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Immunoglobulin Adsorption in Patients With Idiopathic Dilated Cardiomyopathy

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Background—Idiopathic dilated cardiomyopathy (IDC) frequently is a progressive disease without causative therapy options. Following the hypothesis that in certain patients autoantibodies against cardiac structures may induce, maintain, or promote the progression of the disease, we investigated whether the elimination of these autoantibodies through immunoadsorption would improve cardiac function.

Methods and Results—This prospective case-control study included 34 patients with IDC. Each patient presented with moderate to severe heart failure and evidence of autoantibodies directed against β_1 -adrenoceptors (β_1 -AABs). Seventeen patients received standard medical therapy (control group), whereas 17 were also treated with immunoadsorption (treatment group) to eliminate β_1 -AABs. A 1-year follow-up included echocardiographic assessment of left ventricular ejection fraction and internal diameters, β_1 -AAB levels, and clinical status every 3 months. Within 1 year, the mean \pm SD left ventricular ejection fraction rose from $22.3 \pm 3.3\%$ to $37.9 \pm 7.9\%$ ($P=0.0001$) in the treatment group, with a relative increase of 69.9%. However, in the control group, no overall increase was seen (from $23.8 \pm 3.0\%$ to $25.2 \pm 5.9\%$, $P=0.3154$). Left ventricular diameter in diastole decreased by 14.5% from 74.5 ± 7.1 to 63.7 ± 6.0 mm in the treatment group ($P=0.0001$) and by 3.8% ($P=0.2342$) in the control group. In the treatment group, the NYHA functional rating improved after immunoadsorption ($P=0.0001$). β_1 -AABs did not increase anew.

Conclusions—In IDC, the use of immunoadsorption is superior to the use of standard medical therapy. It significantly improves cardiac performance and clinical status. (*Circulation*. 2000;101:385-391.)

Key Words: dilated cardiomyopathy ■ autoantibodies

Nonischemic idiopathic dilated cardiomyopathy (IDC) is one of the principle reasons for heart transplantation.¹ Because little is known concerning the pathogenesis of the disease, therapy is usually focused on the symptomatic treatment of heart failure. However, several autoantibodies, which react against cardiac cellular proteins, have been detected that may represent important pathogenetic factors.²⁻⁸ In vitro receptor and animal studies indicate that autoantibodies contribute to the progressive deterioration of cardiac function in a subset of patients with IDC.⁹⁻¹¹ Rabbits developed the clinical entity of IDC after being immunized with sequences of the β_1 -adrenoceptor that induced the production of anti- β_1 -adrenoceptor autoantibodies (β_1 -AABs). Those β_1 -AABs, which were first detected in patients with Chagas' disease, were present in 80% of our patients with end-stage IDC and in all patients who required mechanical cardiac support.^{12,13} In 20% of the mechanically supported patients, cardiac function recovered to near-normal values concomitant with a gradual disappearance of β_1 -AABs.

From these observations, we derived the hypothesis that cardiac antibodies are of importance for the induction, main-

tenance, and progression of IDC and that their elimination through extracorporeal IgG adsorption (immunoadsorption) should improve or at least stabilize the function of the heart in an analogous manner to other autoimmune diseases.¹⁴⁻¹⁶ A first report of positive short-term effects with this treatment in 7 patients with IDC was published by Wallukat et al.¹⁷

To evaluate the long-term effects of immunoadsorption on cardiac function in patients with IDC that are more clinically relevant, we conducted a prospective clinical case-control trial with a 1-year follow-up period.

The β_1 -AABs, which are of the IgG fraction, were used as a marker for autoantibody presence to identify patients who might benefit from immunoadsorption.⁵ Echocardiographic evaluation of left ventricular ejection fraction (LVEF) and internal diameters were chosen to determine cardiac performance, and the NYHA functional class was correspondingly assessed.¹⁸

Methods

Patients and Group Allocation

Thirty-four patients (32 men and 2 women) were enrolled in the study on their routine follow-up visits to our institution. Principally,

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TABLE 1. Baseline Characteristics of Study Patients

