Cardiomyopathy

Hemodynamic Effects of Immunoadsorption and Subsequent Immunoglobulin Substitution in Dilated Cardiomyopathy
Three-Month Results from a Randomized Study

Stephan B. Felix, MD,* Alexander Staudt, MD,* Wolf V. Dörffel, MD,* Verena Stangl, MD,*
Kurt Merkel, MD,‡ Manfred Pohl, PHD,§ Wolf D. Döcke, MD,‖ Stanislao Morgera, MD,†
Hans H. Neumayer, MD,† Klaus D. Wernecke, PHD,¶ Gerd Wallukat, PHD,# Karl Stangl, MD,*
Gert Baumann, MD*

Berlin, Germany

OBJECTIVES   The objective of our study was to assess the hemodynamic effects of immunoadsorption (IA) and subsequent immunoglobulin G (IgG) substitution in comparison with the effects of conventional medical treatment in patients with dilated cardiomyopathy (DCM).

BACKGROUND   Various circulating cardiac autoantibodies have been detected among patients suffering from DCM. These antibodies are extractable by IA.

METHODS   Patients with DCM (n = 18, New York Heart Association III–IV, left ventricular ejection fraction <30%) and who were on stable medication participated in the study. Hemodynamic measurements were performed using a Swan-Ganz thermodilution catheter. The patients were randomly assigned either to the treatment group with IA and subsequent IgG substitution (IA/IgG group, n = 9) or to the control group without IA/IgG (n = 9). In the IA/IgG group, the patients were initially treated in one IA session daily on three consecutive days. After the final IA session, 0.5 g/kg of polyclonal IgG was substituted. At one-month intervals, IA was then repeated for three further courses with one IA session daily on two consecutive days, until the third month.

RESULTS   After the first IA course and IgG substitution, cardiac index (CI) increased from 2.1 (±0.1) to 2.8 (±0.1) L/min/m² (p < 0.01) and stroke volume index (SVI) increased from 27.8 (±2.3) to 36.2 (±2.5) ml/m² (p < 0.01). Systemic vascular resistance (SVR) decreased from 1,428 (±74) to 997 (±55) dyne·s·cm⁻⁵ (p < 0.01). The improvement in CI, SVI and SVR persisted after three months. In contrast, hemodynamics did not change throughout the three months in the control group.

CONCLUSIONS   Immunoadsorption and subsequent IgG substitution improves cardiovascular function in DCM. (J Am Coll Cardiol 2000;35:1590–8) © 2000 by the American College of Cardiology

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by progressive depression of myocardial contractile function and by ventricular dilatation (1).

Disturbances in both humoral and cellular immunity have been described in patients with myocarditis and DCM (2,3). A number of antibodies against cardiac cell proteins have been identified in DCM; these include antibodies against mitochondrial proteins (4), contractile proteins (5) and cardiac beta-receptors (6,7). For some cardiac antibodies, it has been possible to demonstrate direct cardiodepressant effects (4,8). The functional role of cardiac autoantibodies in DCM is still unclear. They may reflect an inflammatory response to myocyte necrosis, thereby representing an epiphenomenon. On the other hand, cardiac autoantibodies may also play an active role in the pathogenesis of DCM by initiating the disease process or by

---

Abbreviations and Acronyms

ACE	=	angiotensin converting enzyme
BPmean	=	mean arterial blood pressure
CI	=	cardiac index
DCM	=	dilated cardiomyopathy
HR	=	heart rate
IA	=	immunoadsorption
Ig	=	immunoglobulin
IgG	=	immunoglobulin G
IL	=	interleukin
LVEDD	=	left ventricular end-diastolic diameter
LVEF	=	left ventricular ejection fraction
NYHA	=	New York Heart Association
PAPmean	=	mean pulmonary arterial pressure
PCWP	=	pulmonary capillary wedge pressure
PVR	=	pulmonary vascular resistance
RAP	=	right atrial pressure
sIL-2R	=	soluble IL-2 receptor
sTNF-R1 and -R2	=	soluble tumor necrosis factor receptor I and II
SVI	=	stroke volume index
SVR	=	systemic vascular resistance
TNFalpha	=	tumor necrosis factor alpha

---

contributing to the progression of myocardial contractile malfunction. If cardiac autoantibodies do, in fact, contribute to cardiac malfunction in DCM, their removal would be expected to improve the hemodynamics of patients with DCM. Cardiac antibodies are extractable by immunoadsorption (IA) (9).

We recently performed the first uncontrolled pilot study conducted to ascertain the short-term hemodynamic effects of IA in patients with DCM (9). Immunoadsorption induced significant decrease in immunoglobulin (Ig) G (IgG) serum levels, as well as consistent reduction of beta-receptor antibodies. The cardiac index (CI) rose and systemic vascular resistance (SVR) simultaneously fell. These data suggest that removal of antibodies improves hemodynamics in DCM.

