Interferon-ß Treatment Eliminates Cardiotropic Viruses and Improves Left Ventricular Function in Patients With Myocardial Persistence of Viral Genomes and Left Ventricular Dysfunction

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Background—Viral infections are important causes of myocarditis and may induce cardiac dysfunction and finally lead to dilated cardiomyopathy. We investigated whether interferon (IFN)-β therapy is safe and may achieve virus clearance and prevent deterioration of left ventricular (LV) function in patients with myocardial virus persistence.

Methods and Results—In this phase II study, 22 consecutive patients with persistence of LV dysfunction (history of symptoms, 44±27 months) and polymerase chain reaction–proven enteroviral or adenoviral genomes were treated with 18×10^6 IU/week IFN-β (Beneferon) subcutaneously for 24 weeks. Histological and immunohistological analysis of endomyocardial biopsies was used to characterize myocardial inflammation. LV diameters and ejection fraction were assessed by echocardiography and angiography, respectively. During the treatment period, IFN-β was well tolerated by all patients. No patient deteriorated. Clearance of viral genomes was observed in 22 of 22 of patients after antiviral therapy. Virus clearance was paralleled by a significant decrease of LV end diastolic and end systolic diameters, decreasing from 59.7±11.1 to 56.5±10.0 mm ($P<0.001$) and 43.2±13.6 to 39.4±12.1 mm ($P<0.001$), respectively. LV ejection fraction increased from 44.6±15.5% to 53.1±16.8% ($P<0.001$).

Conclusions—A 6 months, IFN-β treatment was safe in patients with myocardial enteroviral or adenoviral persistence and LV dysfunction and resulted in elimination of viral genomes (22 of 22 patients) and improved LV function (15 of 22 patients). **(*Circulation*. 2003;107:2793-2798.)**

Key Words: cardiomyopathy ■ viruses ■ biopsy ■ heart failure

Advances in pharmacological therapy resulted in improved survival in patients with heart failure attributable to dilated cardiomyopathy (DCM).[1] However, current therapy is symptomatic and does not influence specific underlying pathomechanisms. Many DCM patients progress to terminal heart failure, and DCM represents the most common heart failure entity requiring heart transplantation.[1–3] Enteroviruses and adenoviruses are the most frequently implicated pathogens in Western countries.[4–11] The detection of enteroviral genomes in the myocardium is associated with an adverse prognosis and an independent predictor of clinical outcome.[12,13] These observations led to a search for a specific antiviral therapy in this subgroup of patients with DCM. The antiviral potential of interferon (IFN)-β against coxsackieviruses has been demonstrated in vitro[14] and in animal models.[15] The effect of IFNs against adenoviral infection has not yet been elucidated. The present study was undertaken to investigate whether IFN-β treatment of patients with biopsy-proven myocardial persistence of enteroviral or adenoviral genomes is safe and if it may achieve elimination of viral genomes, which could possibly prevent progression of cardiac dysfunction. To include patients most likely to benefit from antiviral therapy, we used 2 inclusion criteria (virus persistence and long-term cardiac dysfunction despite conventional heart failure therapy) reported to be associated with an adverse prognosis.[12,13]

Methods

Patients and Clinical Assessment

Among 1518 consecutive patients who were admitted to our clinic between December 1989 and October 2002 for the investigation and treatment of heart muscle disease and who underwent complete biopsy-based virological analysis, enteroviral and adenoviral genomes were detected in 15% and 7%, respectively. The patient population studied comprised 22 consecutive enterovirus- or adenovirus-positive patients with a long history of persisting cardiac symptoms. Other causes of left ventricular (LV) dysfunction (ie, coronary heart disease, valvular and hypertensive heart disease, and the familiar forms of cardiomyopathies) had been excluded in all

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TABLE 1. Demographic and Clinical Characteristics of 22 Virus-Positive Patients With Persistent LV Dysfunction Included in the IFN-β Treatment Study