	Group			P*
	Patients (n=34)	Treatment (n=17)	Control (n=17)	
Age, y	47.5±10.6	45.9±10.7	49.1±10.5	0.3998†
Sex, n (%)				
Male	32 (94.1)	15 (88.2)	17 (100)	0.4850‡
Female	2 (5.9)	2 (11.8)	0 (0)	
Body surface area, m ²	1.96±0.2	1.92±0.2	1.99±0.2	0.2245†
Duration of symptoms, y	3.4±2.0	3.4±1.8	3.5±2.2	0.9343†
Duration of routine visits before study enrollment, mo	10.6±7.0	10.4±7.2	10.9±7.0	0.9955†
β ₁ -AAB, LU	5.4±1.4	6.0±1.4	4.7±1.0	0.0104†
LVEF, %	23.1±3.2	22.3±3.3	23.8±3.0	0.2182†
LVIDd, mm	75.4±6.4	74.5±7.1	76.2±5.7	0.7235†
Indexed LVIDd, cm/m ²	3.87±0.4	3.89±0.3	3.84±0.3	0.6584†
LVIDs, mm	66.8±6.1	65.9±6.1	67.7±6.1	0.7347†
Left ventricular end-diastolic volume, mL	318±81.4	308±87.0	328±77.3	0.4968†
NYHA class, n (%)				
II	7 (20.6)	3 (17.6)	4 (23.5)	...
III	26 (76.5)	13 (76.5)	13 (76.5)	0.4838§
IV	1 (2.9)	1 (5.9)
Serum IgG, mg/dL	1112±257	1164±257	1060±247	0.7696†

Values are mean±SD.

*P for differences between groups.

†P by ANOVA.

‡P by Fisher's exact test.

§P by Kruskal-Wallis test.

they were all accepted as candidates for heart transplantation. Inclusion criteria were diagnosis of IDC with no evidence of coronary artery disease or any other cardiac disease, NYHA functional class of II or worse, left ventricular ejection fraction (LVEF) of below 0.29, left ventricular internal diameter in diastole (LVIDd) of above 64 mm, and evidence of β₁-AABs of above 3.0 laboratory units (LU).¹⁹

Exclusion criteria were atrial fibrillation, infectious diseases, alcohol-induced cardiomyopathy, previous allergic reactions to sheep proteins, and signs of malignancy.

The study commenced with the first consecutive 17 patients who presented for their routine follow-up to our pretransplantation outpatient department. They were assigned to the treatment group and were treated with immunoadsorption. Seventeen control group patients were recruited from the pool of patients to match the criteria of age, body surface area, duration of symptoms, and follow-up before the start of the study, LVEF, LVIDd, indexed LVIDd, left ventricular internal diameter in systole (LVIDs), left ventricular end-diastolic volume, and NYHA functional class. They were not treated with immunoadsorption (Table 1).

Medical therapy was standardized at baseline and continued throughout the study in both groups. Termination criterion was an occurrence of adverse side effects during immunoadsorption treatment or a deterioration in the clinical status of the patient with the subsequent need for heart transplantation. Written informed consent was obtained from all patients. The study protocol was approved by the Human Ethics Committee of the Humboldt University (Berlin, Germany).

The design of a matched control study instead of a randomized trial was chosen to minimize group differences with regard to the matching criteria. Without matching, it would have been necessary to include all the essential variables for statistical analysis with a required consequent increase in patient number. The use of a greater

number of patients would have prolonged the study and multiplied costs (the cost for each patient amounted to \$30 600). Such an extension seemed unreasonable in view of the use of a new therapeutical approach and the lack of clinical long-term experience. The randomization of 34 patients, however, would have involved a considerable risk of group differences, which could have impeded proper statistical analysis and thus weakened the results.

Protocol

All patients were assessed at baseline and subsequently at 3-, 6-, 9-, and 12-month intervals. Evaluation included an echocardiographic assessment of LVEF, LVIDd, and LVIDs; clinical examination; and laboratory tests that included IgG, IgM, IgA, IgE, and β₁-AAB levels.

Primary end points were a relative increase in LVEF by 40% and a decrease in LVIDd by 10%. Secondary end points were LVIDs, NYHA functional class, and level of β₁-AABs.

Medical Treatment

At baseline, medical treatment for heart failure was standardized in that all patients were administered maximal tolerated dosages of ACE inhibitors, digitalis, diuretics, and oral anticoagulants. In addition, all patients were treated with β-blockers (bisoprolol) for the first time. Dosage adjustments were made to achieve a systolic blood pressure of 100 to 110 mm Hg and a heart rate of 60 to 80 bpm within 3 months. All patients received a supplement of a moderate dose of vitamins, minerals, and trace elements (OrthoCorPlus; Orthomol GmbH) for oxidative stress reduction.