Intravenous Ig treatment also influences the immune system by means of various mechanisms (10). The autoantibodies are neutralized by the anti-idiotypic properties of intravenous IgG, thus preventing binding of the autoantibodies to its antigen. Furthermore, binding of anti-idiotypic Igs to B-cell Fc receptors decreases the production of autoantibodies in B cells. Finally, intravenous IgG modulates cellular immunity and cytokine metabolism (10). Immunoglobulin treatment as well as IA have been successfully used for treatment of various autoimmune diseases such as immune thrombocytopenic purpura, myasthenia gravis and polyneuropathy in the form of Guillain-Barré syndrome (11–16). Interestingly, recent data from an uncontrolled study published by McNamara et al. (17) indicate that intravenous Ig therapy may also be useful in treatment of

patients with new-onset dilated cardiomyopathy. In patients with myocarditis and acute cardiomyopathy, application of intravenous IgG was associated with significant improvement in left ventricular performance as a long-term effect. The combination of IA and intravenous IgG administration may, therefore, represent a promising therapeutic principle in treatment of DCM. This study investigated the acute and prolonged effects of IA and subsequent IgG substitution in patients with DCM, in comparison with a control group without IA and IgG substitution.

METHODS

Study patients. Included in this study were male patients suffering from DCM (n = 18) and left ventricular dysfunction (left ventricular ejection fraction [LVEF] <30%, as assessed by cineangiography or two-dimensional echocardiography), as well as from severe chronic heart failure (New York Heart Association [NYHA] III–IV) refractory to medical therapy. The hemodynamic inclusion criteria assessed by a Swan-Ganz thermodilution catheter were CI ≤ 2.5 liters/min/m^2 or pulmonary capillary wedge pressure (PCWP) ≥ 16 mm Hg. Coronary heart disease—in particular, relevant stenoses (>40% of vessel diameter) of the coronary arteries—were excluded by coronary angiography, and the possibility of acute myocarditis was eliminated by histological examination of myocardial biopsies in all cases studied. Serum levels of cholesterol and low-density lipoprotein cholesterol did not differ significantly at baseline between the treatment group and the control group. Three patients from the control group and one from the treatment group had elevated levels of cholesterol (>200 mg/dl, <280 mg/dl). Three patients (one from the control group and two from the treatment group) had noninsulin-dependent diabetes mellitus and two (one from the control group and one from the treatment group) had insulin-dependent diabetes mellitus. All patients had received stable oral medication for more than three months before this study. All patients were treated with angiotensin converting enzyme (ACE) inhibitors, digitalis and diuretics. Thirteen patients received nitrates. Four patients were treated with a beta-adrenergic blocking agent. In these four cases the doses of the beta-blockers had been stable at least six months before this study. At baseline, medication and dosage for the treatment group did not differ significantly from that for the control group. Patients were excluded if they had a history of arterial hypertension, myocardial infarction, heart failure due to other known origins (e.g., primary valvular disease) or if they had suffered from active infectious diseases, cancer or chronic alcoholism.

Written consent was obtained from each patient, and the protocol was approved by the Charité Hospital Ethics Committee.

Hemodynamics. The patients were transferred to the intensive care unit for hemodynamic monitoring. We conducted right-heart catheterization with a Swan-Ganz ther-

modilution catheter to evaluate patient hemodynamics. Measurements were carried out four times a day. The interval between two hemodynamic measurements was at least 3 h. The hemodynamic values given in tables and figures represent the mean values from hemodynamic measurements performed four times a day. We employed two-dimensional echocardiography. The readings were recorded, and assessment of LVEF took place off-line by a reader blinded to the treatment group. Left ventricular ejection fraction was measured according to the Simpson rule.

Immunoadsorption. After completion of baseline measurements, Ig extraction from the plasma was performed using Ig Therasorb (Baxter, Munich, Germany), an immunoadsorber for immunoglobulin. The extracorporeal IA system (Microsorb Dialyse Technik, Ettlingen, Germany) consisted of conventional plasmapheresis and immunoapheresis systems. We used a Hemaplex-BT 900/B plasma filter (Dideco, Mirandola, Italy) for conventional plasmapheresis. The plasma was separated at a maximum flow rate of 30 ml/min, passed through the IA column, and then reinfused. The IA system employed consisted of two parallel columns. Plasma passes through one of the columns (plasma filling volume = 100 ml) while the other is being regenerated. During IA, anticoagulation was performed with intravenous infusion of heparin.

Study protocol. All patients who had been on long-term oral medication on a stable basis for more than three months were transferred to the intensive care unit. Hemodynamic baseline measurements were carried out four times on day 1. On the following day (day 2), the patients were randomly assigned either to treatment with IA and subsequent IgG substitution (IA/IgG group, n = 9) or to the control group without IA or IgG treatment (n = 9) on the basis of closed-label randomization. On days 2 through 5, hemodynamic measurements were continued four times a day in the IA/IgG group as well as in the control group.

