Potential Role of Humoral Immunity in Cardiac Dysfunction of Patients Suffering From Dilated Cardiomyopathy

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OBJECTIVES

This research was conducted to evaluate the role played by the humoral immune system in cardiac dysfunction among dilated cardiomyopathy (DCM) patients, as enabled by immunoadsorption therapy (IA) that effectively removes functionally active cardiac autoantibodies from plasma.

BACKGROUND

Various circulating autoantibodies have been detected among patients suffering from DCM. Before IA, antibodies were purified from plasma of 45 DCM patients (left ventricular ejection fraction [LVEF] <30%). We analyzed the functional effects of antibodies (300 mg/ml) on calcium transients and on systolic cell shortening in adult rat cardiomyocytes. After this in vitro analysis, IA was performed in four courses at one-month intervals until month 3.

RESULTS

Antibodies from 29 patients induced a reduction (>10%) in calcium transients (mean reduction: −16.5 ± 1.9%) and a simultaneous reduction (>10%) of cell shortening (mean reduction: −21.2 ± 1.8%) on cardiomyocytes (p < 0.001) (cardiodepressant group). Antibodies from 16 patients did not significantly influence calcium transients and cell shortening (<10%) (non-cardiodepressant group). During the first IA course, the cardiodepressant group demonstrated an acute increase in cardiac index (CI) from 2.2 ± 0.1 l/min/m² to 2.9 ± 0.1 l/min/m² (p < 0.001). In the non-cardiodepressant group, hemodynamics did not significantly change throughout three months. After three months before the final IA course (prolonged effects), the CI was 2.1 ± 0.1 l/min/m² in the non-cardiodepressant group and 2.9 ± 0.1 l/min/m² in the cardiodepressant group (p < 0.001). After three months LVEF increased only in the cardiodepressant group: from 20.8 ± 1% to 30.5 ± 1% (p < 0.01).

CONCLUSIONS

In the majority of DCM patients, disturbances of humoral immunity with production of cardiodepressant antibodies may play a functional role in cardiac dysfunction. Evidence of cardiodepressant antibodies predicts hemodynamic benefits during IA. (J Am Coll Cardiol 2004;44:829–36) © 2004 by the American College of Cardiology Foundation

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by progressive depression of myocardial contractile function and by ventricular dilatation (1). Disturbances of humoral immunity have been described in DCM patients. A number of antibodies against various cardiac cell proteins have been identified in DCM (e.g., antibodies against mitochondrial proteins, contractile proteins, beta-1-receptors, and muscarinergic receptors) (2–6). Recent data indicate that cardiac antibodies play an active role in the pathogenesis of DCM and may contribute to cardiac dysfunction in these patients (2,3). Removal of antibodies by immunoadsorption (IA) consequently improves cardiac function and alleviates myocardial inflammation in patients with heart failure (HF) due to DCM (7–11). Recent evidence, furthermore, has enabled confirmation that IA removes functional active antibodies from the plasma of patients with DCM (10). These antibodies induce a negative inotropic effect in isolated rat cardiomyocytes through depression of calcium transients. This cardiodepressant effect of the eliminated antibodies correlates with the acute hemodynamic improvement induced by IA (10). The presence of cardiodepressant antibodies in plasma of DCM patients before IA may accordingly predict the potential efficacy of this therapeutic approach.

The detection of cardiodepressant antibodies by means of this bioassay system may be of essential therapeutic relevance because the contribution of humoral activity with production of cardiodepressant antibodies may differ among DCM patients. For treatment of patients with severe HF due to DCM, it is important to identify those patients who will receive hemodynamic benefit from IA. In addition, IA represents an invasive and expensive treatment. Hence, predictors for hemodynamic improvement are necessary. The present study accordingly investigated systematically for the first time, and in a larger number of patients, whether detection of cardiodepressant effects of antibodies received from patients’ plasma before IA may be predictive for short- and long-term hemodynamic improvement during IA. Because, furthermore, IA effectively removes cardiac

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autoantibodies from plasma, such a procedure enables evaluation of the role played by the humoral immune system in cardiac dysfunction among DCM patients.

