Journal of the American College of Cardiology
© 2002 by the American College of Cardiology
Published by Elsevier Science Inc.

Vol. 39, No. 4, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(01)01794-6

Removal of Cardiodepressant Antibodies in Dilated Cardiomyopathy by Immunoadsorption

Stephan B. Felix, MD,* Alexander Staudt, MD,* Martin Landsberger, PHD,* Yvonne Grosse, MD,* Verena Stangl, MD,† Thomas Spielhagen, MD,† Gerd Wallukat, PHD,‡ Klaus D. Wernecke, PHD,§ Gert Baumann, MD,† Karl Stangl, MD†

Greifswald and Berlin, Germany

OBJECTIVES The objective of this study was to investigate whether immunoadsorption (IA) removes cardiodepressant antibodies from the plasma of patients with dilated cardiomyopathy (DCM), as well as to describe their effects on isolated rat cardiomyocytes.

BACKGROUND Immunoadsorption induces early hemodynamic improvement in patients with DCM. The mechanisms for this improvement remain to be elucidated.

METHODS Patients with DCM (n = 11; left ventricular ejection fraction <30%, cardiac index [CI] <2.5 l/min per m^2) were treated with IA on three consecutive days, with one IA session daily, by application of specific antibody columns directed against human immunoglobulin (Ig). Immunoadsorption was also conducted on 500 ml of blood taken from nine healthy donors (control subjects). After passage of plasma, the IA columns were regenerated. Column eluent (CE) was collected and dialyzed (100 kD). Confocal laser scanning microscopy was used to analyze the effects of CE on cell contraction and on Ca^{2+}-dependent fluorescence in isolated, field-stimulated adult rat cardiomyocytes loaded with cell-permeable Fluo-3. Immunoprecipitation with different preparations of myocardial protein fractions was used for characterization of cardiotropic antibodies.

RESULTS During IA, the IgG plasma level decreased from 10.7 ± 0.6 to 2.4 ± 0.1 g/l (mean ± SEM), and the CI increased from 2.2 ± 0.1 to 2.7 ± 0.2 l/min per m^2 ($p < 0.01$). The CE obtained from control subjects did not influence Ca^{2+} transients or cell shortening of cardiomyocytes. In contrast, in patients with DCM, the CE collected during the first regeneration cycle of the first IA session caused an immediate and dose-related decrease of Ca^{2+} transients (dilution 1:5; −22.7 ± 5.5%; $p < 0.01$) and cell shortening (dilution 1:5; −29.9 ± 6.0%, $p < 0.01$). Early hemodynamic improvement among the patients correlated with the cardiodepressant effect of CE on the isolated cardiomyocytes. Purification of CE by protein A adsorption indicated that the cardiodepressant substances are antibodies. Immunoprecipitation revealed that the eliminated antibodies are capable of binding to various myocardial proteins.

CONCLUSIONS Cardiac autoantibodies play a functional role in DCM, and their removal may induce early hemodynamic improvement. (J Am Coll Cardiol 2002;39:646–52) © 2002 by the American College of Cardiology

Dilated cardiomyopathy (DCM) is a chronic myocardial disease characterized by progressive ventricular enlargement and myocardial contractile malfunction (1). Disturbances in humoral and cellular immunity have been described in cases of myocarditis and DCM (2,3). A number of autoantibodies against cardiac cell proteins have been identified in DCM. These include antibodies against contractile proteins, the cardiac beta-receptor, mitochondrial proteins, the calcium channel, the muscarinic receptor and the sarcoplasmic reticulum (4–8). The functional role of cardiac autoantibodies in DCM is still unclear. The possibility exists that antibodies play an active role in the pathogenesis and progression of the disease (9). If autoantibodies contribute to cardiac dysfunction in DCM, their removal would be expected to improve the hemodynamic variables. Cardiac autoantibodies that belong to the immunoglobulin G (IgG) fraction are extractable by immunoadsorption (IA). We recently reported that IA induces an early and prolonged hemodynamic improvement in patients with DCM, which manifests itself by a significant increase in the cardiac index (CI) (10,11). Immunoadsorption and subsequent IgG substitution also ameliorate myocardial inflammation in patients with DCM (12). Immunoadsorption may represent an additional therapeutic approach in patients suffering from DCM (10–13). The underlying mechanisms of the early hemodynamic effects of IA were not further investigated. The objectives of this study were therefore to investigate whether IA removes cardiodepressant antibodies from the plasma of patients with DCM and to describe their effects on isolated rat cardiomyocytes.