In the IA/IgG group, IA was performed in four courses, at one-month intervals, until month 3, as indicated in Figure 1. During the first course (course I), all patients of the IA/IgG group underwent one IA session daily on three consecutive days. Each session was continued until 5 liter of plasma had passed the IA columns: a procedure that resulted in decrease of IgG plasma levels by approximately 40% during each session. After the final IA session, the patients received 0.5 g/kg polyclonal IgG (Venimmun N) over a period of 6 h, in order to restore the IgG plasma levels. Immunoadsorption courses 2 through 4 were performed until month 3, at one-month intervals and with one session daily on two consecutive days (Fig. 1). After the final session of each course, 0.5 g/kg of polyclonal IgG was again substituted intravenously. The second and third courses (courses II and III) were performed without hemodynamic
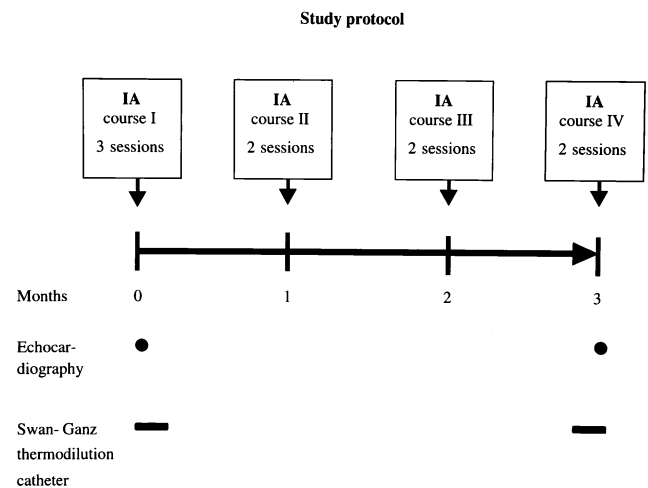
Study protocol

Figure 1. Time schedule of immunoadsorption (IA) and cardiovascular monitoring; IA was performed in four courses until the third month.

monitoring. During the fourth course (course IV), hemodynamic monitoring with a Swan-Ganz thermodilution catheter again took place four times a day: one day before IA and proceeding until one day after IA. Left ventricular ejection fraction was determined by two-dimensional echocardiography, performed at baseline and after three months. In addition to echocardiography, hemodynamic measurements were carried out among control patients after three months, at baseline throughout one day.

For all patients, relief of symptoms and results of clinical examination were documented by independent attending physicians unaware of whether the patients belonged to the control group or to the IA/IgG group.

Histological examination. As an initial step, five right ventricular biopsies were obtained from all patients from the interventricular septum. Biopsies were fixed in 4% formalin and embedded in paraffin. The sections were 2 μm thick. In all cases, we excluded acute myocarditis according to the Dallas criteria. Immunohistochemical staining took place in addition to conventional histology. The immunohistochemical staining procedure with the labeled streptavidin-biotin method was used for identification of inflammatory cells. The cells were counted as described elsewhere (18). The following antibodies were used: anti CD3; anti CD4; anti CD8. The antibodies were obtained from DAKO (Hamburg, Germany) and Novacastra (Newcastle upon Tyne, United Kingdom).

Blood rheology. We tested blood samples within 3 h after venous puncture. A plasma-viscometer (Fresenius, Bremen, Germany) was used for measurement of plasma viscosity. Whole-blood viscosity was determined with a Ubbelhode Microviscometer (DIN 51 562, Schott, Germany) at a wall shear rate of 800 s^{-1}, corresponding to shear rates in arteries and capillaries. The measurements with this capillary vis-

cometer took place in a water bath at 25 ± 0.2°C, with precision of ±2%. A Sysmex K 100 Cell Counter (TOA Medical Electronics Co., Kobe, Japan) was used for determination of hematocrit.

Immunological parameters. Serum supernatants were harvested from native venous blood samples and were stored at −70°C until analysis of soluble immune mediators by commercially available enzyme immunoassays. Tumor necrosis factor alpha (TNFalpha), interleukin (IL)-6 and soluble IL-2 receptor (sIL-2R, CD25) concentrations were determined using the automated Immulite chemiluminescence enzyme immunoassay system (DPC Biermann, Bad Nauheim, Germany). Serum levels of soluble tumor necrosis factor receptor I (sTNF-R1) and II (sTNF-R2) were analyzed by the respective Quantikine ELISA kits (DPC Biermann). Determination of beta-receptor autoantibodies took place under the direction of Gerd Wallukat, as previously described (19). Assessment of all laboratory data took place in a blinded fashion without knowledge of the patients' treatment.

Statistics. Results are expressed as mean ± SEM. Changes in interesting clinical outcomes over time were analyzed using nonparametric repeated measures analysis of variance with data alignment. The analyses performed involved comparisons between the treatment groups (with IA vs. without IA), as well as within the groups. After overall testing within the groups, post-hoc analyses (Wilcoxon tests) took place in order to detect specific differences between certain periods of treatment. In addition, differences between the two treatment groups with respect to certain interesting times were investigated by means of the Mann-Whitney U test. Adjustments for multiple comparisons were carried out using a sequentially rejective test procedure (Bonferroni-Holm) for the periods of follow-up after course I, before course IV and after course IV, which are of primary clinical interest. Changes in NYHA classification after treatment were analyzed by singly ordered 2×4 contingency tables (two treatments, four classes) using the Kruskal-Wallis test of identically distributed rows (treatments). Significance was assessed at the $p < 0.05$ level.

RESULTS

Characteristics of patients. Table 1 lists the clinical characteristics, the hemodynamic data and the immunohistochemical findings at baseline. In the control group and the IA/IgG group, the following were comparable: age, disease duration, NYHA classification, immunohistochemical findings and hemodynamic parameters at baseline. Left ventricular ejection fraction and left ventricular end-diastolic diameter (LVEDD) were also similar in both groups.