METHODS

Study protocol. A total of 45 DCM patients were admitted to the study. Five patients had participated in earlier IA studies (12,13). All patients demonstrated left ventricular (LV) dysfunction (left ventricular ejection fraction [LVEF] <30%, as assessed by two-dimensional echocardiography), as well as symptoms of severe chronic HF (New York Heart Association [NYHA] functional class III to IV). We excluded coronary heart disease by angiography, as well as acute myocarditis among all patients by myocardial biopsy, in accordance with Dallas criteria. An additional immunohistological analysis of CD3-positive lymphocytes, furthermore, was performed in endomyocardial biopsies. Patients were excluded if they had suffered from active infectious diseases, cancer, chronic alcoholism, or HF due to known origins (e.g., primary valvular disease). Patients with postpartum cardiomyopathy were also excluded. All patients were treated with angiotensin-converting enzyme inhibitors/angiotensin receptor antagonists, digitalis, and diuretics. All patients had received stable oral medication for more than three months before the study. Thirty-nine patients were treated with a beta-blocker. In these cases, doses of beta-blockers had been stable for at least six months before the present study. The hemodynamic inclusion criteria, as established by a Swan–Ganz thermodilution catheter, were cardiac index (CI) ≥2.5 l/min/m² and/or pulmonary capillary wedge pressure ≥16 mm Hg.

Written consent was obtained from each patient, and the protocol was approved by the Ethics Committees of the University Hospital of Greifswald and of the Charité University Hospital of Berlin, Germany. Preparation of plasma IgG. Before IA, antibodies were isolated from plasma by immunoaffinity purification using anti-IgG-coupled sepharose (PlasmaSelect, Teterow, Germany). Purification of antibodies was followed by dialysis (MWCO 100 KD, 1:100,000) against experimental buffer (in mmol/l: 117 NaCl, 2.8 KCl, 0.6 MgCl₂, 1.2 KH₂PO₄, 1.2 CaCl₂, 20 glucose, and 10 HEPES; pH = 7.3) for 30 h to remove molecules <100 KD. Antibodies were additionally heated (56°C, 30 min) for inactivation of complement (10). Aliquots of purified antibodies were stored at −70°C before testing in isolated cardiomyocytes. Measurement of intracellular Ca²⁺ transients and of systolic cell shortening. Ventricular cardiomyocytes from adult Wistar rats (180 to 200 g) were isolated as described elsewhere (10,14). The cardiomyocytes were suspended in experimental buffer and stained with the Ca²⁺ fluorescent probe Fura 2-AM (1 μmol/l) for 10 min at 25°C. Single cardiomyocytes were field-stimulated (1 Hz, 5 ms) and superfused continuously with experimental buffer (2 ml/min) (10). Fluorescence measurements were recorded with a dual-excitation, single-emission fluorescence multiplier system (IonOptix, Milton, Massachusetts). Changes in intracellular Ca²⁺ transients were inferred from the ratio of the fluorescence intensity at two wavelengths (15). A video-imaging edge detector system (IonOptix) was used to measure systolic cell shortening, as described (15). The relative change of Ca²⁺ transients and systolic cell shortening after superfusion of antibodies diluted with experimental buffer (IgG 300 mg/l) were analyzed in isolated rat cardiomyocytes as described recently (10,13).

Cardiodepressant effects were defined by a reduction of the Ca²⁺ transient (>10%, ~mean ± 2 SDs of controls) and simultaneous reduction of systolic cell shortening (>10%, ~mean ± 2 SDs of controls). Each purified antibody sample from one DCM patient was measured in blinded fashion, together with an age- and gender-matched control on the same cardiomyocyte preparation. The purified antibodies of controls and DCM patients were measured on at least six different cardiomyocytes of six different cardiomyocyte preparations. The values given in the present study express the mean obtained from these experiments. Each cardiomyocyte was used for only a single investigation of one sample. After this in vitro analysis (<4 weeks before IA), IA was performed in all patients in four courses at one-month intervals until month 3.

Immunoadsorption. IgG extraction including all IgG subclasses from the plasma took place with IgG-Therasorb (PlasmaSelect, Teterow, Germany), an anti-IgG immunoadsorbent. Immunoadsorption was performed in four courses, at one-month intervals until month 3, as described elsewhere (8). During the first course (course I), all patients underwent one IA session daily on three consecutive days. After the final IA session of each course, the patients received for safety reasons 0.5 g/kg polyclonal IgG (Ven-immun N) to restore IgG plasma levels (8).