Demographic	
No. of patients	22
Mean (SD) age, y	51.8±13.6*
Sex, male/female	13/9
Clinical characteristics	
NYHA functional class	
I	1 (5)†
II	10 (45)
III	10 (45)
IV	1 (5))
Fatigue	18 (82)
Angina	7 (32)
Dyspnea	18 (82)
Arrhythmias	11 (50)
History of cardiac symptoms, mo	44±27
Endomyocardial biopsy	
Enterovirus/adenovirus	14/8
Active/borderline myocarditis	0/0
Immunohistology (CD3-T-lymphocytes/mm^2)	5.7±5.5
Hemodynamic and echocardiographic measurements	
LVEF	44.6±15.5
LVEDD	59.7±11.1
LVESD	43.3±13.6
Systolic blood pressure, mm Hg	121±16
Diastolic blood pressure, mm Hg	76±12
Medication	
ACE inhibitors	73%
β-Blockers	64%
Glycosides	64%
Diuretics	59%
Warfarin	50%

*Mean±SD; †Number (percent).

patients. A full history was obtained from all patients before IFN-β treatment, and the following details were recorded: a history (44±27 months) of fatigue, chest pain, dyspnea, or arrhythmias. Routine ECGs, echocardiograms, and Holter monitoring were performed in all patients. Echocardiographic follow-up demonstrated global (ejection fraction [EF] <50%, n=12) or regional (EF >50%, n=10) LV dysfunction and lack of LV improvement during the last 22 months before the start of IFN-β treatment, despite constant ACE inhibitors, β-blockers, diuretics, and glycoside medication. Polymerase chain reaction (PCR) analysis revealed enteroviral genomes in biopsies of 14 of 22 patients (63.6%) and adenoviral genomes in 8 of 22 (36.4%). Demographic and clinical data of the patients are given in Table 1.

Study Design

LVEF was evaluated by LV angiography. Endomyocardial biopsies were obtained from the right ventricular septum within 2 days after angiographic evaluation of LVEF using standard techniques.[11,16] Two biopsies were used for the histologic evaluation (paraffin-embedded) according to the Dallas criteria[17] and the immunohistological analysis of inflammation (frozen sections), respectively.[18] Four biopsies were subjected to DNA and RNA extraction for the amplification of adenoviral and enteroviral genomes, respectively. DNA and RNA were extracted simultaneously from biopsy specimens, and the amplification of adenoviral and enteroviral genomes was conducted by nested PCR as described.[16] For the detailed histological, immunohistological, and molecular biological methods, see the Data Supplement.

All patients underwent M-mode and 2D echocardiography to evaluate LV end diastolic diameter (LVEDD) and LV end systolic diameter (LVESD). M-mode measurements followed the leading edge-to-edge principle as recommended by the American Society of Echocardiography.[19] Angiographic scans and echocardiographic measurements were analyzed independently in a blinded fashion by 2 experienced operators.

Physical examination, clinical assessments including echocardiography and ECG, as well as laboratory controls were conducted every second month to monitor IFN-β–associated side effects and adverse cardiac events. The assessment of clinical complaints (patient diary) and heart failure symptoms according to the NYHA classification and the completion of a questionnaire inquiring both IFN-β–associated adverse effects (flu-like symptoms, headache, and signs of inflammation) and specific cardiac adverse symptoms (eg, angina and dyspnea) were carried out at every visit. Follow-up biopsies and final clinical assessment were done within 6 weeks after cessation of the IFN-β treatment.

IFN-β Treatment

The IFN-β therapy (Beneferon) followed a stepped regimen to reduce the flu-like side effects typical of the initial phase of an IFN therapy. The subcutaneous administration was initiated at a dose of 2×10^6 U IFN-β 3 times a week on alternate days and was increased to 12×10^6 U during the second and 18×10^6 U during the third week. By the end of week 24, the IFN-β treatment was discontinued. The Human Research Committee of the Freie Universität Berlin approved the protocol, and all patients gave written informed consent before treatment.

Statistical Analysis

Statistical analysis was performed using JMP Statistical Discovery Software, Version 4.0.2 (SAS Institute, Inc). Qualitative data were analyzed by the χ^2 test, and continuous data were compared with ordinal ones by the Student's t test. All results were expressed as mean±SD, unless stated otherwise. $P<0.05$ was considered statistically significant.

Results

Clinical Course

During a 22-month period before inclusion into the IFN-β study, symptoms and hemodynamics had not improved in any of the 22 patients. LV diameters had slowly increased, despite constant heart failure medication. LVEDD and LVESD diameters (n=22) increased from 58.2±9.9 to 59.7±11.1 mm ($P<0.01$) and from 41.1±11.7 to 43.4±13.6 ($P<0.01$), respectively. At the time of initiation of IFN-β treatment, LVEF was 44.7±15.5% (Table 2).