Hemodynamic and clinical findings. In the control group, no change was determined for the following during follow-up tests: LVEF, CI, stroke volume index (SVI),

Table 1. Clinical, Immunohistochemical and Hemodynamic Characteristics of Patients at Baseline

	Controls	IA/IgG Group
Age (yrs)	55.3 ± 2.4	49.7 ± 3.2
Disease duration (yrs)	4.3 ± 0.7	4.1 ± 0.5
NYHA classification III (n)/ IV (n)	8/1	6/3
Immunohistology (cells/mm²)		
CD3	3.6 ± 1.0	3.1 ± 0.8
CD4	1.4 ± 0.2	1.2 ± 0.3
CD8	1.9 ± 0.6	1.8 ± 0.4
Hemodynamics		
HR (/min)	84.5 ± 3.1	79.5 ± 4.5
CI (liter/min/m²)	2.3 ± 0.1	2.1 ± 0.1
SVI (ml/m²)	27.6 ± 1.3	27.8 ± 2.3
PCWP (mm Hg)	17.6 ± 2.7	16.0 ± 2.7
RAP (mm Hg)	6.4 ± 1.6	6.0 ± 1.7
BPmean (mm Hg)	83.2 ± 1.6	84.7 ± 2.0
PAPmean (mm Hg)	26.5 ± 3.1	27.1 ± 2.6
SVR (dyne·s·cm⁻⁵)	1,400 ± 38	1,428 ± 74
PVR (dyne·s·cm⁻⁵)	160 ± 13	200 ± 16
Echocardiography		
LVEDD (mm)	69.0 ± 2.1	69.0 ± 2.5
LVEF (%)	20.1 ± 1.2	22.6 ± 1.7

BPmean = mean arterial blood pressure; CD = cluster of differentiation; CI = cardiac index; HR = heart rate; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; PAPmean = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; RAP = right atrial pressure; SVI = stroke volume index; SVR = systemic vascular resistance.

heart rate (HR), mean arterial blood pressure (BPmean), mean pulmonary arterial pressure (PAPmean), PCWP, right atrial pressure (RAP), SVR and pulmonary vascular resistance (PVR). After three months, upon final hemodynamic monitoring, none of the measured hemodynamic and echocardiographic parameters differed from those at baseline when the study was started (Fig. 2, A to D).

All patients tolerated IA and subsequent IgG substitution well. No major complications such as infection, major bleeding or worsening of renal function occurred. During the first IA course, IgG and beta-receptor autoantibody levels (Fig. 3) decreased in the IA/IgG group from 10.9 ± 0.8 to 2.2 ± 0.3 g/L (p < 0.01) and from 4.83 ± 0.21 to 0.38 ± 0.06 relative U, respectively. Simultaneously, CI significantly increased in the IA/IgG group from 2.1 ± 0.1 to 2.8 ± 0.1 liter/min/m² (p < 0.01) (Fig. 2A). Since heart rate remained stable, SVI also significantly increased: from 27.8 ± 2.3 to 36.2 ± 2.5 ml/m² (p < 0.01) (Fig. 2B). Systemic vascular resistance decreased from 1,428 ± 74 to 997 ± 55 dyne · s · cm⁻⁵ (p < 0.01) (Fig. 2C), and PVR fell from 200 ± 16 to 135 ± 11 dyne·s·cm⁻⁵ (p < 0.01). Mean arterial blood pressure, PAP mean, PCWP and RAP did not change significantly in the IA/IgG group. Hemodynamic improvement already occurred after the first IA session—before IgG substitution (Fig. 2, A to C).

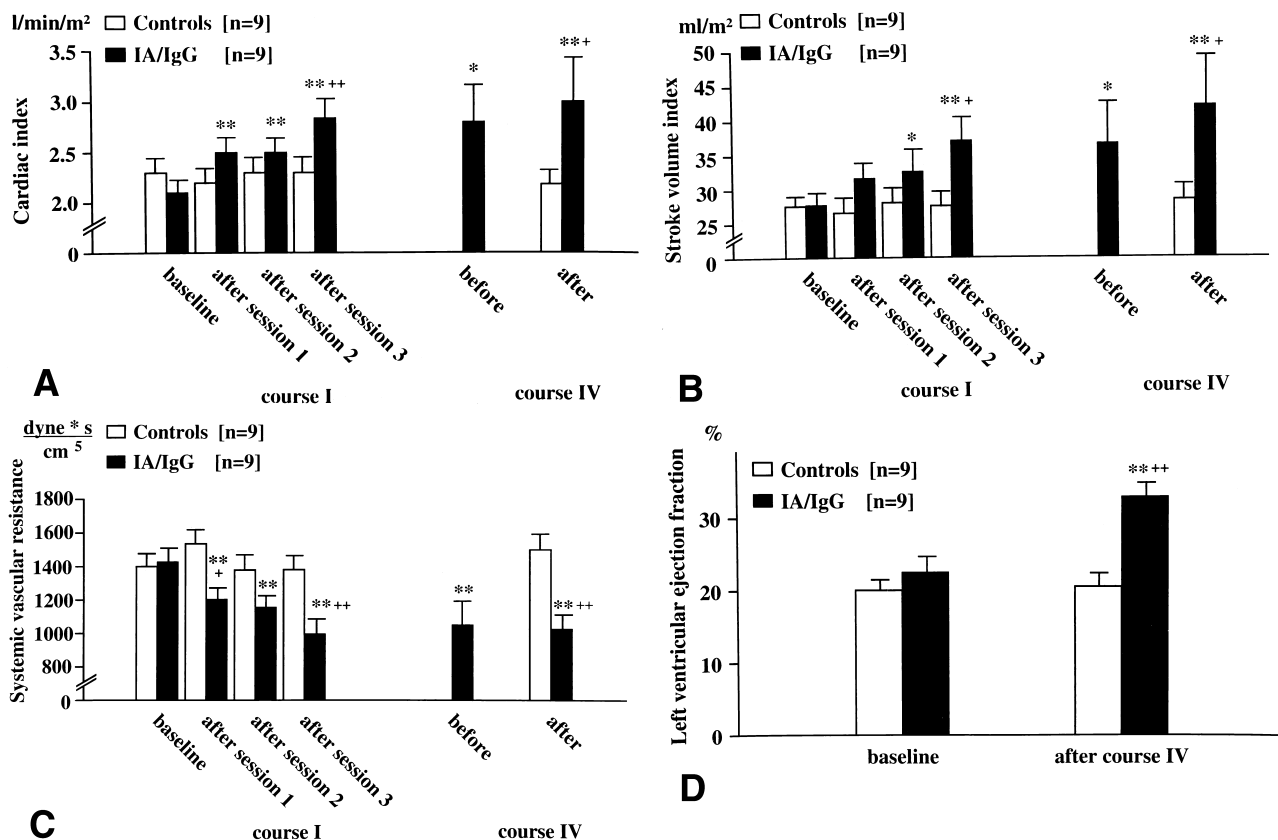During follow-up as well, hemodynamic improvement