From the *Klinik für Innere Medizin B, Universität Greifswald, Greifswald; †Medizinische Klinik, Charité, Campus-Mitte, Berlin; ‡Max Delbrück Zentrum für Molekulare Kardiologie, Berlin; and §Institut für Medizinische Biometrie, Charité, Humboldt-Universität, Berlin, Germany. This study was supported, in part, by grant NBL3 from the German Ministry of Education and Research (BMBF).

Manuscript received May 21, 2001; revised manuscript received November 9, 2001, accepted November 30, 2001.

Abbreviations and Acronyms

CE = column eluent
CI = cardiac index
DCM = dilated cardiomyopathy
IA = immunoadsorption
Ig = immunoglobulin
IL = interleukin
LVEF = left ventricular ejection fraction
rfu = relative fluorescence units
TNF = tumor necrosis factor

METHODS

Study patients. Included in the study were male patients (n = 11; mean age 51.5 ± 4.3 years; mean disease duration 3.7 ± 0.6 years) with DCM and severe left ventricular dysfunction (New York Heart Association functional class III/IV; left ventricular ejection fraction [LVEF] <30%; CI <2.5 l/min per m^2). All patients had been taking a stable dosage of oral medications (e.g., angiotensin-converting enzyme inhibitors, digitalis, diuretics) for at least three months. Six patients received a beta-blocker in a stable dosage for at least six months. Coronary heart disease was excluded by angiography. From all patients, we obtained myocardial biopsies from the interventricular septum of the right ventricle. In all cases, acute myocarditis was excluded, according to the Dallas criteria (14). Patients were excluded if they had active infectious diseases, cancer, chronic alcoholism or heart failure due to other known origins. Immunoadsorption was likewise conducted on 500 ml of blood from healthy, male, age-matched donors (control subjects: n = 9; mean age 49.7 ± 3.3 years).

Immunoadsorption and hemodynamic measurements. Immunoadsorption was performed on 11 patients with DCM, with one session daily on three consecutive days. Immunoglobulin extraction took place with a specific immunoadsorber (Ig-Therasorb, Baxter, Munich, Germany), as described recently (11). Each session was continued until 5 l of plasma had passed through the IA columns. Hemodynamic measurements were performed four times a day with a Swan-Ganz thermodilution catheter, at baseline, one day before IA (day 0) and after each IA session (days 1–3). The interval between two hemodynamic measurements was at least 3 h. The hemodynamic values given in Figure 1 and Table 1 represent the mean values from hemodynamic measurements performed four times a day. After completion of hemodynamic measurements (day 3), polyclonal IgG (0.5 g/kg intravenously) was substituted for safety reasons—to reduce the risk of infection and to prevent rebound of antibody production.

Collection and purification of column eluent (CE). Regeneration of the IA columns was performed by irrigation with different solutions: 1) 120 ml of 0.9% NaCl; 2) 230 ml of glycin-HCl (0.2 mmol; pH 2.8); 3) 300 ml of phosphate- buffered saline (pH 7.2); and 4) 250 ml of 0.9% NaCl. Collection of 230 ml of CE took place during the first regeneration cycle of each IA session, followed by dialyzation (molecular weight cut-off 100 kD, 1:100,000) against experimental buffer (in mmol/l: 117 NaCl, 2.8 KCl, 0.6 $MgCl_2$, 1.2 KH_2PO_4, 1.2 $CaCl_2$, 20 glucose and 10 HEPES; pH 7.3) for 30 h to remove molecules <100 kD. The CE was then heated (56°C, 30 min) for inactivation of the complement. Aliquots of CE were stored at −70°C before testing in isolated cardiomyocytes.

In a further series of experiments, the CE was dialyzed (MWCO 0.5 kd, 1:100,000) for 30 h against the experimental buffer, heated (56°C, 30 min) and passed through protein A sepharose CL-4B columns (Pharmacia Biotech, Sweden) for Ig extraction.

Measurement of Ca^{2+}-dependent fluorescence and cell length in isolated cardiomyocytes. Ventricular cardiomyocytes from adult Wistar rats weighing 180 to 200 g (n = 24) were isolated as described elsewhere (15). The cardiomyocytes were suspended in experimental buffer and stained with the Ca^{2+} fluorescent cell-permeable Fluo-3 (5 μmol/l; F 6142 Sigma, Deisenhofen, Germany).