Extracorporeal Immunoglobulin Adsorption

Immunoadsorption was performed according to the established method of LDL elimination with the use of adsorption columns that

contained polyclonal anti-human immunoglobulin antibodies produced in sheep.²⁰ Immunoabsorption was performed on 5 consecutive days, and serum IgG levels were monitored. A reduction of IgG to below 120 mg/dL was planned to eliminate β_1 -AABs with an acceptable risk of infection.

Measurement of Anti- β_1 -AABs

A description of the bioassay for β_1 -AAB measurement has been previously published.²¹ The underlying principle is the registration of the chronotropic effect of β_1 -AABs on primary cultures of neonatal rat cardiomyocytes. The increase in the number of contractions after the addition of the IgG fraction prepared from the patient’s serum is defined and given in LU; values of below 1.5 LU are considered to be negative, and values of above 3.0 LU are considered to be positive.

Echocardiographic Evaluation

Transthoracic echocardiography was performed according to the guidelines of the American Society of Echocardiography.²² All examinations were performed by the same operator at nearly the same time of day with an Aloka SS 2200 ultrasonograph. The operator was blinded to the group allocation of the patients. Intracavitary dimensions were measured with use of the motion mode, whereas LVEF was calculated from 2 reliable orthogonal views with the biplanar Simpson’s rule approach.²³

Statistical Analysis

Our model contained 4 dependent variables (LVEF, LVIDd, LVIDs, and β_1 -AABs) and 2 independent variables (group allocation and repeated measures).

The overall therapy effect on the completeness of all dependent variables was tested with MANOVA.²⁴ Two-way ANOVA was performed to test the overall effects of the 2 factors on each dependent variable. One-way ANOVA was performed to calculate the time course results within each group (contrast transformation and profiles), followed by 1-way ANOVA to evaluate the differences between the groups at each follow-up visit. Because the requirements for ANOVA were not completely satisfied for all variables, the results of 1-way ANOVA were confirmed with the use of nonparametric tests (Kruskal-Wallis and Friedmann).²⁵⁻²⁷

Sample size was planned with the assumption of a strong therapy effect for a model with 1 *df*. The analyses were performed with the SPSS 7.0 and SAS 6.10 for Windows packages.

Results

Baseline Characteristics

According to our matching criteria, there were no statistically significant differences between the patient groups at baseline except for the β_1 -AAB level (Table 1).

TABLE 2. IgG Levels Before and After Each of the Five Treatment Days

Treatment Day	Value Before, mg/dL	Value After, mg/dL
1	1164±257	366±254
2	556±241	149±94
3	384±279	108±63
4	246±133	112±30
5	222±73	84±48

Values are mean±SD. n=17.

Extracorporeal Immunoglobulin Adsorption

Immunoabsorption was tolerated without reported side effects. All 17 patients spontaneously reported an impressive improvement in their subjective well-being.

A reduction of IgG to below 120 mg/dL was achieved in all patients after 5 treatment days (Table 2). The mean amount of plasma treated was 26.1±5.7 L (range 15.2 to 35.6 L) per patient.

Each session lasted a mean±SD of 5.6±2.1 hours (range 3.2 to 7.0 hours) for treatment of 17.4±2.8 L (range 12.3 to 22.8 L) of whole blood.

Anti- β_1 -AABs

The β_1 -AABs were eliminated through immunoabsorption to values of below 1.0 LU, which is within the normal range. This mean reduction by 93.2% after 3 months was highly significant ($P=0.0001$). During the 1-year follow-up, no significant reincrease was seen (Figure 1).

In the control group, mean±SD β_1 -AAB levels remained unchanged (4.7±1.0 LU at baseline compared with 5.0±1.3 LU after 1 year).

Cardiac Performance

At 1 year after immunoabsorption in the treatment group, LVEF increased from 0.223±0.33 to 0.379±0.79, LVIDd decreased from 74.5±7.1 to 63.7±6.0 mm, and LVIDs decreased from 65.9±6.1 to 55.4±6.0 mm. All of these changes were significant ($P=0.0001$).