Figure 2. Changes in hemodynamics in the immunoadsorption/immunoglobulin group (IA/IgG group, **filled bars,** n = 9) and in the control group (controls, **open bars,** n = 9). **(A)** Cardiac index. **(B)** Stroke volume index. **(C)** Systemic vascular resistance. **(D)** Changes in left ventricular ejection fraction, as assessed by echocardiography. *p < 0.05; **p < 0.01 significantly different from baseline; +p < 0.05; ++p < 0.01 significantly different from controls.

persisted in the IA/IgG group. At month 3, before the last IA course, CI 2.8 ± 0.2 liter/min/m^2 (p < 0.05 vs. baseline), SVI 36.7 ± 4.4 ml/m^2 (p < 0.05 vs. baseline) and SVR 1,050 ± 52 dyne·s·cm^{-5} (p < 0.01 vs. baseline) were at levels comparable with those measured after the first

Figure 3. Changes in beta-receptor antibody level in the immunoadsorption/immunoglobulin group (IA/IgG group, **filled bars,** n = 9) and in the control group (controls, **open bars,** n = 9). *p < 0.05; **p < 0.01 significantly different from baseline; +p < 0.05; ++p < 0.01 significantly different from controls.

course of IA. The fourth IA course subsequently induced moderate further increase of CI and SVI to 3.0 ± 0.3 liter/min/m^2 (p < 0.01 vs. baseline, p < 0.05 vs. controls, p = 0.054 vs. results before last course) and to 42.2 ± 5.4 ml/m^2 (p < 0.01 vs. baseline, p < 0.05 vs. controls, p < 0.05 vs. results before last course) (Fig. 2, A and B). Changes in these hemodynamic outcomes over the entire study period were analyzed using nonparametric repeated measures analysis of variance with data alignment. This analysis yielded significant differences for the analyzed parameters between the groups (p < 0.05), as well as within the IA/IgG group (p < 0.01). No significant changes were found in the control group. For the periods of interest, multiple comparisons (Bonferroni-Holm) of the hemodynamic parameters were significant within the IA/IgG group (Wilcoxon test). Comparisons between the two groups (U tests) provided results with multiple significance.

After three months, improvement in hemodynamic findings was paralleled by a comparable increase in LVEF (p < 0.01) (Fig. 2D). Before the fourth IA course, anti-beta-receptor autoantibodies had again increased; they decreased subsequently during the last IA course (Fig. 3).

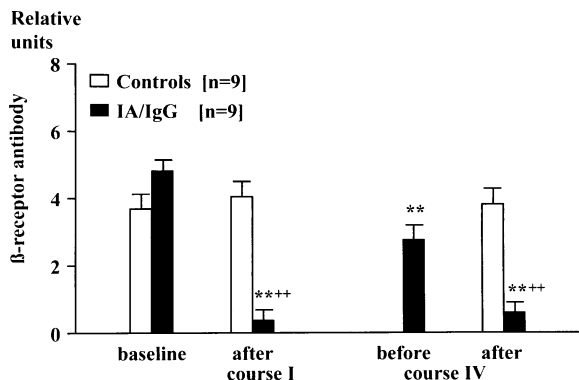The patients' functional state paralleled their hemody-

Table 2. New York Heart Association Classification of the Control Group and of the IA/IgG Group at Baseline and After Course IV (After Three Months)

NYHA	Controls		IA/IgG Group	
	Baseline	After Three Months	Baseline	After Three Months
I	—	—	—	2
II	—	1	—	2
III	8	5	6	5
IV	1	3	3	—

IA/IgG = immunoadsorption/immunoglobulin G serum; NYHA = New York Heart Association.

namic response. Examination after three months disclosed that symptoms improved in the IA/IgG group, as indicated by improvement in NYHA heart-failure classification (p < 0.05 vs. baseline, Table 2). In contrast, the patients in the control group obtained no relief from symptoms.