Single cardiomyocytes were field-stimulated (1 Hz, 5 ms) and superfused continuously with experimental buffer (2 ml/min). The Ca^{2+}-dependent fluorescence—expressed by relative fluorescence units (rfu)—and cell length were simultaneously measured (488 nm, 120 images/s) in the cardiomyocytes by confocal laser scanning microscopy (Odyssey XL, Noran Instruments, Middleton, Wisconsin), as described elsewhere (15). Changes in Ca^{2+} transients were calculated as: peak systolic rfu − diastolic rfu, without the effort for calibration, due to the uncertain subcellular compartmentation of the probes (16). Under control conditions, the cardiomyocytes (n = 90) shortened during stimulation by 8.2 ± 0.5% (mean ± SEM), and Ca^{2+}-dependent fluorescence increased from 31.3 ± 0.9 rfu (diastolic) to 80.9 ± 3.1 rfu (peak systolic). After equilibration for 2 min, control data were recorded, and different dilutions of CE from patients with DCM and control subjects were superfused. The measured variables were evaluated for each dilution once the effect of CE had reached a steady state. Each cardiomyocyte was used for only a single investigation of one of five different dilutions of CE. The experiments were performed in blinded fashion, and the investigators were unaware of whether the eluent originated from patients or control subjects. All data were stored on a hard disk, and changes in Ca^{2+} transient and cell shortening were analyzed off-line by a computer program, as described elsewhere (15).

Immunologic variables. Concentrations of tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-6, IL-8, IL-10 and IL-1-beta were determined using the automated Immulite chemiluminescence enzyme immunoassay system (DPC Biermann, Bad Nauheim, Germany) to determine the role of cardioactive cytokines in CE. Determination of