Clinical findings. Echocardiographic parameters were determined in all patients by two-dimensional echocardiography, performed at baseline and after three months. The results were recorded, and a blinded reader performed off-line assessment of LVEF and left ventricular internal diameter in diastole (LVDiDd); LVEF was measured according to the Simpson rule.
Hemodynamics. We conducted right-heart catheterization with a Swan-Ganz thermodilution catheter to evaluate the hemodynamics of the patients. Measurements were carried out four times per day. The interval between two hemodynamic measurements was at least three hours. Hemodynamic measurements were carried out four times a day, one day before and one day after the first IA course. In addition, before and after the fourth course (course IV), hemodynamic monitoring again took place four times a day.

Statistics. Results are expressed as mean ± SEM. Changes in clinical outcomes were analyzed with respect to time by applying nonparametric multivariate analysis of variance for repeated measurements, in a two-factorial design (group: cardiodepressant vs. non-cardiodepressant and repetitions as factors) (16). All four time points were accordingly compared simultaneously on the corresponding response curves. After overall testing, post hoc analyses (Wilcoxon tests) were performed to detect specific differences between certain times of treatment as well as between treatment and baseline. Differences were further analyzed between the two groups with respect to certain time points by employing the Mann-Whitney U test. Adjustments were carried out for multiple comparisons (n = 3) by conducting a sequentially rejective test procedure according to Bonferroni-Holm (17). Fisher exact test was performed to detect differences with respect to gender between the subgroups. Changes were analyzed in NYHA classification before and after treatment by means of singly ordered 2 × 3 (before) and 2 × 4 (after) contingency tables, with use of the exact Mantel-Haenszel test of identically distributed rows (for treatments). Significance was assessed at the p < 0.05 level. All numerical calculations were performed using the packages SAS Version 8.2 (SAS Institute Inc., Cary, North Carolina), SPSS Version 11 (SPSS Inc., Chicago, Illinois), and StatXact 5 (CYTEL Software Corp., Cambridge, Massachusetts).

RESULTS

Clinical findings of all patients. A total of 45 DCM patients was admitted to the study from 1998 to 2002. The first IA course induced hemodynamic improvement as follows: CI increased from 2.2 ± 0.1 l/min/m² to 2.7 ± 0.1 l/min/m²; and stroke volume index (SVI) increased from 29.6 ± 1 ml/m² to 36.0 ± 1 ml/m²; (p < 0.001 vs. baseline). After three months, the hemodynamic improvement persisted: before the last IA course, CI 2.6 ± 0.1 l/min/m²; SVI 35.0 ± 1 ml/m² (p < 0.001 vs. baseline). The last IA course induced moderate improvement in hemodynamic parameters: after the last IA course, CI 2.8 ± 0.1 l/min/m²; SVI 37.0 ± 2 ml/m² (p < 0.001 vs. baseline; p < 0.05 vs. before last IA course). After three months, LVEF increased from 21.4 ± 1% to 28.1 ± 1% (p < 0.001 vs. baseline). One female patient in the non-cardiodepressant group died suddenly after the second IA course.

In vitro findings. In contrast with antibodies obtained from age- and gender-matched healthy blood donors (controls, n = 45), antibodies from DCM patients induced a reduction in Ca²⁺ transients (DCM: −11.1 ± 1.5%; controls: 0.6 ± 0.7%; p < 0.001 vs. controls) and in cell shortening (DCM: −14.7 ± 1.7%; controls: −1.3 ± 0.7%; p < 0.001 vs. controls). However, the inotropic effects of the antibodies on the isolated cardiomyocytes were not uniform among the patients. The antibodies obtained from 29 patients (cardiodepressant group) induced a significant reduction (>10%) in Ca²⁺ transients (mean reduction: −16.5 ± 1.9%) and cell shortening (mean reduction: −21.2 ± 1.8%) (p < 0.001 vs. controls; p < 0.001 vs. non-cardiodepressant group) on cardiomyocytes (Fig. 1). In contrast, antibodies from 16 patients (non-cardiodepressant group) did not significantly influence calcium transients (mean reduction: −1.3 ± 0.6%) or cell shortening (mean reduction: −2.9 ± 1.2%) (Fig. 1).