After 6 months of IFN-β treatment, the LVEDD (59.7±11.1 versus 56.5±10.0 mm; $P<0.001$) and LVESD (43.3±13.6 versus 39.4±12.1 mm; $P<0.001$) had decreased significantly. This decrease of LV diameters was associated with a significant increase of LVEF from 44.7±15.5% to 53.1±16.8% ($P<0.001$) (Table 2). Improvement of LVEF and decrease of LV diameters occurred both in patients with regional (baseline EF, 58.0±6.7%; n=10) and with global (baseline EF, 33.6±11.2%; n=12) wall motion abnormalities (Table 3). However, the extent of the changes was more pronounced in patients with global dysfunction compared with those with regional dysfunction (Figure 1). Noticeably,

TABLE 2. Clinical, Hemodynamic, Virological, and Immunohistological Data of Patients Before and After IFN-β Treatment

	Before IFN-β	After IFN-β	P
Echocardiography			
LVEDD (n=22)	59.7±11.1*	56.5±11.1*	<0.001
LVESD (n=22)	43.4±13.6*	39.4±12.1*	<0.001
LV angiography			
LVEF (n=22)	44.7±15.5*	53.1±16.8*	<0.001
Endomyocardial biopsy			
Molecular biology (PCR)			
Enterovirus	15	0	<0.001
Adenovirus	7	0	<0.05
Histology			
Myocarditis	0	0	
Borderline myocarditis	0	0	
Immunohistology			
Inflammation (n=7) CD3, cells/mm^2	19.2±4.8*	6.0±4.6*	<0.05
No inflammation (n=15) CD3, cells/mm^2	2.6±1.8*	2.9±3.1*	NS
NYHA	2.5±0.6*	1.7±0.7*	<0.05

*Mean±SD.

no IFN-β–treated patient deteriorated. These findings were independent of the duration of symptoms before the onset of antiviral therapy. In detail, LVEF normalized completely in 9 patients and improved significantly (increase of LVEF >5%) in another 5 patients. Most of these 14 patients had initially displayed a mildly to moderately decreased LVEF. In contrast, patients whose LVEF did not improve substantially under IFN-β therapy had presented initially with severely impaired LVEF, and 2 of those had an LVEF ≤20% and were awaiting heart transplantation. Fifteen of the 22 IFN-β treated patients (68%) reported a decrease of clinical complaints such as angina, dyspnea, or fatigue after β-IFN treatment, resulting in an improvement in NYHA classification by 1 class (Figure 2).

Adverse Drug Effects

Adverse cardiac effects of the IFN-β therapy such as deterioration of LV function or induction of arrhythmias did not ensue in any of the patients during or after 6 months of treatment. Virtually all patients reported flu-like side effects of the IFN-β medication during the first 3 weeks of treatment. When necessary, flu-like side effects could be efficiently suppressed by nonsteroidal anti-inflammatory drugs during the first weeks of treatment (n=2). IFN-β administration was overall well tolerated, and no patient wished to discontinue the treatment.

Biopsy Results

After 24 weeks of IFN-β treatment, neither enteroviral nor adenoviral genomes were traceable in any biopsy specimens by nested PCR. According to the histologic Dallas criteria, none of the endomyocardial biopsies was graded as active or borderline myocarditis, neither before nor after the IFN-β treatment. In the immunohistochemical analysis, 7 patients had an increased number of CD3-positive T-lymphocytes quantitated at 19.2±4.8 cells/mm^2, consistent with myocardial inflammation.[18] After IFN-β treatment, T-lymphocyte counts had decreased to 6.0±3.1 cells/mm^2 ($P<0.05$) (Table 2). Lymphocyte numbers in the 15 patients without myocardial inflammation (baseline) did not change significantly (Table 2).