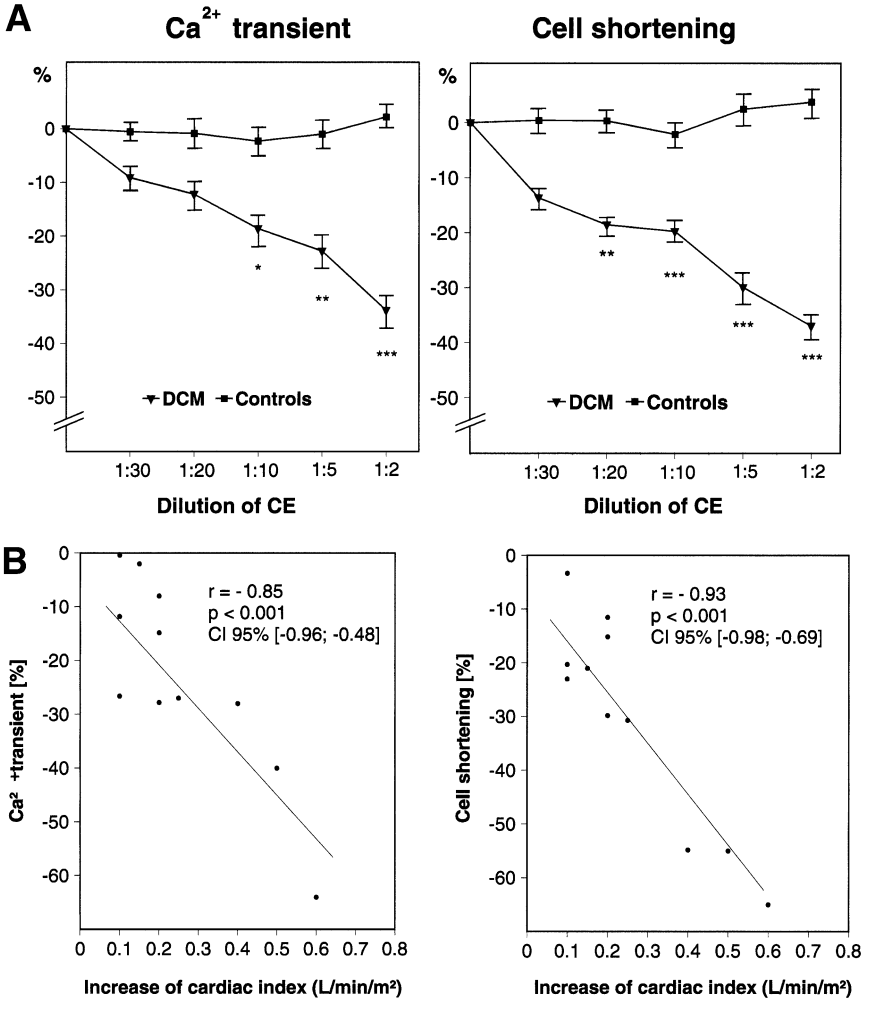

Figure 1. (A) Effects of the indicated dilutions of CE on isolated, field-stimulated rat cardiomyocytes. The CE was collected on day 1 during the first regeneration cycle of IA, session 1. Changes of Ca^{2+} transients (peak systolic rfu − diastolic rfu) **(left plot)** and systolic cell shortening **(right plot)** during superfusion of CE from healthy blood donors (control subjects = **solid squares,** n = 9) and of CE from patients with DCM **(solid triangles,** n = 11). Data (percent changes from baseline) are presented as the mean value ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control subjects. **(B)** Correlation of effects of CE (collected on day 1, dilution 1:5) from patients with DCM on Ca^{2+} transients and systolic cell shortening of isolated, field-stimulated rat cardiomyocytes, with the increase in CI during session 1 of IA. CI = confidence interval.

beta-receptor antibodies in the patients' serum and CE took place as previously described (8).

Preparation of myocardial protein fractions and immunoprecipitation. Explanted hearts of adult Wistar rats were homogenized in buffer A (50 mmol/l Tris/HCl [pH 7.5], 150 mmol/l sodium chloride, 10 mmol/l magnesium chloride) and were centrifuged at 20,000 *g* for 1 h at 4°C. After resuspension of the pellet in buffer B (50 mmol/l Tris/HCl [pH 7.5], 0.5 mol/l NaCl, 10 mmol/l EDTA) and stirring at 4°C for 30 min, centrifugation was carried out under the same conditions as described previously. The supernatant consisting of the myocardial membrane-associated protein fraction was saved, and the pellet containing the total myocardial membrane protein fraction was

Table 1. Hemodynamic Effects of Immunoadsorption in Patients With Dilated Cardiomyopathy and Effects of Column Eluent (Dilution 1:5) on Ca^{2+} Transient and Systolic Cell Shortening of Isolated, Field-Stimulated Rat Cardiomyocytes

	Day			
	0	**1**	**2**	**3**
IA session		1	2	3
Cardiac index (liters/min per m^2)	2.2 ± 0.1	2.5 ± 0.2	2.6 ± 0.2	2.7 ± 0.2
Decrease of Ca^{2+} transients (% change)		−22.7 ± 5.5	−7.9 ± 1.1	−4.8 ± 2.8
Decrease of systolic cell shortening (% change)		−29.9 ± 6.0	−9.4 ± 2.6	−6.1 ± 3.6

Data are presented as the mean value ± SEM.
IA = immunoadsorption.

resuspended in buffer A. Protein quantification was performed according to the method of Bradford (17). Antibodies obtained from CE were coupled to sepharose by Biogenes, Berlin, Germany. Twenty micrograms of antibodies coupled to sepharose were used with 100 μg of proteins for immunoprecipitation. Immunoprecipitation took place for 12 h at 4°C on a rotary wheel in buffer C containing 50 mmol/l Tris/HCl [pH 7.5], 150 mmol/l NaCl, 2 mmol/l EDTA, 0.2% tergitol NP40 (Sigma-Aldrich Chemie, Taufkirchen, Germany) and a mixture of proteinase inhibitors (Complete, Roche Diagnostics, Mannheim, Germany). The immunoprecipitated proteins were washed five times (10 min, 4°C, 20,000 *g*) with 1 ml of buffer C. Gel electrophoresis of proteins under denaturing conditions was carried out according to Laemmli (18).

Ethics. The protocol of IA in patients with DCM was approved by the Charité Hospital Ethics Committee. The in vitro investigation conforms to the "Position of the American Heart Association on Research Animal Use," as adopted by the AHA on November 11, 1984.

Statistics. Data are expressed as the mean value ± SEM for the number of calculations. The effects of the indicated dilutions of CE were analyzed using nonparametric, repeated-measures analysis of variance (ANOVA), with data alignment. The analyses involved comparisons between the groups (control subjects vs. patients with DCM) and within the groups. Univariate post-hoc analyses were performed with the Mann-Whitney *U* test and the Wilcoxon test, after overall testing. Accounts for multiple comparisons were carried out using the sequentially rejective Bonferroni-Holm procedure. The correlation of hemodynamic data with the effects of CE on the isolated cardiomyocytes was examined by Pearson's product moment correlation.