Characteristics of the subgroups at baseline. Table 1 lists clinical characteristics and echocardiographic parameters at baseline. In the cardiodepressant group and in the non-cardiodepressant group the following data were comparable: age, disease duration, NYHA functional classification, infiltration with lymphocytes in myocardial tissue, as well as medical treatment (e.g., angiotensin-converting enzyme inhibitors and beta-blockers). Left ventricular ejection fraction and LVIDd were also similar in both groups. In four patients of the cardiodepressant group (13.8%), and in three patients of the non-cardiodepressant group (18.8%), an increased number of lymphocytes (> 7 lymphocytes/mm²) in endomyocardial biopsies were detectable. There were more female patients in the non-cardiodepressant group than in the cardiodepressant group (p < 0.05).

Clinical findings of the subgroups. During IA, total IgG levels were effectively reduced in both groups by anti-IgG IA (IgG reduction in cardiodepressant group: −80.7 ± 2%; non-cardiodepressant group: −81.7 ± 2%). During the first IA course, hemodynamic parameters in the non-cardiodepressant group did not alter significantly (Figs. 2, 3, and 4). No significant changes were determined for the following parameters: CI, SVI, heart rate, and systemic vascular resistance (SVR).

In contrast, CI in the cardiodepressant group increased from 2.2 ± 0.1 l/min/m² to 2.9 ± 0.1 l/min/m² (p < 0.001 vs. baseline; p < 0.001 vs. non-cardiodepressant group) during the first IA course (Fig. 2). Because heart rate remained stable, SVI also significantly increased: from 30.8 ± 1 ml/m² to 38.3 ± 2 ml/m² (p < 0.001 vs. baseline; p < 0.001 vs. non-cardiodepressant group) (Fig. 3). Systemic vascular resistance decreased from 1,343 ± 45 dyne·s·cm⁻⁵ to 1,010 ± 39 dyne·s·cm⁻⁵ (p < 0.001 vs. baseline; p < 0.001 vs. non-cardiodepressant group) (Fig. 4). Mean arterial blood pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and right atrial pressure did not change significantly in either group.
The prolonged benefits of both groups were also different. After three months and before the last IA course in the non-cardiodepressant group, none of the measured hemodynamic parameters differed from those at baseline (Figs. 2, 3, and 4). In contrast, hemodynamic improvement persisted in the cardiodepressant group: after three months, before the final IA course, CI was 2.9 ± 0.1 l/min/m² in the cardiodepressant group (p < 0.001 vs. baseline; p < 0.001 vs. non-cardiodepressant group) (Fig. 2). Stroke volume index and SVR were,

The prolonged benefits of both groups were also different. After three months and before the last IA course in the non-cardiodepressant group, none of the measured hemodynamic parameters differed from those at baseline when the study began (Figs. 2, 3, and 4). In contrast, hemodynamic improvement persisted in the cardiodepressant group: after three months, before the final IA course, CI was 2.9 ± 0.1 l/min/m² in the cardiodepressant group (p < 0.001 vs. baseline; p < 0.001 vs. non-cardiodepressant group) (Fig. 2). Stroke volume index and SVR were,

### Table 1. Characteristics of Patients at Baseline

<table>
<thead>
<tr>
<th></th>
<th>All Patients (n = 45)</th>
<th>Cardiodepressant Group (n = 29)</th>
<th>Non-Cardiodepressant Group (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>51.8 ± 1.4</td>
<td>50.3 ± 1.8</td>
<td>54.5 ± 2.0</td>
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<td>Gender (male/female)</td>
<td>39/6</td>
<td>28/1</td>
<td>11/5*</td>
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<td>Disease duration (yrs)</td>
<td>4.7 ± 0.5</td>
<td>4.5 ± 0.6</td>
<td>5.0 ± 0.9</td>
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<td>NYHA classification III/IV</td>
<td>33/12</td>
<td>22/7</td>
<td>11/5</td>
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<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
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<tr>
<td>LVIDd (mm)</td>
<td>73.2 ± 2</td>
<td>72.8 ± 2</td>
<td>73.9 ± 3</td>
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<tr>
<td>LVEF (%)</td>
<td>21.4 ± 1</td>
<td>20.8 ± 1</td>
<td>22.4 ± 2</td>
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<tr>
<td>Hemodynamics</td>
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<tr>
<td>CI (l/min/m²)</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.0 ± 0.1</td>
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<tr>
<td>SVI (ml/m²)</td>
<td>29.6 ± 1</td>
<td>30.8 ± 1</td>
<td>27.4 ± 1</td>
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<tr>
<td>SVR (dyne·cm⁻⁵)</td>
<td>1,379 ± 34</td>
<td>1,343 ± 45</td>
<td>1,442 ± 45</td>
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<td>Medication</td>
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<tr>
<td>ACE inhibitor/AT-1 antagonist (n)</td>
<td>45</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>Beta-blocker (n)</td>
<td>39</td>
<td>25</td>
<td>14</td>
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<tr>
<td>Immunohistology</td>
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<td></td>
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<tr>
<td>CD3 (cells/mm³)</td>
<td>3.9 ± 0.5</td>
<td>3.8 ± 0.6</td>
<td>4.0 ± 0.8</td>
</tr>
</tbody>
</table>