In the control group, the values at 1 year did not significantly differ from their baseline values: LVEF changed from 0.238±0.30 to 0.252±0.59, LVIDd decreased from 76.2±5.7

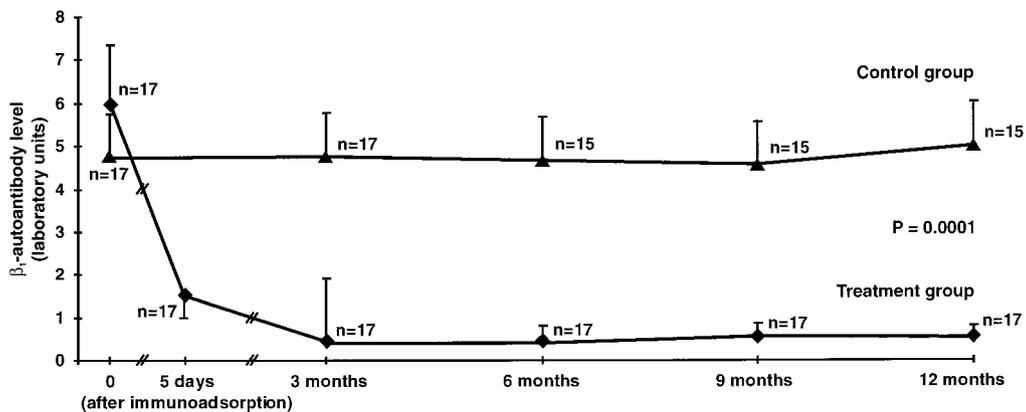


Figure 1. One-year repeated measures (mean±SD) of β_1 -AAB level with treatment and control groups.

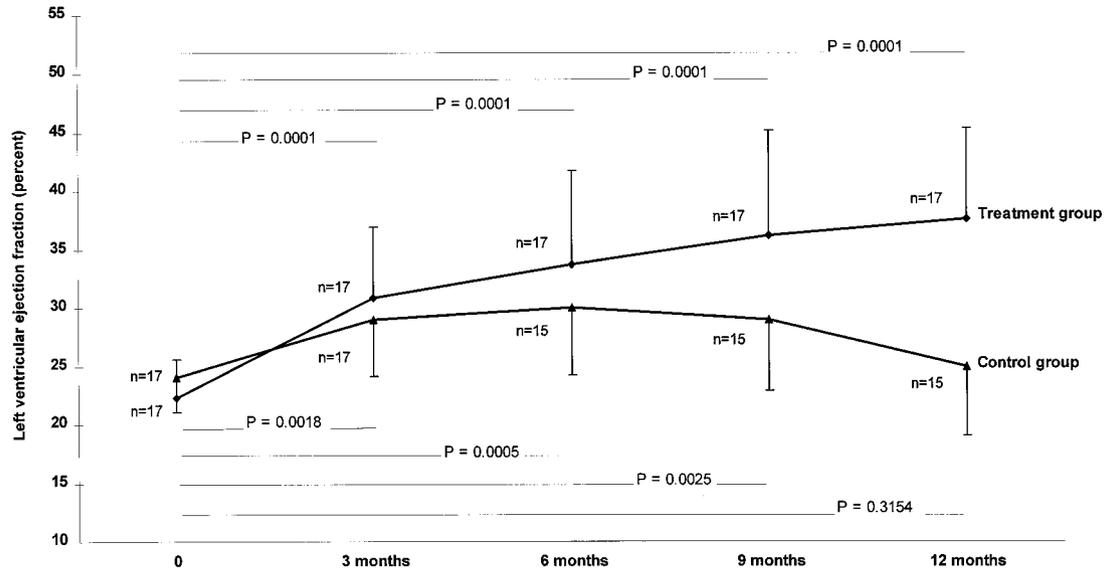


Figure 2. One-year repeated measures (mean±SD) of LVEF within 2 groups. *P* values were derived through 1-way ANOVA (contrast transformation).

to 73.3±6.8 mm, and LVIDs decreased from 67.7±6.1 to 63.9±7.1 mm.

The significance was calculated with the use of repeated-measures ANOVA and is presented in Figures 2, 3, and 4, which show the time courses of LVEF, LVIDd, and LVIDs within both groups. LVEF increased and LVIDd and LVIDs decreased in both groups until month 3. In the further course, LVEF continued to increase and LVIDd and LVIDs continued to decrease in the treatment group, whereas a regression toward baseline values was observed in the control group. Contrast transformation revealed that the changes in LVEF, LVIDd, and LVIDs between baseline and month 3 were significant within each group.