RESULTS

Effects of the eluent collected from IA columns on isolated rat cardiomyocytes. In patients with DCM, IA induced an increase in CI, from 2.2 ± 0.1 (day 0, baseline) to 2.7 ± 0.2 l/min per m^2 (day 3) ($p < 0.01$) (Table 1). The stroke volume index increased in parallel: from 31 ± 2 to 37 ± 2 ml/m^2 ($p < 0.01$). The heart rate did not change significantly. Immunoglobulin G and beta-receptor antibody serum levels decreased in the IA group from 10.7 ± 0.6 to 2.4 ± 0.1 g/l ($p < 0.01$) and from 4.4 ± 0.2 to 0.3 ± 0.1 relative units ($p < 0.01$), respectively. Superfusion of the rat cardiomyocytes with CE obtained from the blood of healthy donors ($n = 9$) did not influence Ca^{2+} transients or cell contraction (Fig. 1A). However, when the cells were superfused with CE collected on day 1 from patients with DCM, Ca^{2+} transients and cell contraction decreased in parallel. The reduction of systolic cell shortening and Ca^{2+} transients was a function of the dilution of CE with experimental buffer (Fig. 1A). Nonparametric repeated-measures ANOVA with data alignment yielded significant changes in cell shortening and Ca^{2+} transients ($p < 0.001$ within DCM group; $p < 0.001$ between DCM group and control group). When CE was diluted to 1:5, Ca^{2+} transients and systolic cell shortening decreased by −22.7 ± 5.5% and −29.9 ± 6.0% (Fig. 1A, Table 1). The effect produced by the eluent occurred within 2 min and remained unchanged after superfusion with fresh experimental buffer. Diastolic Ca^{2+}-dependent fluorescence did not change significantly. The decline of Ca^{2+} transients was therefore primarily the result of a decrease of systolic Ca^{2+}-dependent fluorescence.

Hemodynamic improvement during IA was not uniform among the patients with DCM. However, there was a significant correlation of the patients' hemodynamic benefit from IA, as indicated by the increase in CI, with the negative inotropic effect of their CE on the isolated cardiomyocytes (Fig. 1B). The negative inotropic effect of CE collected on days 2 and 3 was less pronounced: CE collected on day 3 of IA (dilution 1:5) decreased Ca^{2+} transients and systolic cell shortening by −4.8 ± 2.8% and −6.1 ± 3.6%, respectively (Table 1).

Identification of antibodies in CE. Neither TNF-alpha nor IL-1-beta, IL-6, IL-8 or IL-10 were detectable in the CE of control subjects and patients with DCM. The beta-receptor antibody level in CE collected on day 1 of IA was 5.3 ± 0.3 relative units. However, pretreatment with metoprolol (2.8 μmol/l) did not influence the negative inotropic effects of CE (dilution 1:5) obtained from patients with DCM on the rat cardiomyocytes, which indicates that the inotropic effects of CE were not modulated by beta-receptor antibodies.

After adsorption to protein A sepharose columns, the CE (dilution 1:5) from the patients with DCM did not significantly influence Ca^{2+} transients (−3.3 ± 1.5%, $p > 0.05$ vs. baseline) or cell shortening (−6.8 ± 3.8%, $p > 0.05$ vs. baseline) in the isolated myocytes. After elution of the protein A sepharose columns, the negative inotropic effect of CE obtained from the patients with DCM was again detectable: superfusion of the rat cardiomyocytes with CE (dilution 1:5) eluted from the protein A columns significantly decreased Ca^{2+} transients (−15.6 ± 4%, $p < 0.05$ vs. baseline) and cell shortening (−26.3 ± 6%, $p < 0.05$ vs. baseline).

The CE was furthermore used for immunoprecipitation with different protein fractions of myocardial tissue. Use of the fraction of membrane-associated proteins for immunoprecipitation resulted in more precipitated proteins, as compared with use of the membrane protein fractions. The amount of precipitated proteins varied among the patients; however, protein patterns did not differ between control subjects and patients with DCM (Fig. 2).