*p < 0.05.

ACE = angiotensin-converting enzyme; AT-1 = angiotensin II type 1 receptor; CI = cardiac index; LVEF = left ventricular ejection fraction; LVIDd = left ventricular internal diameter in diastole; NYHA = New York Heart Association; SVI = stroke volume index; SVR = systemic vascular resistance.
likewise, significantly different compared with baseline (Figs. 3 and 4).

The final IA course induced moderate improvement of CI, SVI, and SVR in the cardiodepressant group \( (p < 0.05 \) vs. before last IA course) (Figs. 2, 3, and 4). After the final IA course, CI was 2.2 ± 0.1 l/min/m² in the non-cardiodepressant group and 3.1 ± 0.1 l/min/m² in the cardiodepressant group \( (p < 0.001 \) vs. baseline; \( p < 0.001 \) vs. non-cardiodepressant group) (Fig. 2).

In addition, multivariate analyses regarding the entire time period from the first to the last course of IA revealed significant differences between the two groups \( (p < 0.001) \), as well as significant changes in time for the cardiodepressant group \( (p < 0.001) \) with respect to CI, SVI, and SVR.

After three months, moreover, LVEF did not significantly increase in the non-cardiodepressant group: from 22.4 ± 2% to 23.3 ± 2% \( (p = \text{NS}) \). In the cardiodepressant group, improvement in hemodynamic parameters was paralleled by a comparable increase in LVEF: from 20.8 ± 1% to 30.5 ± 1% \( (p < 0.001 \) vs. baseline; \( p < 0.01 \) vs. non-cardiodepressant group) (Fig. 5).

After three months, examinations for NYHA HF classification revealed improvement in the cardiodepressant group \( (p < 0.05 \) vs. baseline/vs. non-cardiodepressant group). In contrast, non-cardiodepressant patients obtained no relief from symptoms. The NYHA HF classification improved in the cardiodepressant group \( (p < 0.001) \) in comparison with the non-cardiodepressant patients after treatment.

**DISCUSSION**

Abnormalities of the humoral immune system are present in patients with myocarditis and DCM. Several studies have disclosed that IA, which removes autoantibodies from plasma, may represent an additional therapeutic approach in patients with HF due to DCM \( (7–11) \). The findings of the present study have revealed that abnormalities of humoral immunity with production of circulating cardiodepressant antibodies play a potential role in cardiac dysfunction in only a subgroup of DCM patients. The present study investigated whether detection of cardiodepressant effects of antibodies received from patients’ plasma before IA may be predictive for short- and long-term hemodynamic improvement during IA. Identification of those patients with DCM and severe HF who will receive hemodynamic benefit from
IA is important, because most of these patients are potential candidates for heart transplantation. Furthermore, IA is an expensive and invasive therapeutic method.

**Clinical findings and the detection of functionally active cardiac antibodies.** At baseline, no differences existed between the subgroups with regard to age, disease duration, NYHA class, LV function, infiltration by lymphocytes in myocardial tissue, or hemodynamics. These findings indicate that the detection of cardiodepressant antibodies was not associated with these clinical parameters. However, it is important to point out that only patients with advanced HF (NYHA III/IV and ejection fraction <30%; CI ≤2.5 l/min/m² and/or pulmonary capillary wedge pressure ≥16 mm Hg) were included in the present study. Owing to the strict inclusion criteria of the present study, a relatively homogeneous patient group was admitted to the study. On the other hand, these findings indicate that the prevalence of cardiodepressant antibodies does not reflect severity of myocardial damage in DCM but may rather imply reversible impairment of cardiac function with the potential of recovery upon removal of these antibodies. Furthermore, the comparable baseline findings indicate that other mechanisms are additionally involved in the pathogenesis of DCM. The potential of the different pathogenic factors involved may differ among patients. The literature includes contradictory findings on associations between disturbances of humoral immunity and clinical findings. A recent study demonstrated that detection of the specific antibody against the beta-1 receptor was more common among DCM patients with poorer LV function (18). On the other hand, Cañorio et al. (19) concluded that cardiac-specific autoantibodies in DCM become undetectable with disease progression. The proportion of female patients was higher in the non-cardiodepressant group that did not receive hemodynamic benefit during IA. However, a conclusion concerning gender-based differences is limited by the small number of female patients included in the present study. On the other hand, clinical data on gender-based differences in the pathophysiology, treatment results, and outcomes of HF are well known (20).