Discussion

Myocardial Virus Persistence in Dilated Cardiomyopathy

Viral infections have been associated with the development of myocarditis for many years.[20,21] A correlation of viral myocarditis with the development of dilated cardiomyopathy has been reported.[22,23] Using molecular genetic methods, enteroviral genomes have been detected in 10% to 35% of endomyocardial biopsies of patients with DCM.[4,6,8–11,24] Fur-

TABLE 3. Hemodynamic Parameters in Patients With Regional (EF >50%, n=10) Versus Global (EF <50%, n=12) LV Dysfunction Before and After IFN-β Treatment

	$LVEF_{baseline}$ <50% (n=12)			$LVEF_{baseline}$ >50% (n=10)		
N=22	Baseline	Follow-Up	*P*	Baseline	Follow-Up	*P*
LVEF, %	33.6±11.2	43.9±17.2	<0.001	58.0±6.7	64.1±7.2	<0.05
LVEDD	67.2±9.4	62.2±10.0	<0.001	50.8±4.4	49.7±4.2	0.20
LVESD	52.3±11.9	47.0±10.9	<0.001	32.6±4.8	30.3±5.0	<0.05

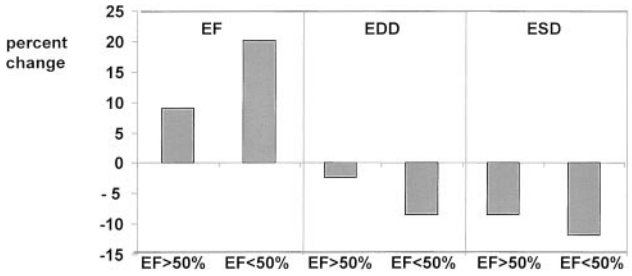

Figure 1. Hemodynamic changes in patients with global (EF <50%, n=12) versus regional (EF >50%, n=10) LV dysfunction during IFN-β therapy.

thermore, adenoviruses emerged as relevant infectious pathogens in DCM.[16] During the natural course of viral heart disease, an early local and systemic immune response limits uncontrolled virus spreading and usually achieves virus elimination from the target organ. However, viruses may escape immune surveillance and establish persistent myocardial infections. The causes for the development of virus persistence and the mechanisms by which viruses cause progression of myocardial dysfunction are incompletely understood.

Natural Course and Molecular Pathogenesis of the Disease

With respect to the natural course of the disease, we have found in accordance with other studies[12,13,25] that enteroviral RNA persistence is associated with progression of LV dysfunction and lack of clinical improvement (for details, see the Data Supplement). Why et al[12] have reported the presence of enteroviral RNA in 34% of 120 consecutively studied patients with heart muscle disease. At follow-up, the virus-positive patients had an increased mortality compared with virus-negative patients. Similarly, echocardiography demonstrated lack of improvement during the 22 months before initiation of the IFN-β therapy in our 22 study patients. Concerning the underlying pathomechanism, Wessely et al[26] have demonstrated that myocardial enteroviral persistence and restricted viral replication is sufficient for the maintenance of chronic inflammation, structural alterations of the myocardium, and interference with cardiomyocyte function in animal models of coxsackievirus B3–infected mice. The prognostic significance of enteroviral infection and replication has also been demonstrated in humans.[12,13] By using strand-specific PCR analyses, we and others have demonstrated that enteroviral genomes may actively replicate in the myocardium of DCM patients, suggesting that enteroviruses may exert an ongoing myocytopathic effect in patients with chronic viral persistence.[11,26,27] This observation is in line with a recent report by Fujioka et al,[13] who provided evidence for the importance of active viral RNA replication as a prognostic marker for poor clinical outcome. Moreover, Frustaci et al[25] reported that most patients not responding to immunosuppressive therapy were enterovirus positive in a retrospective PCR study, demonstrating virus-induced myocyte damage during immunosuppressive treatment. Because enteroviruses may actively replicate even in late stages of infection,[11,22,28] direct myocytopathic effects could perpetuate LV dysfunction in patients with virus persistence, eg, by interference with cardiomyocyte energy delivery and transmission, matrix integrity, or inflammatory cytokine release.[29–31]

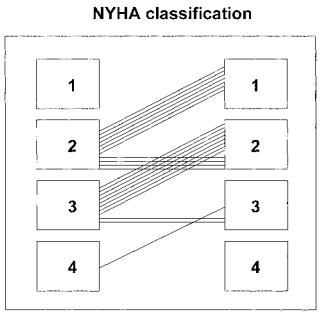

Figure 2. Changes of NYHA functional class during IFN-β treatment.

Searching for Novel Treatment Strategies

Optimal treatment with ACE inhibitors, glycosides, β-blockers, and diuretics does not directly influence specific underlying pathomechanisms of chronic viral heart disease and therefore may delay but not prevent progression of the disease. This therapy is unspecific, however, and virus-positive patients may possibly benefit from additional specific antiviral strategies.