DISCUSSION

Immunoadsorption removes cardiodepressant substances from the plasma of patients with DCM. Immunoadsorption induced an early increase in CI, an increase similar to

JACC Vol. 39, No. 4, 2002
February 20, 2002:646–52

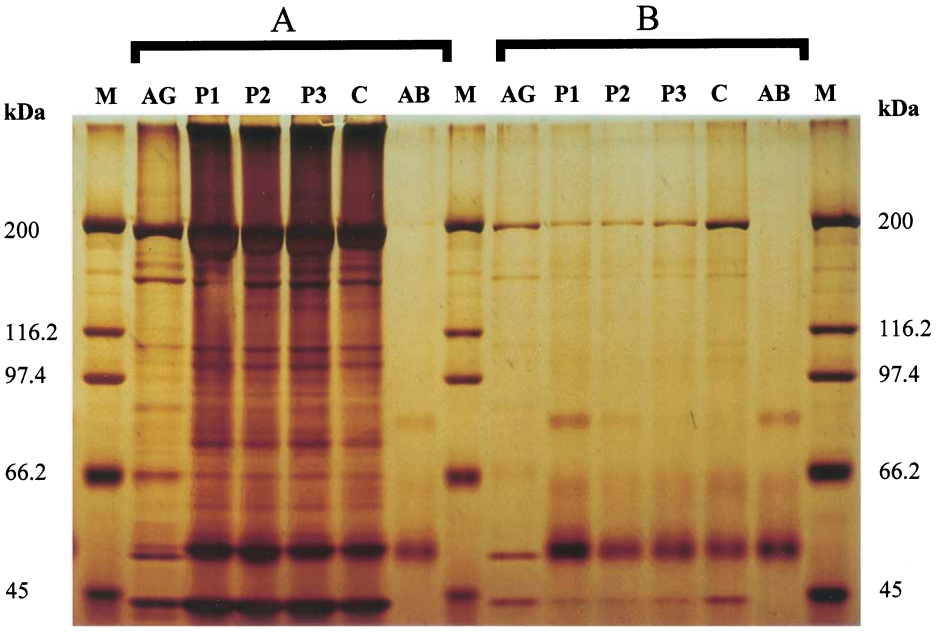

Figure 2. Equal amounts of immunoprecipitated myocardial proteins were loaded onto 5% to 20% sodium dodecyl sulfate–polyacrylamide gradient gels and visualized by silver staining. Immunoprecipitation was performed with sepharose-coupled antibodies collected during the first regeneration cycle of session 1 of IA from the CE of three patients with DCM and with sepharose-coupled antibodies obtained from the combined CE of two healthy blood donors. Immunoprecipitation with myocardial membrane-associated protein fraction **(A)** or with myocardial membrane protein fraction **(B)**. M = molecular weight marker; AG = antigen (myocardial protein fraction) alone, no antibodies added; P1–3 = sepharose-coupled antibodies obtained from the CE of a patient with DCM plus myocardial protein fraction; C = sepharose-coupled antibodies obtained from the CE of control subjects (combined CE of two healthy male blood donors) plus myocardial protein fraction; AB = sepharose-coupled antibodies alone.

the rise in the stroke volume index. The heart rate did not change significantly. The increase in CI was therefore primarily related to parallel improvement in the stroke volume index. The aim of the present study was to investigate the mechanisms involved in the early beneficial hemodynamic effects of IA in DCM, by means of an in vitro bioassay system using isolated cardiomyocytes. Our results indicate that the CE of patients with DCM contains substance(s) that decrease cell contraction in isolated rat cardiomyocytes by depression of Ca^{2+} transients. In contrast, the CE from healthy control subjects did not modulate cell contraction and Ca^{2+}-dependent fluorescence. In addition, the early hemodynamic benefit seen in patients with DCM during IA correlated with the negative inotropic effects of their CE: the higher the increase in CI, the greater the decrease in systolic cell shortening and Ca^{2+} transients in the cardiomyocytes induced by CE (Fig. 1B).