The differences in LVEF between the 2 groups became statistically significant at month 9 (*P*=0.0171) and were highly significant after 1 year (*P*=0.0001). The group differ-

ences in LVIDd were statistically significant after 1 year (*P*=0.0004), as were the differences in LVIDs (*P*=0.0038).

Clinical Status

After 1 year, NYHA functional class was significantly better in the treatment group than in the control group (Table 3).

In the control group, the study was discontinued for 2 patients after month 3 due to rapidly deteriorating cardiac function that required heart transplantation. Statistical analysis included 15 patients from month 6 on.

Discussion

With this prospective case-control study, we were able to confirm our hypothesis that immunoabsorption improves cardiac performance and NYHA functional class after 1 year. In the treatment group, LVEF increased and LVIDd and

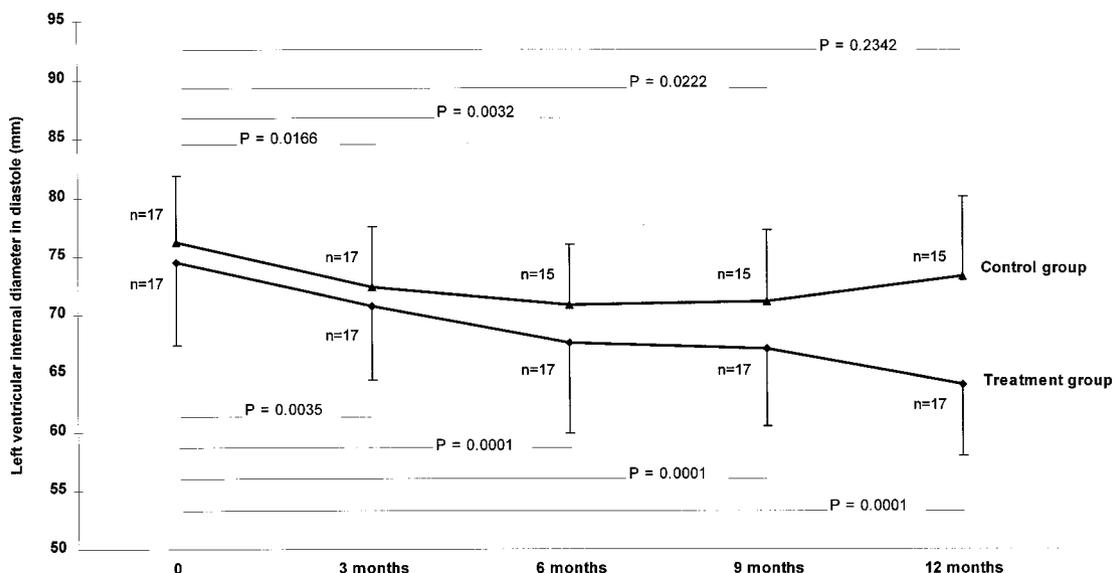


Figure 3. One-year repeated measures (mean±SD) of LVIDd within 2 groups. *P* values were derived through 1-way ANOVA (contrast transformation).