The doses of ACE inhibitors, nitrates, digitalis and beta-blockers were modified neither in the control group nor in the IA group throughout the entire study period. If necessary, dosage of the diuretics was changed on discretion of the attending physicians. However, the mean dosage of the diuretics did not change significantly for either group throughout the entire study period.

The Ethics Committee of the Charité Hospital terminated the study after 18 patients had undergone the final hemodynamic evaluation and after significant improvement in hemodynamics was documented for the IA/IgG group.

Assessment of laboratory data. The combination of IA and IgG treatment had no significant influence on plasma and whole-blood viscosity, fibrinogen or hematocrit (Table 3). Plasma levels of TNFalpha, sTNF-R1 and -R2, IL-6 and sIL-2R were comparable in the control group and in the IA/IgG group at baseline (Table 3). No significant changes in proinflammatory cytokines or in soluble receptors became

apparent during further follow-up tests until month 3 in the control group or in the IA/IgG group. For neither of the groups, furthermore, were laboratory signs of inflammation observed. Erythrocyte sedimentation rate and C-reactive protein remained stable. Immunoadsorption and immunoglobulin G substitution likewise produced no significant influence on the differential blood count. Clinical examination, in addition, did not disclose signs of infections in the study patients during follow-up. Serum levels of cholesterol and LDL cholesterol were not modulated by IA in combination with IgG substitution. Immunoadsorption and immunoglobulin G substitution also did not affect the activity of serum glutamate oxalacetate transaminase or of glutamate pyruvate transaminase. Immunoadsorption and immunoglobulin G substitution did not modulate renal function. In the control group and in the IA/IgG group, serum creatinine and creatinine clearance remained unchanged in both groups.

DISCUSSION

Abnormalities of the humoral immune system in DCM. An association between myocarditis and DCM has been hypothesized for a subset of patients with DCM (18,20). Abnormalities of the cellular and humoral immune system are present in patients with myocarditis and DCM (2,3). A number of different cardiac autoantibodies against cardiac cellular proteins have been identified in patients with myocarditis and DCM. Among patients with DCM, various cardiac cellular proteins have been identified as antigens: e.g., cardiac myosin, the beta$_1$-receptor, the Ca^{2+} ATPase, the Ca^{2+} channel, the muscarinergic M2 receptor and the adenine nucleotide translocator (4–8). It is unclear, however, whether these antibodies are directly pathogenic (21) or whether they merely represent humoral markers of autoimmunity in myocarditis and DCM. In vitro data indicate for certain antibodies a negative effect on cardiac performance (4,8). If cardiac autoantibodies, in fact, play a role as causal agents in initiating or triggering the disease

Table 3. Course of Selected Parameters of Blood Rheology and of Cytokine Metabolism at Baseline and After Three Months in the Control Group and in the Immunoadsorption/Immunoglobulin G (IA/IgG) Group

	Baseline		After Three Months	
	Controls	IA/IgG Group	Controls	IA/IgG Group
Blood viscosity (mPa·s)	4.01 ± 0.1	3.94 ± 0.2	4.04 ± 0.02	3.84 ± 0.14
Plasma viscosity (mPa·s)	1.19 ± 0.03	1.55 ± 0.24	1.31 ± 0.09	1.27 ± 0.13
Hematocrit	0.42 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.4 ± 0.01
Hematocrit/blood viscosity (/mPa·s)	0.11 ± 0.004	0.11 ± 0.004	0.11 ± 0.002	0.10 ± 0.003
Fibrinogen (mg/dl)	3.27 ± 0.23	3.0 ± 0.24	3.68 ± 0.26	3.09 ± 0.13
TNFalpha (pg/ml)	13.9 ± 2.6	14.0 ± 1.4	14.6 ± 2.7	18.2 ± 3.8
IL-6 (pg/ml)	19.2 ± 3.0	23.2 ± 3.4	10.8 ± 1.7	22.4 ± 6.7
sIL-2R (U/ml)	679 ± 117	928 ± 252	698 ± 154	1,175 ± 548
sTNF-RI (pg/ml)	1,169 ± 74	1,144 ± 80	987 ± 92	1,228 ± 144
sTNF-RII (pg/ml)	2,974 ± 293	2,924 ± 331	2,330 ± 234	3,505 ± 401

IL = interleukin; sIL-2R = soluble IL-2 receptor; sTNF-R = soluble tumor necrosis factor receptor; TNFalpha = tumor necrosis factor alpha.

process, their elimination or blockade would be expected to improve myocardial function.

Therapeutic effects of IA and IgG substitution. The data obtained in this study clearly indicate that IA and subsequent IgG substitution may represent an additional therapeutic tool for stabilization of the cardiovascular function of patients with severe heart failure due to DCM. This therapy induced short-term hemodynamic improvement as shown by pronounced increase in CI and SVI (Fig. 2, A and B). Furthermore, the beneficial hemodynamic effects have proved to be of prolonged duration. Symptomatic relief paralleled the patients' hemodynamic response.