Chemical nature of cardiodepressant substances. The present study yields further information on the chemical nature of cardiodepressant substances. Different cardioactive cytokines were not detected in CE. Dialysis of CE indicates that the molecular size of the substances is >100 kD. A possible role played by the complement system was excluded by heat inactivation. Passage of CE through protein A columns that bind to the Fc region of Ig molecules prevented the negative inotropic effect of CE, an effect that was again detectable in the eluent of the protein A columns. Therefore, we deduce that removal of circulating cardiodepressant antibodies from plasma is possibly the primary underlying mechanism of the early beneficial hemodynamic effects of IA in patients with DCM. Immunoprecipitation disclosed that the antibodies obtained from CE are able to bind to different myocardial proteins. However, no differences were detected between control subjects and patients with DCM. The apparent lack of different myocardial protein patterns between patients and control subjects may be due to the fact that the amount of cardiac antibodies is too low to demonstrate a specific myocardial protein pattern for the patients with DCM. Furthermore, it is possible that IA removed negative inotropic antibodies of low affinity. This conclusion is supported by the fact that the first IA session already significantly improved the patients' hemodynamic variables, a development paralleled by the negative inotropic effects of CE (Fig. 1, Table 1). This finding suggests that the putative antibodies diffuse easily from their binding to the cardiac antigens. Finally, the functional activity of cardiac antibodies is not paralleled by their binding properties. Jahns et al. (19) showed that only a subgroup of antibodies that bind to the peptide of $beta_1$ receptor can realize functional activity on the native receptor.

Therapeutic interventions in immune disorders for patients with heart failure and DCM. Immune-mediated damage to the myocardium is thought to be a primary cause of DCM. Experimental and clinical data suggest a causal relationship between viral myocarditis and DCM (20,21).

In a major subset of patients with DCM, immunohistologic methods have been introduced for the diagnosis of myocardial inflammation, which allows identification of inflammatory cellular infiltration, as well as increased expression of cell adhesion molecules (22,23). Proinflammatory cytokines play an important role in the development of myocardial inflammation (21). Cytokines are also recognized as essential markers of immune responses in heart failure. Clinical studies have shown that proinflammatory cytokines—in particular, TNF—are released in congestive heart failure (24).

Recent therapeutic approaches to the treatment of heart failure have implicated the factors reported in these immunologic findings. Modulation of cytokine metabolism or anticytokine therapy may represent a promising therapeutic target for the treatment of chronic congestive heart failure. A recent phase-1 study indicates that anticytokine therapy may lead to improvement in the functional status of patients with heart failure (25). Furthermore, administration of high doses of Ig has a positive influence on the balance between inflammatory and anti-inflammatory cytokines in patients with chronic, symptomatic heart failure (26). This effect has been correlated to an improvement of LVEF. In contrast, a recent controlled trial did not demonstrate evidence of therapeutic efficacy in Ig therapy of patients with recent-onset DCM (27). Immunosuppression should not be used in the routine treatment of myocarditis (20). However, for a subset of patients with inflammatory DCM and immunohistologically proven myocarditis, immunosuppressive therapy has a long-term benefit (28). Our data indicate that disorders of the humoral immune system, in conjunction with production of cardiac autoantibodies, play a functional role in DCM. Removal of cardiodepressant antibodies by IA may, accordingly, represent an additional therapeutic approach to patients with DCM.

Study limitations. Several antibodies have been described for DCM. The present study included only a small number of patients, and therefore did not investigate the contribution of a particular antibody to cardiac malfunction in DCM. Further studies are necessary to elucidate the specific antigens of the various cardiac autoantibodies obtained in the CE of patients with DCM and to ascertain their effects on cell contraction and signal transduction of the cardiomyocytes.

Conclusions. Removal of circulating negative inotropic autoantibodies from plasma may contribute to the early beneficial hemodynamic effects of IA in patients with DCM. Our in vitro data indicate that these antibodies do not reflect an epiphenomenon associated with DCM; rather, they play a functional role in this disease.

Reprint requests and correspondence: Dr. Stephan B. Felix, Klinik für Innere Medizin B, Universität Greifswald, Friedrich-Loeffler-Str. 23a, 17487 Greifswald, Germany. E-mail: felix@uni-greifswald.de.