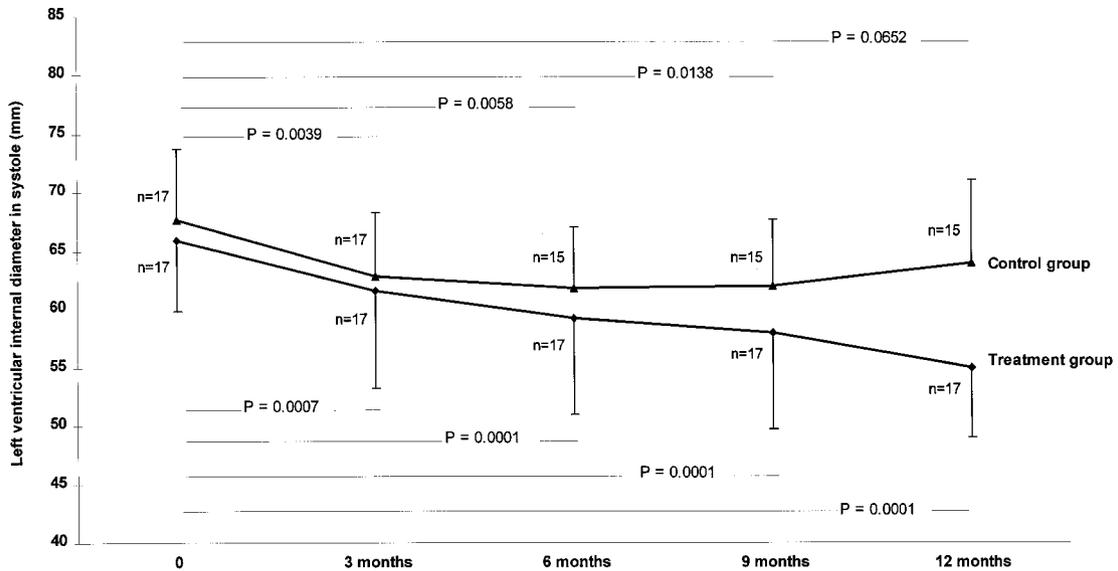


Figure 4. One-year repeated measures (mean±SD) of LVIDs within 2 groups. *P* values were derived through 1-way ANOVA (contrast transformation).

LVIDs decreased significantly, whereas in the control group, the 1-year values of these parameters did not significantly differ from baseline values. A relative increase in LVEF by 69.9% was considerably beyond the anticipated 40% increase (primary end point). The same applies for the relative decrease in LVIDd by 14.5%.

One notable result of the study is the revelation that an ostensible initial improvement in cardiac function in the control group patients was followed by a return to the baseline values after 6 months. In contrast, the treatment group data continuously improved (Figures 2 to 4). The significant improvement in cardiac performance in both groups during the first 3 months may be explained by the administration of a β -blocker, the optimization of medical therapy (ACE inhibitor dosage adjustments), and the closer follow-up in our outpatient clinic. In the control group, no further decrease in LVIDd ($P=0.1963$) or LVIDs ($P=0.3405$) was seen, and LVEF indeed decreased ($P=0.0027$) from month 3 to month 12. This course explains why the group difference in LVEF was not significant until month 9 and why those changes in LVIDd and LVIDs were not significant until month 12.

TABLE 3. NYHA Functional Class at Baseline and After 1 Year

NYHA Class	At Baseline†		After 12 mo‡	
	Treatment (n=17)	Control (n=17)	Treatment (n=17)	Control* (n=15)
I	8 (47.1)	...
II	3 (17.6)	4 (23.5)	9 (52.9)	7 (46.7)
III	13 (76.5)	13 (76.5)	...	6 (40.0)
IV	1 (5.9)	2 (13.3)

Values are given as number of patients (%).

*Two patients underwent transplantation due to cardiac deterioration after months 2 and 3.

†Difference between the groups was calculated with Kruskal-Wallis test: † $P=0.4838$; ‡ $P=0.0001$.

However, our data did not confirm the positive short-term effect on cardiac output in patients after immunoadsorption as recently reported by Wallukat et al¹⁷ and Dörffel et al.²⁸

Echocardiography

Echocardiography was selected for the assessment of cardiac performance because it is a noninvasive, easy, and frequently applicable method.

With regard to parameter selection, there is no completely load-independent index with which to appropriately measure the contractile state.²⁹ LVEF measurements were used because this parameter is generally accepted and is appropriate as long as its load dependence is considered.^{29–33} To assess whether the observed increase in ejection fraction in the treatment group might have been caused by afterload reduction instead of an improvement in contractile state, the meridional end-systolic wall stress was calculated with the use of cuff systolic blood pressure in a generally accepted and invasively validated formula according to Poiseuille’s law.^{34–36} These calculations showed a significant decrease in wall stress at month 3 in the treatment group ($-14.4\pm 20.7\%$, $P=0.0111$) and in the control group ($-14.7\pm 15.7\%$, $P=0.003$). Between months 3 and 12, no further significant decrease in wall stress was seen in either the treatment ($-11.1\pm 27.3\%$, $P=0.1148$) or in the control ($-10.3\pm 25.9\%$, $P=0.8074$) group. Therefore, even when the initial improvement in LVEF may be, at least in part, an effect of afterload reduction achieved through the use of optimized medical therapy, we consider the 1-year results to be an effect of increased myocardial contractility.^{37–41}

The impact of preload changes on LVEF is difficult to assess. However, in consideration of the significant reduction in LVIDd as well as an absence of significant changes in transmitral peak E-wave velocity and E/A ratio throughout the study (E-wave velocity: treatment group $+11.9\pm 38.9\%$, $P=0.2380$; control group $+3.1\pm 27.9\%$, $P=0.7355$; E/A ratio: treatment group $+17.15\pm 77.5$, $P=0.3903$; control

group -6.3 ± 36.6 , $P=0.5310$), a significant contribution of preload changes to the increase in LVEF also cannot be justifiably assumed.^{42,43}

Autoantibodies

In the treatment group, β_1 -AABs were effectively eliminated through immunoadsorption and did not reincrease within the observation period. In the control group, β_1 -AAB levels remained unchanged.