A former study by Parrillo et al. (21) showed that immunomodulatory treatment using prednisone, which primarily influences the cellular immune system, did not induce general improvement in LV function of DCM patients. Only a subgroup of DCM patients with various inflammatory markers in blood and in myocardial tissue experienced improvement in LVEF from prednisone (21). In accordance with the findings of the present study, differences concerning general clinical characteristics between the subgroups at baseline (e.g., age, NYHA functional class, and disease duration) did not exist in the study by Parrillo et al. (21).

**Pathogenic importance of humoral immunity in DCM.** A recent study has analyzed the pathogenic role of autoantibodies in the development of ventricular arrhythmias. In a subgroup of patients with DCM, antibodies against the sarcolemmal Na-K-ATPase were detectable (22). Ventricular tachycardias were more common in patients with antibodies against the Na-K-ATPase than in those without such antibodies. Autoantibodies could therefore be responsible for the electrical instability in a subgroup of DCM patients (22). A model of DCM was created by immunizing rabbits with peptides corresponding to the sequence of the second extracellular loop of either beta-1 adrenoceptors or M2-muscarinic receptors. High titers of anti-peptide antibodies were found in the sera. Both groups of immunized rabbits demonstrated morphologic heart alterations similar to those found in human DCM (23). These findings also indicate that autoantibodies represent factors of clinical importance, and not an epiphenomenon.

The present study disclosed that, in the majority of DCM patients, humoral immunity may play an important role in cardiac dysfunction. In contrast with the non-cardiodepressant group, hemodynamic improvement in the cardiodepressant group was immediately detectable after the first IA course (after three consecutive days of IA). It is unlikely that patients with a long history of HF and without change of medical treatment would experience such spontaneous hemodynamic improvement. In comparable control patients who suffered from chronic HF caused by DCM, and who were included in former randomized studies, LV function remained stable for three months (8,11).

**Potential mechanisms of the cardiodepressant effects.** Before testing of plasma on cardiomyocytes, antibodies were purified by various methods. The antibodies were isolated from plasma by immunoaffinity purification by means of anti-IgG-coupled sepharose. To remove smaller molecules, purification of antibodies was followed by dialyzation against experimental buffer. Antibodies were additionally heated for inactivation of complement. In an earlier study, various cytokines were measured after such purification (10). Neither TNF-alpha nor interleukin-1-beta, -6, -8, or -10 were detectable. In a following study, the functional effects of antibodies belonging to the IgG-3 subclass on cardiomyocytes were analyzed. Findings of this study disclosed that the cardiodepressant effects on cardiomyocytes are mediated by antibodies belonging to IgG-3 subclass (13). Therefore, the removal of antibodies belonging to the IgG-3 subclass may represent one essential mechanism of IA. A previous study analyzed the time course of cardiodepressant effects induced by the eliminated antibodies during IA (10). Antibodies obtained during the first IA course on day 1 induced a pronounced cardiodepressant effect on cardiomyocytes. Parallel to the reduction of total IgG during IA, the negative inotropic effect of the eliminated antibodies collected during days 2 and 3 was less pronounced (10).

A number of autoantibodies against cardiac cell proteins have been identified in DCM (2–6). However, the known cardiac autoantibodies cannot explain the major in vitro findings of the present study (i.e., impairment of systolic cell shortening by reduction of Ca²⁺ transients without a change in intracellular diastolic Ca²⁺ level). Therefore,
further studies are necessary to elucidate the specific antigens of the cardiac autoantibodies responsible for the functional effects.