REFERENCES

1. 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;93:841–2.
2. Limas CJ, Goldenberg IF, Limas C. Soluble interleukin-2 receptor levels in patients with dilated cardiomyopathy: correlation with disease severity and cardiac autoantibodies. Circulation 1995;91:631–4.
3. Klappacher G, Mundigler G, Papousek A, et al. Elevated circulating levels of beta-2 microglobulin in patients with idiopathic dilated cardiomyopathy. Am J Cardiol 1993;71:119–22.
4. Schulze K, Becker BF, Schauer R, Schultheiss HP. Antibodies to ADP-ATP carrier—an autoantigen in myocarditis and dilated cardiomyopathy—impair cardiac function. Circulation 1990;81:959–69.
5. Caforio AL, Grazzini M, Mann JM, et al. Identification of alpha- and beta-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. Circulation 1992;85:1734–42.
6. Limas CJ, Goldenberg IF, Limas C. Autoantibodies against beta-adrenoceptors in human idiopathic dilated cardiomyopathy. Circ Res 1989;64:97–103.
7. Magnusson Y, Wallukat G, Waagstein F, Hjalmarson A, Hoebeke J. Autoimmunity in idiopathic dilated cardiomyopathy: characterization of antibodies against the $beta_1$-adrenoceptor with positive chronotropic effect. Circulation 1994;89:2760–67.
8. Wallukat G, Wollenberger A. Effects of the serum gamma globulin fraction of patients with allergic asthma and dilated cardiomyopathy on chronotropic beta-adrenoceptor function in cultured neonatal rat heart myocytes. Biomed Biochem Acta 1987;46:S634–9.
9. Fu ML, Schulze W, Wallukat G, Hjalmarson A, Hoebeke J. A synthetic peptide corresponding to the second extracellular loop of the human M2 acetylcholine receptor induces pharmacological and morphological changes in cardiomyocytes by active immunization after 6 months in rabbits. Clin Immunol Immunopathol 1996;78:203–7.
10. Dörffel WV, Felix SB, Wallukat G, et al. Short-term hemodynamic effects of immunoadsorption in dilated cardiomyopathy. Circulation 1997;95:1994–7.
11. Felix SB, Staudt A, Dörffel WV, et al. Hemodynamic effects of immunoadsorption and subsequent immunoglobulin substitution in dilated cardiomyopathy: three-month results from a randomized study. J Am Coll Cardiol 2000;35:1590–8.
12. Staudt A, Schäper F, Stangl V, et al. Immunohistological changes in dilated cardiomyopathy induced by immunoadsorption therapy and subsequent immunoglobulin substitution. Circulation 2001;103:2681–6.
13. Müller J, Wallukat G, Dandel M, et al. Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. Circulation 2000;101:385–91.
14. Aretz HT. Myocarditis: the Dallas criteria. Hum Pathol 1987;18:619–24.
15. Felix SB, Stangl V, Pietsch P, et al. Soluble substances released from postischemic reperfused rat hearts reduce calcium transient and contractility by blocking the L-type calcium channel. J Am Coll Cardiol 2001;37:668–75.
16. Spurgeon HA, Stern MD, Baartz G, et al. Simultaneous measurement of Ca^{2+}, contraction, and potential in cardiac myocytes. Am J Physiol 1990;258:H574–86.
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
19. Jahns R, Boivin V, Siegmund C, Inselmann G, Lohse MJ, Boege F. Autoantibodies activating human $beta_1$-adrenergic receptors are associated with reduced cardiac function in chronic heart failure. Circulation 1999;99:649–54.
20. Feldman AM, McNamara DM. Myocarditis. N Engl J Med 2000;343:1388–98.
21. Liu PP, Mason JW. Advances in the understanding of myocarditis. Circulation 2001;104:1076–82.
22. Kühl U, Noutsias M, Schultheiss HP. Immunohistochemistry in dilated cardiomyopathy. Eur Heart J 1995;16 Suppl:100–6.
23. Noutsias M, Seeberg B, Schultheiss HP, Kühl U. Expression of cell

adhesion molecules in dilated cardiomyopathy: evidence for endothelial activation in inflammatory cardiomyopathy. Circulation 1999;99: 2124–31.
24. Mann DL. Recent insights into the role of tumor necrosis factor in the failing heart. Heart Fail Rev 2001;6:71–80.
25. Deswal A, Bozkurt B, Seta Y, et al. Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, etanercept) in patients with advanced heart failure. Circulation 1999;99:3224–6.
26. Gullestad L, Aass H, Fjeld JG, et al. Immunomodulating therapy with intravenous immunoglobulin in patients with chronic heart failure. Circulation 2001;103:220–5.
27. McNamara DM, Holubkov R, Starling RC, et al. Controlled trial of intravenous immune globulin in recent-onset cardiomyopathy. Circulation 2001;103:2254–9.
28. Wojnicz R, Nowalany-Kozielska E, Wojciechowska C, et al. Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. Circulation 2001;104:39–45.