Antiviral effects of IFN in an in vitro model of persistent enteroviral infection and its efficacy in preventing viral myocarditis in susceptible rodents are well known.[14,15,32–34] However, because IFNs were administered before or simultaneously with enteroviral infections in these experiments, they do not adequately represent the situation in humans, in whom antiviral treatment is mostly feasible not before the stage of latent viral persistence. Apart from the direct virostatic effect of IFN-β, immunomodulatory effects are induced that may suppress virus spreading and facilitate virus clearance.[35] Antigen-specific cytotoxic T-lymphocytes, natural killer cells, and cytokines are involved in this process.[36–38] A retrospective study by McCarthy et al[39] reported that a fulminant inflammatory process is associated with a better long-term outcome compared with nonfulminant presentation of myocarditis patients. This may indicate that local intramyocardial inflammation is not detrimental per se and moreover that inflammation may reflect an attempt of the immune system to eliminate cardiotropic viruses. The lack of substantial myocardial inflammation in our patients with viral heart disease may therefore indicate inefficient immune responses to the enteroviral and adenoviral cardiac infections. Although efficacy of an immunomodulatory treatment of enteroviral infections was demonstrated in animal myocarditis models, reported applications in humans are anecdotal.[32,33,40,41] Successful treatment of myocarditis with antiviral agents has been reported in case reports.[42] A 6-month treatment with IFN-α2a in 4 patients with enteroviral infection led to hemodynamic improvement in all patients, but virus was still detected in 2 of 4 patients.[35] In an open-label randomized study, Miric et al[41] provided data providing a possible beneficial effect of IFN-α in patients with DCM. LV

function improved in 77% of patients after a 6-month IFN-α treatment (3 million U/m^2) compared with improvement in 66% of conventionally treated patients at 2 years of follow-up. The increase of natural killer cell activity and the concurrent decrease in neutralizing antibody viral titers in the IFN-α–treated cohort was interpreted as induction of antiviral immune activation.[41] However, no proof of virus elimination was presented, because viral genomes were not documented before and after treatment.

IFN-β Therapy in Viral Heart Disease

The aim of the present study was to elucidate the effects of an antiviral IFN-β therapy in patients with myocardial virus persistence with respect to safety of the treatment regimen and virus clearance. To include patients most likely to benefit from an antiviral treatment, we selected 22 consecutive, persistently virus-positive patients with a long history (44±27 months) of persistent or progressive LV dysfunction despite medication with ACE inhibitors, β-blockers, glycosides, and diuretics. We found that a 6-month IFN-β treatment was associated with myocardial virus clearance in all (22 of 22) IFN-β–treated patients. Virus clearance was associated with hemodynamic (14 of 22) improvement in these patients; no IFN-β–treated patient deteriorated. These observations suggest that antiviral therapy with IFN-β is safe and may achieve virus clearance in association with hemodynamic improvement in patients with viral heart disease.

On IFN-β treatment, both adenoviral and enteroviral genomes disappeared, and the number of myocardial T-lymphocytes decreased. Patients with regional as well as global contractile dysfunction improved, but improvement was more pronounced in patients with global LV dysfunction. These findings on INF-β treatment taken together with the lack of improvement before treatment suggest that some of the ventricular dysfunction and wall motion abnormalities were caused by the persistent viral infection and resolved after elimination of the responsible agents. Our data furthermore suggest that the beneficial clinical effect of IFN-β based on the elimination of cardiotropic viruses may occur even in DCM patients presenting with a long history. The partial reversibility of cardiac dysfunction in these patients additionally suggests that the progression of LV dysfunction in patients with virus persistence is not solely caused by an irreversible loss of cardiomyocytes but may in part be attributable to interference of virus-encoded proteins or mRNAs with cardiomyocyte function and matrix integrity.[29,30] In addition, virus-induced negative inotropic cytokines may contribute to the hemodynamic deterioration.[43] All of these molecular pathomechanisms should be reversible if the etiologic agent can be eliminated and may thus explain the positive effects of the IFN-β therapy reported here. Although the improvement of LV function did not primarily depend on the duration of the symptoms before treatment, complete recovery of cardiac function can obviously be achieved only if irreversible myocardial damage does not exceed a certain limit, highlighting the importance of early diagnosis and treatment of these patients.

Conclusion

We conclude that antiviral IFN-β treatment may result in virus elimination and prevent progression of LV dysfunction in DCM patients with persistent cardiac viral infections. Because no cardiac-specific adverse effects occurred, the stepped treatment regimen used seems to be safe even in patients with severely depressed cardiac contractility.