Evidence of β_1 -AABs as a marker for autoantibody presence was required in all patients to identify those who would most likely benefit from immunoadsorption. The different ($P=0.0104$) β_1 -AAB levels at baseline were therefore not considered to be important provided the level was >3.0 LU.

β_1 -AABs react against the first or second extracellular loop of the β_1 -adrenoceptor. Findings that affinity-purified β_1 -AABs from sera of patients with IDC increased the spontaneous beating rate of isolated cultured cardiomyocytes and that this positive chronotropic effect was selectively blocked by β_1 -adrenoceptor antagonists indicate that β_1 -AABs realize their effect via the β_1 -adrenoceptor cascade.^{11,44} In these experiments, their stimulating effect lasted >6 hours, whereas stimulation with β -adrenergic agonists, such as isoproterenol, induced a downregulation of the β_1 -adrenoceptors within 1 hour.⁴⁵ Therefore, it can be hypothesized that β_1 -AABs cause chronic adrenergic stimulation. We further speculate that myocardial inflammation may be induced. In our study, the serial endomyocardial biopsies to test markers of inflammation such as myocardial infiltration, cytokine and immune activation, expression of MHC1/2, adhesion molecules, or increased immunoglobulin binding were, however, not performed because of insufficient sensitivity and specificity of the method and the limited number of patients.

Previous findings, together with study data, do not conclusively prove the pathogenetic effect of β_1 -AABs. Because immunoadsorption is neither antigen nor class selective, the positive effect cannot be attributed to the elimination of 1 specific class of autoantibodies.

The mechanisms of β_1 -AAB occurrence and lack of reoccurrence after elimination are still unclear. In other autoimmune diseases, elimination treatment must be periodically repeated to maintain the therapeutic effect.

We hypothesize that antigen presentation is markedly reduced when heart function improves such that the disease-inducing process is attenuated. Our observations made in a study of patients with a temporary mechanical assist device support this hypothesis.¹² Furthermore, we speculate that the ex juvantibus taking of antioxidants prevented reincrease in β_1 -AABs. This speculation is based on the study of Wallukat et al¹⁷ of patients who were not supplemented with antioxidants after immunoadsorption; in these patients, β_1 -AABs increased again after elimination.

In patients with IDC, intravenous immunoglobulin is administered with the anticipated effect of reduced IgG levels through the acceleration of IgG catabolism.⁴⁶ Although convincing success has not yet been described, we readily observed a reincrease in β_1 -AABs when only immunoglobulins had been administered after immunoadsorption.¹⁷

Study Limitations, Questions, and Implications

Although the results of the present study are highly significant, the use of echocardiography, which is associated with a potential for subjectivity instead of objective or invasive methods (eg, scintigraphy, right heart catheterization), may be considered a limitation.

The design of a matched control study was chosen instead of randomization to allow a statistically strong trial to be carried out with a limited number of patients and to provide comparability of the influencing variables between the groups. Because the long-term effects of this new treatment for IDC could not be anticipated in advance, the larger patient number required for proper randomization was considered unreasonable. After our study results, a randomized multicenter study has legitimacy regardless of costs. It should include the use of endomyocardial biopsies and immunohistology to detect active myocarditis and to evaluate predictive factors or mediators to differentiate between potential responders and nonresponders to immunoadsorption.

The pathogenetic relevance of the β_1 -AABs could not be proved by this study, but the results indicate that autoantibodies do play a role in the development, progression, or maintenance of IDC and therefore support our initial hypothesis. Whether other humoral mediators of IDC were removed by immunoadsorption was not explored. The first results of our tests show that immunoadsorption did not remove cytokines like tumor necrosis factor- α .

Because of the simple applicability of immunoadsorption, this method can be applied to the general population of patients with IDC as long as relevant autoantibodies are detected and all cost-effective issues are considered.

In conclusion, these data imply that, at least for a subset of patients with IDC, immunoadsorption offers an effective and low-risk treatment that has the potential to postpone or even avoid heart transplantation, which otherwise would be the midterm perspective for patients with this disease.

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