in developing specific immunomodulatory treatments.

Current standards for diagnostic procedures require routine histological staining, which allows analysis of histomorphological changes and may depict myocytolysis (active myocarditis). For analysis of chronic inflammatory processes, however, immunohistological methods are recommended. Detection of viral genome and replication requires molecular biological techniques such as polymerase chain reaction (PCR) and in situ hybridization. According to the data obtained using these methods, clinically suspected DCM can be subclassified to different disease entities (Fig. 2)\[^{22,23}\]. Further information is derived from cytokine analysis of patients. Preliminary data suggest that dysregulation of the host defence mechanisms against viral infections, associated with an inappropriate T-helper-2-like immune response that lacks antiviral IFN-gamma, favours enteroviral RNA persistence and subsequent progression of left ventricular dysfunction (Kühl et al., unpublished data). A rise in pro-inflammatory antiviral T-helper-1 cytokines such as IFN-gamma appears to be necessary to control and eliminate viral RNA, thereby limiting progression of the disease.

**Figure 4** Molecular biological analysis (polymerase chain reaction [PCR]) of enteroviral RNA in endomyocardial biopsies. Lanes 1 and 7, molecular weight markers; lane 2, positive control; lanes 3 and 4, negative biopsy; lanes 5 and 6, enterovirus RNA-positive biopsies.

### Treatment of patients with virus-induced inflammatory cardiomyopathy

In spite of progress made in heart failure therapy since the introduction of angiotensin-converting enzyme inhibitors, beta-blockers, spironolactone and amiodarone/implantable cardioverter-defibrillator treatment, mortality associated with DCM is still 10%/year\[^{24–26}\]. Current heart failure therapy is symptomatic and does not impact on specific underlying pathogenic mechanisms. Progression of left ventricular dysfunction therefore cannot be halted by therapy with digoxis, diuretics, angiotensin-converting enzyme inhibitors and beta-blocking agents. Thus, specific treatment strategies that are directed against the underlying pathogenetic causes are required if myocarditis and its sequela, namely inflammatory cardiomyopathy, are to be successfully treated.

**Immunosuppressive and immunomodulatory therapy**

Potential therapeutic efficacy of immunosuppressive therapy was suggested by previous uncontrolled studies\[^{27–32}\]. However, it has not proved efficacious in previous randomized trials in patients with recent-onset cardiomyopathy because spontaneous recovery occurred both in patients treated with conventional therapy and in those treated with anti-inflammatory (methylprednisolone, cyclosporine A, intravenous immune globulin) therapy\[^{33–35}\]. Selection of patients on histological rather than immunohistological and molecular biological diagnostic criteria, and failure to account for the high spontaneous healing rate of recent-onset myocarditis may therefore account for those findings\[^{36–38}\]. Moreover, Frustaci et al. reported that most patients who did not respond to immunosuppressive therapy were enterovirus-positive in a retrospective PCR analysis, demonstrating virus-induced myocyte damage during immunosuppressive treatment (see the review by Frustaci et al., in the present supplement). In order to avoid such diagnostic shortcomings future trials must focus on reliable diagnostic criteria, which should include histological and immunohistological evaluation of myocardial inflammatory processes, as well as molecular biological analysis of myocardial virus infection (Fig. 5).

Immunosuppressive treatment regimens include corticosteroids, azathioprine or cyclosporin A. Alpha-methylprednisolone is generally initially given at a rate of 1 mg .kg\(^{-1}\) body weight (for children 1–2 mg . kg\(^{-1}\)) for 4 weeks, after which the dosage is tapered biweekly in increments of 8–12 mg until a maintenance dose of 12 mg is reached. The treatment should last for 6 months. In cases of persistent inflammation (35–40% of patients are affected by this), azathioprine may be administered in addition to low-dose corticosteroid. However, this appears to be effective only if a sufficient level of immunosuppression is achieved, which is signalled by a reduction in peripheral...

lymphocytes levels to around 1000/µl. Provided that adequate molecular biology and immunohistochemical characterization of patients is conducted, both clinical and haemodynamic improvement can be achieved in 65% of cases with immunosuppressive treatment (Fig. 6).

**Antiviral therapy with interferon-beta**

Three types of IFNs have been identified, which differ both in structure and in antigenic properties; IFN-alpha is derived from leucocytes, IFN-beta from fibroblasts and IFN-gamma from lymphocytes. The important role played by the IFNs as a natural defence mechanism against viruses is documented by three lines of experimental and clinical evidence: in many viral infections a strong correlation has been established between IFN production and natural recovery; inhibition of production or action of IFN enhances the severity of infection; and treatment with IFN protects against viral infection. The antiviral effect is independent of virus type and results in an intracellular block of the viral replication cycle. IFNs increase resistance toward viral replication, even in cells that neighbour infected cells but have not yet been infected. Because effective concentrations of IFN-beta can be attained in vivo, IFN-beta may become useful in the treatment of patients with viral cardiomyopathy.

Analyzing virus positive patients in a phase II pilot study, we demonstrated that patients with persistence of viral genomes and left ventricular dysfunction benefit from antiviral treatment with IFN-beta (manuscript submitted). Before IFN-beta treatment, clinical complaints and haemodynamic course did not improve and left ventricular diameters slowly increased, in spite of constant heart failure medication before IFN-beta treatment and during therapy. After 6 months of IFN-beta treatment, viral genomes were no longer detectable in the biopsies of all treated patients, indicating that this treatment had led to a complete elimination of both adenoviral and enteroviral genomes. Virus clearance was accompanied by a reduction in clinical complaints such as angina pectoris, dyspnoea, palpitations and fatigue, resulting in improvement in New York Heart Association functional class. This clinical improvement was associated with a significant increase in left ventricular ejection fraction and a decrease in left ventricular diameters. Because viral genomes were no longer detectable in any patient after completion of IFN-beta treatment, our data suggest that the beneficial clinical effect of IFN-beta is based on elimination of cardiotropic viral genomes, which occurred even in DCM patients with a long history of disease.

**Conclusion**

Myocarditis is caused by a wide variety of pathological conditions, in particular viral infections, and often precedes the development of DCM. The infecting virus initiates the disease process and then may persist as an insidious molecular pathogen, causing ongoing cardiac damage (viral heart disease). If the host immune system can effectively clear the viral infection, then cardiac inflammation often resolves. Dysregulation of the immune response, however, may result in a persisting pathological (auto)immune process (chronic myocarditis/inflammatory cardiomyopathy), causing progression of left ventricular dysfunction. Cytokines play a critical role in the development of viral persistence and chronic inflammation. Because pathological/anatomical and, especially, clinical diagnosis is fraught with numerous problems, the current standard of biopsy-based diagnostic procedures means that routine histological staining, which allows analysis of histo-
morphologic changes and may demonstrate myocytolysis (active myocarditis), is quite appropriate. For the analysis of chronic inflammatory processes, immunohistological methods are recommended. Detection of viral genome and replication requires molecular biology techniques such as PCR and in situ hybridization. The aetiological classification thus obtained may not only contribute toward improving our understanding of underlying pathological mechanisms, but ultimately may also assist in developing specific immunomodulatory treatments.

Preliminary results from ongoing treatment trials suggest that specific antiviral or anti-inflammatory treatment strategies are successful in patients who have been carefully selected and characterized according to biopsy-based diagnostic criteria. At present, the optimal timeframe for starting antiviral or immunosuppressive therapy is speculative because of lack of data. However, treatment should certainly begin as early as possible and before the emergence of serious ventricular dysfunction, because complete normalization of ventricular function is unlikely to be achieved once serious myocardial damage has occurred.

References