Possible mechanisms of hemodynamic improvement. The underlying mechanisms of the beneficial effects of IA and IgG substitution remain to be elucidated. At least theoretically, physical inactivity during the procedure may have additionally contributed to the observed stabilization of cardiovascular function. Bed rest, however, did not contribute to hemodynamic improvement in the IA/IgG group. This conclusion is based on the following: during the first course, the randomly assigned control patients were subjected to bed rest over a period of five consecutive days, without observed changes in hemodynamic parameters. The beneficial effects of IA in combination with IgG substitution are related neither to reduction of left ventricular preload caused by loss of plasma volume nor to increased urine production due to primary improvement of renal function. Throughout a period of three months, PCWP and hematocrit remained stable, and creatinine clearance in the IA/IgG group did not change significantly. Immunoadsorption and IgG substitution may also have modulated plasma and whole-blood viscosity. Compared with the control group, however, the IA/IgG group demonstrated no significant changes in plasma, whole-blood viscosity or hematocrit (Table 3).

Infections or line sepsis may have induced a decrease in vascular resistance and a concomitant increase of CI. However, neither clinical examination nor laboratory data revealed any signs of infection in the study patients during the study period. At least theoretically a decrease of serum levels of cholesterol may have contributed to the observed improvement of vascular resistance. However, IA did not influence serum levels of LDL cholesterol. We cannot, however, definitively exclude the possibility that peripheral vascular events are responsible for the cardiac effects of IA and IgG substitution. It remains unclear whether the hemodynamic response to IA is characterized by direct improvement of myocardial contractile function—or whether it is due to vasodilation and a decrease in systemic vascular resistance.

Finally, IA and IgG substitution may have nonspecifically affected the immune system: in particular, cytokine metabolism. Cytokines are recognized as essential markers of immune responses in heart failure of various etiologies, including DCM. In patients with congestive heart failure, increased levels of circulating cytokines have been earlier described (22–25). Tumor necrosis factor alpha has been reported to depress myocardial contractility (26). It is, therefore, conceivable that the beneficial effects of IA are caused, at least in part, by a decrease in cytokine serum levels. During IA, however, there was no significant alteration in the serum levels of the measured proinflammatory cytokines TNFalpha and IL-6, of the soluble TNF receptors I and II or of the IL-2 receptors. This result concurs with a previous report on the relevance of TNFalpha and TNFalpha 1 and 2 receptors in decompensated heart failure and the effects of clinical interventions on short-term elaboration of this cytokine (27). Despite clinical improvement, the cited report noted no alteration of the high levels of TNFalpha.

Immunohistochemical examination of the endomyocardial biopsies was comparable in the IA/IgG and control groups. The present data do not allow conclusions on whether the efficacy of IA and IgG substitution depend on a persistent latent inflammatory process in the myocardial tissue. Large-scale studies are necessary to clarify this question.

Functional role of cardiac autoantibodies. The functional role of a particular cardiac autoantibody is still speculative. This study provides no data that allow conclusions on whether the acute hemodynamic effects of IA were related to removal of a specific cardiac autoantibody. Since the IA columns used in this study were oriented to human IgG in general, no conclusion is possible on whether the observed beneficial effects were due to extraction of a specific antibody from the sera. In particular, the contribution of the beta-receptor autoantibodies to cardiac malfunction in DCM is unclear. We, therefore, used these antibodies as an immunological marker of DCM and, in turn, as a means of evaluating the efficacy of IA in removing IgG-type antibodies. It was recently shown in patients with DCM who were treated with mechanical cardiac support systems that beta$_1$-receptor autoantibodies gradually decreased (28). This implies that this type of cardiac autoantibody may be a marker of functional recovery. It remains to be elucidated whether cardiac autoantibodies represent an epiphenomenon in DCM, whether they play a causal role in initiating cardiac injury or whether they contribute to cardiac malfunction after the disease has developed. In any case, a recent report by Matsui et al. (29) is of particular interest. These authors provided evidence that peptides derived from cardiovascular G-protein-coupled receptors induce morphological cardiomyopathic changes in the myocardium of immunized rabbits, which are similar to those found in humans with DCM.

Possible effects of IgG substitution. Hemodynamic measurements carried out during the first IA course revealed significant hemodynamic improvement, which had already

taken place before IgG substitution. On the other hand, in addition to IA, substitution of IgG also modulates the immune system. Beneficial effects of intravenous Ig therapy have been reported for various autoimmune diseases (11,13,15). Also in DCM, administration of intravenous IgG may improve the disease process. According to a recent report on patients with myocarditis and acute cardiomyopathy, the use of high-dose intravenous IgG was associated with significant increase in left ventricular performance at one-year follow-up (17). Administration of intravenous Ig after IA may, therefore, be a confounding variable. However, we considered IgG substitution necessary—after IA and subsequent IgG depletion—in order to reduce the risk of infection in our patients instrumented with a pulmonary catheter. According to clinical data, risk of acute infection increases when IgG plasma levels fall below 5g/dl (30). Another reason for IgG substitution was to prevent rebound of antibody production in the B cells. Anti-idiotypic IgG inhibits autoantibody production in B cells by binding of the Ig to B-cell Fc-receptors (10). Furthermore, substitution with intravenous IgG causes idiotype-anti-idiotype reactions and neutralization of the remaining autoantibodies.