Limitations of the Study

The primary objective of this nonrandomized phase 2 pilot study was to determine the efficacy of IFN-β treatment on myocardial virus clearance and its safety in patients with LV dysfunction. Accordingly, a relatively small number of patients with viral heart disease and various degrees of LV dysfunction were treated. A randomized placebo-controlled multicenter study (Betaferon in Chronic Viral Cardiomyopathy [BICC]) was started in November 2002 to verify the promising results reported here.

References

1. Elliot P. Diagnosis and management of dilated cardiomyopathy. *Eur Heart J*. 2000;84:106–112.
2. Feldmann AM, McNamara D. Myocarditis. *N Engl J Med*. 2000; 343: 1388–1398.
3. Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation*. 1996;93:841–842.
4. Bowles NE, Richardson PJ, Olsen EG, et al. Detection of Coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. *Lancet*. 1986;1: 1120–1123.
5. Tracy S, Wiegand V, McManus B, et al. Molecular approaches to enteroviral diagnosis in idiopathic cardiomyopathy and myocarditis. *J Am Coll Cardiol*. 1990;15:1688–1694.
6. Jin O, Sole MJ, Butany JW, et al. Detection of enterovirus RNA in myocardial biopsies from patients with myocarditis and cardiomyopathy using gene amplification by polymerase chain reaction. *Circulation*. 1990;82:8–16.
7. Archard LC, Bowles NE, Cunningham L, et al. Molecular probes for detection of persisting enterovirus infection of human heart and their prognostic value. *Eur Heart J*. 1991;12:56–59.
8. Koide H, Kitaura Y, Deguchi H, et al. Genomic detection of enteroviruses in the myocardium: studies on animal hearts with coxsackievirus B3 myocarditis and endomyocardial biopsies from patients with myocarditis and dilated cardiomyopathy. *Jpn Circ J*. 1992;56:1081–1093.
9. Grasso M, Arbustini E, Silini E, et al. Search for Coxsackievirus B3 RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction. *Am J Cardiol*. 1992;69:658–664.
10. Baboonian C, Treasure T. Meta-analysis of the association of enteroviruses with human heart disease. *Heart*. 1997;78:539–543.
11. Pauschinger M, Doerner A, Kuehl U, et al. Enteroviral RNA replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. *Circulation*. 1999;99:889–895.
12. Why HJ, Meany BT, Richardson PJ, et al. Clinical and prognostic significance of detection of enteroviral RNA in the myocardium of patients with myocarditis or dilated cardiomyopathy. *Circulation*. 1994;89: 2582–2589.
13. Fujioka S, Kitaura Y, Ukimura A, et al. Evaluation of viral infection in the myocardium of patients with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 2000;36:1920–1926.
14. Heim A, Canu A, Kirschner P, et al. Synergistic interaction of interferon-beta and interferon-gamma in coxsackievirus B3-infected carrier cultures of human myocardial fibroblasts. *J Infect Dis*. 1992;166: 958–965.
15. Matsumori A, Tomioka N, Kawai C. Protective effect of recombinant alpha interferon on coxsackievirus B3 myocarditis in mice. *Am Heart J*. 1988;115:1229–1232.