Removal of beta-1-receptor autoantibodies and hemodynamic improvement during IA. A recent study elucidated the association between removal of beta-1-receptor autoantibodies and the hemodynamic benefit during IA. Independently of the analytical method, however, patients positive and negative for beta-1-receptor antibody received hemodynamic benefits during IA (12). The beneficial hemodynamic effects induced by IA are consequently not directly associated with the removal of beta-1-receptor autoantibodies (12). Other cardiac antibodies may consequently be likewise involved in the hemodynamic effects of IA. We have recently shown that antibodies eliminated by IA induce a negative inotropic effect on cardiomyocytes. Furthermore, immunoprecipitation has revealed that antibodies eliminated by IA are able to bind to different myocardial proteins (10). These results, however, disclosed no differences between controls and DCM patients. The evident absence of a difference in myocardial protein patterns between patients and controls may arise from the fact that the amount of cardiac antibodies is not great enough to evidence a myocardial protein pattern for DCM patients. It is further possible that IA removes functional active antibodies of low affinity (10). This hypothesis suggests that the putative antibodies diffuse easily from their binding to the cardiac antigens. The removal of antibodies by IA accordingly induced immediate hemodynamic improvement. Finally, the functional activity of cardiac antibodies is not paralleled by their binding properties. Jahns et al. (18) showed that only a subgroup of antibodies that bind to the peptide of beta-1-receptor can realize functional activity on the native receptor. Further studies are therefore necessary to elucidate the specific antigens of the cardiac autoantibodies that are responsible for the functional and clinical effects. Additional mechanisms may likewise be involved in the hemodynamic effects of IA. Because direct influence of IA on peripheral blood vessels cannot be excluded, the role of the eliminated factors on SVR remains to be elucidated.

Potential role of IgG substitution. After plasma IgG depletion induced by IA, IgG was substituted for safety reasons (8). In addition to IA, IgG treatment additionally influences the immune system through various mechanisms (24). Gullestad et al. (25) showed that IgG treatment may induce beneficial effects in patients with HF. The improvement of LVEF has correlated with changes in inflammatory and anti-inflammatory mediators. In contrast, McNamara et al. (26) showed that patients with acute cardiomyopathy who received IgG therapy (2 g/kg) obtained no further relief from symptoms than did placebo patients. In comparison with IgG treatment (2.0 g/kg), furthermore, substitution of IgG plasma level after IA was performed in our study with a low dosage of polyclonal IgG (0.5 g/kg). After substitution, the IgG level was comparable with the level before IA (data not shown). Hence, IgG substitution must be distinguished from IgG treatment. Moreover, in a recent study involving patients treated with protein-A IA and subsequent IgG substitution (0.5 g/kg), hemodynamic parameters did not change significantly (13). These data indicate that IgG substitution with low dosage of IgG performed after IA does not influence the hemodynamic parameters measured.

Our results indicate that the antibodies of DCM patients are functionally active, by virtue of the evidence that they induce a decrease of cell contraction in isolated rat cardiomyocytes by depression of calcium transients. These effects, however, are not detectable in all DCM patients. The short- and long-term hemodynamic benefit among DCM patients during IA was associated with the negative inotropic effects of their antibodies received from patient plasma before IA. The long-term effects of IA therapy on clinical outcome of both subgroups remain to be elucidated.

Study limitations. In the present study, there was no significant difference in CD3+ lymphocytic infiltration between the cardiodepressant and the non-cardiodepressant group. An association, however, cannot be excluded between the detection of functional active antibodies and additional markers of inflammatory cardiomyopathy such as further phenotypes of immunocompetent infiltrates or endothelial expression of cell adhesion molecules in myocardial tissue. Consequently, a higher prevalence of functional active antibodies in patients with inflammatory cardiomyopathy cannot be definitely excluded.

Conclusions. In the majority of DCM patients, functionally active autoantibodies are detectable that may play an important role in cardiac dysfunction. Immunoadsorption accordingly induces beneficial effects only in this subgroup of DCM patients. Evidence of cardiodepressant antibodies predicts short- and long-term hemodynamic benefit during IA. Findings of the present study suggest that the humoral immune system, with production of circulating antibodies, may play an important role in cardiac dysfunction of a subgroup of DCM patients. On the other hand, the humoral immune system may exert only a minor influence on myocardial function among remaining DCM patients.

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