In summary, IA—in combination with IgG substitution and with repetition at monthly intervals—induces stable hemodynamic improvement and may, therefore, represent an additional beneficial therapeutic approach in patients with DCM.

Study limitations. The design of this study was not adequately controlled and blinded, owing to the fact that the control group was not sham treated by plasmapheresis and subsequent reinfusion of the plasma without IA. This treatment procedure, however, was considered inappropriate on ethical grounds.

Conclusions. These data indicate that activation of the humoral immune system, with production of cardiac autoantibodies, may play a functional role in cardiac malfunction of patients with DCM. Modulation of the humoral immune system by IA and subsequent IgG substitution may, accordingly, represent a promising therapeutic approach which intervenes in this autoimmune process.

Reprint requests and correspondence: Dr. Stephan B. Felix, Medizinische Klinik und Poliklinik Charité, Kardiologie, Campus Mitte, Humboldt-Universität zu Berlin, Schumannstr. 20-21, D-10098 Berlin, Germany. E-mail: stephan.felix@charite.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. Schwimmbeck PL, Badorff C, Schultheiss HP, Strauer BE. Transfer of human myocarditis into severe combined immunodeficiency mice. Circ Res 1994;75:156–64.
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;89:2760–7.
8. 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 six month in rabbits. Clin Immunol Immunopathol 1996;78:203–7.
9. Dörffel WV, Felix SB, Wallukat G, et al. Short-term hemodynamic effects of immunoadsorption in dilated cardiomyopathy. Circulation 1997;95:1994–7.
10. Dwyer J. Manipulating the immune system with immune globulin. N Engl J Med 1992;326:107–16.
11. Godeau B, Lesage S, Divine M, Wirquin V, Farcet J, Bierling P. Treatment of adult chronic autoimmune thrombocytopenic purpura with repeated high-dose intravenous immunoglobulin. Blood 1993;82:1415–21.
12. Snyder H, Cochran S, Balint J, et al. Experience with protein A-immunoadsorption in treatment-resistant adult immune thrombocytopenic purpura. Blood 1992;79:2237–45.
13. Arsura E, Bick A, Brunner N, Namba T, Grob D. High-dose intravenous immunoglobulin in the management of myasthenia gravis. Arch Intern Med 1986;146:1365–8.
14. Grob D, Simpson D, Mitsumoto H, et al. Treatment of myasthenia gravis by immunoadsorption of plasma. Neurology 1995;45:338–44.
15. Jackson M, Godwin-Austen R, Whiteley A. High-dose intravenous immunoglobulin in the treatment of Guillain-Barre syndrome: a preliminary open study. J Neurol 1993;240:51–3.
16. Tagawa Y, Yuki N, Hirata K. Ability to remove immunoglobulins and anti-ganglioside antibodies by plasma exchange, double-filtration plasmapheresis and immunoadsorption. J Neurol Sci 1998;157:90–5.
17. McNamara DM, Rosenblum WD, Janosko KM, et al. Intravenous immune globulin in the therapy of myocarditis and acute cardiomyopathy. Circulation 1997;95:2476–8.
18. Kühl U, Noutsias M, Schultheiss HP. Immunohistochemistry in dilated cardiomyopathy. Eur Heart J 1995;16:100–6.
19. Wallukat G, Wollenberger A. Effects of serum gamma globulin fraction in patients with allergic asthma and dilated cardiomyopathy on chronotropic beta-adrenoceptor function in cultured neonatal rat heart myocytes. Biomed Biochem Acta 1987;46:634–9.
20. Jin O, Sole MJ, Butany JW, McLaughlin PR, Liu P, Liew CC. 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.
21. Liao L, Sindhwani R, Rojkind M, Factor S, Leinwand L, Diamond B. Antibody-mediated autoimmune myocarditis depends on genetically determined target organ sensitivity. J Exp Med 1995;181:1123–31.
22. Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. Br Heart J 1994;72:561–6.
23. Torre-Amione G, Kapadia S, Lee J, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996;93:704–11.
24. Ferrari R, Bachetti T, Confortini R, et al. Tumor necrosis factor

soluble receptors in patients with various degrees of congestive heart failure. Circulation 1995;92:1479–86.

25. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL. Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the studies of left ventricular dysfunction (SOLVD). J Am Coll Cardiol 1996;27:1201–6.

26. Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, Mann DL. Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. J Clin Invest 1993;92:2303–12.

27. Milani RV, Mehra MR, Endres S, et al. The clinical relevance of circulating tumor necrosis factor-alpha in acute decompensated chronic heart failure without cachexia. Chest 1996;110:992–5.

28. Müller J, Wallukat G, Wenig Y-G, et al. Weaning from mechanical cardiac support in patients with idiopathic dilated cardiomyopathy. Circulation 1997;96:542–9.

29. Matsui S, Fu ML, Katsuda S, et al. Peptides derived from cardiovascular G-protein-coupled receptors induce morphological cardiomyopathic changes in immunized rabbits. J Mol Cell Cardiol 1997;29:641–55.

30. Roifman C, Levison H, Gelfand E. High-dose versus low-dose intravenous immunoglobulin in hypogammaglobulinemia and chronic lung disease. Lancet 1987;1:1075–7.