16. Pauschinger M, Bowles NE, Fuentes-Garcia FJ, et al. Detection of adenoviral genome in the myocardium of adult patients with idiopathic left ventricular dysfunction. *Circulation*. 1999;99:1348–1354.
17. Aretz HT. Myocarditis: the Dallas criteria. *Hum Pathol*. 1987;18: 619–624.
18. Kuhl U, Noutsias M, Seeberg B, et al. Immunohistological evidence for a chronic intramyocardial inflammatory process in dilated cardiomyopathy. *Heart*. 1996;75:295–300.
19. Echocardiography CoM-mSotASo. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation*. 1978;58:1072–1083.
20. Woodruff JF. Viral myocarditis: a review. *Am J Pathol*. 1980;101: 425–484.
21. Feldman AM, McNamara D. Medical progress: myocarditis. *N Engl J Med*. 2000;343:1388–1398.
22. Kawai C. From myocarditis to cardiomyopathy: mechanisms of inflammation and cell death. Learning from the past for the future. *Circulation*. 1999;99:1091–1100.
23. Liu PP, Mason JW. Advances in the understanding of myocarditis. *Circulation*. 2001;104:1076–1082.
24. Archard LC, Khan MA, Soteriou BA, et al. Characterization of Coxsackie B virus RNA in myocardium from patients with dilated cardiomyopathy by nucleotide sequencing of reverse transcription-nested polymerase chain reaction products. *Hum Pathol*. 1998;29:578–584.
25. Frustaci A, Chimenti C, Calabrese F, et al. Immunosuppressive therapy for active lymphocytic myocarditis: virologic and immunologic profile of responders versus nonresponders. *Circulation*. 2003;107:857–863.
26. Wessely R, Henke A, Zell R, et al. Low-level expression of a mutant coxsackieviral cDNA induces a myocytopathic effect in culture: an approach to the study of enteroviral persistence in cardiac myocytes. *Circulation*. 1998;98:450–457.
27. Liu P, Aitken K, Kong Y, et al. The tyrosine kinase p56Ick is essential in coxsackievirus B3-mediated heart disease. *Nature Med*. 2000;6:429–434.
28. Klingel K, Rieger P, Mall G, et al. Visualization of enteroviral replication in myocardial tissue by ultrastructural in situ hybridization: identification of target cells and cytopathic effects. *Lab Invest*. 1998;78:1227–1237.
29. Badorff C, Lee GH, Lamphear BJ, et al. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med*. 1999;5:320–326.
30. Doerner A, Pauschinger M, Schwimmbeck PL, et al. The shift in the myocardial adenine nucleotide translocator isoform expression pattern is associated with an enteroviral infection in the absence of an active T-cell dependent immune response in human inflammatory heart disease. *J Am Coll Cardiol*. 2000;35:1778–1784.
31. Badorff C, Fichtlscherer B, Rhoads RE, et al. Nitric oxide inhibits dystrophin proteolysis by coxsackieviral protease 2A through S-nitrosylation: a protective mechanism against enteroviral cardiomyopathy. *Circulation*. 2000;102:2276–2281.
32. Yamamoto N, Shibamori M, Ogura M, et al. Effects of intranasal administration of recombinant murine interferon-gamma on murine acute myocarditis caused by encephalomyocarditis virus. *Circulation*. 1998;97: 1017–1023.
33. Wessely R, Klingel K, Knowlton KU, et al. Cardioselective infection with coxsackievirus B3 requires intact type I interferon signaling: implications for mortality and early viral replication. *Circulation*. 2001;103:756–761.
34. Horwitz MS, La Cava A, Fine C, et al. Pancreatic expression of interferon-gamma protects mice from lethal coxsackievirus B3 infection and subsequent myocarditis. *Nature Med*. 2000;6:693–697.
35. Ramshaw IA, Ramsay A, Karupiah G, et al. Cytokines and immunity to viral infection. *Immunol Rev*. 1997;159:119–135.
36. Seko Y, Shinkai Y, Kawasaki A, et al. Evidence of perforin-mediated cardiac myocyte injury in acute murine myocarditis caused by Coxsackie virus B3. *J Pathol*. 1993;170:53–58.
37. Huang S, Hendrix W, Althage A, et al. Immune response in mice that lack the interferon-gamma receptor. *Science*. 1993;259:1742–1745.
38. Liu P, Aitken K, Kong YY, et al. The tyrosine kinase p56lck is essential in coxsackievirus B3-mediated heart disease. *Nat Med*. 2000;6:429–434.
39. McCarthy RE, Boehmer JP, Hruban RH, et al. Long-term outcome of fulminant myocarditis as compared with acute (non-fulminant) myocarditis. *N Engl J Med*. 2000;342:734–735.
40. Figulla HR, Stille-Siegener M, Mall G, et al. Myocardial enterovirus infection with left ventricular dysfunction: a benign disease compared with idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 1995;25: 1170–1175.
41. Miric M, Vasiljevic J, Bojic M, et al. Long-term follow up of patients with dilated heart muscle disease treated with human leucocytic interferon alpha or thymic hormones initial results. *Heart*. 1996;75: 596–601.
42. Baykurt C, Caglar K, Ceviz N, et al. Successful treatment of Epstein-Barr virus infection associated with myocarditis. *Pediatr Int*. 1999;41: 389–391.
43. Bozkurt B, Kribbs SB, Clubb FJ Jr, et al. Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats. *Circulation*. 1998;97: 1382–1391.