Potential Role of Autoantibodies Belonging to the Immunoglobulin G-3 Subclass in Cardiac Dysfunction Among Patients With Dilated Cardiomyopathy

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Background—Immunoadsorption capable of removing circulating autoantibodies represents an additional therapeutic approach in dilated cardiomyopathy (DCM). The role played by autoantibodies belonging to the immunoglobulin (Ig) subclass G-3 in cardiac dysfunction remains to be elucidated.

Methods and Results—Patients with DCM (left ventricular ejection fraction <30%) participated in this case-control study. Nine patients underwent immunoadsorption with protein A (low affinity to IgG-3), and 9 patients were treated with anti-IgG, which removes all IgG subclasses. Immunoadsorption was performed in 4 courses at 1-month intervals until month 3. In the 2 groups, immunoadsorption induced comparable reduction of total IgG (>80%). IgG-3 was effectively eliminated only by anti-IgG adsorption (eg, during the first immunoadsorption course; protein A, −37±4%; anti-IgG, −89±3%; P<0.001 versus protein A). The β1-receptor autoantibody was effectively reduced only by anti-IgG (P<0.01 versus protein A). Hemodynamics did not change in the protein A group. In the anti-IgG group during the first immunoadsorption course, cardiac index increased from 2.3±0.1 to 3.0±0.1 L·min⁻¹·m⁻² (P<0.01 versus protein A). After 3 months, before the last immunoadsorption course, cardiac index was 2.2±0.1 L·min⁻¹·m⁻² in the protein A group and 3.0±0.2 L·min⁻¹·m⁻² in the anti-IgG group (P<0.01 versus protein A). Left ventricular ejection fraction increased only in the anti-IgG group (P<0.05 versus protein A).

Conclusions—Autoantibodies belonging to IgG-3 may play an important role in cardiac dysfunction of DCM. The removal of antibodies of the IgG-3 subclass may represent an essential mechanism of immunoadsorption in DCM. (Circulation. 2002;106:2448-2453.)

Key Words: cardiomyopathy ■ immunoadsorption ■ antibodies

Disturbances in humoral and cellular immunity have been described in patients with dilated cardiomyopathy (DCM).¹ A number of antibodies against cardiac proteins have been identified in DCM, eg, antibodies against mitochondrial proteins, contractile proteins, and β1-receptors.²⁻⁴ Cardiac autoantibodies may play an active role in the pathogenesis of DCM by initiating the disease process⁵ or by contributing to the progression of contractile dysfunction.² Cardiac antibodies belong to the IgG fraction and can be extracted by immunoadsorption.⁶⁻⁹

An initial uncontrolled pilot study and a randomized study were performed to ascertain the hemodynamic effects of immunoadsorption with anti-IgG columns and subsequent IgG substitution in DCM.⁶⁻⁷ In contrast to controls, the cardiac index (CI) in the immunoadsorption/IgG group rose immediately, and systemic vascular resistance (SVR) simultaneously fell. Immunoadsorption was repeated at monthly intervals until month 3. Acute hemodynamic improvement persisted over this period.⁷

A recent study used an in vitro bioassay system to investigate the mechanisms involved in the acute hemodynamic effects of immunoadsorption.¹⁰ The results indicate that column eluent (CE) from immunoadsorption columns containing the eliminated antibodies decreases cell contraction in cardiomyocytes by depression of Ca²⁺ transients. Furthermore, the acute hemodynamic benefit during immunoadsorption correlated with the negative inotropic effects of CE, which demonstrates that removal of circulating negative inotropic autoantibodies may contribute to the acute hemodynamic effects of immunoadsorption in DCM.¹⁰
Protein A and anti-IgG columns are licensed for use in immunoadsorption. Compared with the anti-IgG sepharose raised in immunized sheep, protein A offers the advantage of being synthesized by bacteria. Protein A binds to the Fc fragment of human IgG-1, -2, and -4. However, the affinity of protein A to IgG-3 is low. In contrast, the anti-IgG column containing antibodies from immunized animals has a high affinity for all IgG subclasses. IgG subclasses differ immunologically and functionally from one another. Complement activation, for example, is most effective with IgG-3. The role played by IgG-3 autoantibodies in cardiac dysfunction is unclear. Less effective removal of IgG-3 may demonstrate effects that differ from those for anti-IgG adsorption.

In accordance with previous studies of immunoadsorption with anti-IgG columns, we decided initially to perform a pilot study on the hemodynamic effects of protein A adsorption in 9 patients with heart failure due to DCM. Immunoadsorption with protein A and subsequent IgG substitution was performed at monthly intervals until month 3. We then planned performance of a further randomized prospective study on the effects of protein A adsorption compared with anti-IgG adsorption. The results of this pilot study with protein A demonstrated no significant effect on the evaluated hemodynamic parameters. For this reason, on ethical grounds, we decided against conducting a further randomized study. We therefore applied a case-control study format to compare the laboratory data and the hemodynamic parameters with anti-IgG adsorption.

Methods

Study Protocol

Eighteen DCM patients (left ventricular ejection fraction [LVEF] <30%, New York Heart Association [NYHA] class III or IV) were admitted from October 1999 to July 2001. All patients were treated with immunoadsorption and subsequent IgG substitution at monthly intervals until month 3, as described elsewhere. In 9 consecutive patients (protein A group), immunoabsorption was performed with protein A columns (Fresenius). In this case-control study, 9 individually matched patients (anti-IgG group) were identically treated with anti-IgG columns (PlasmaSelect).

The hemodynamic inclusion criteria were CI ≤2.5 L·min⁻¹·m⁻² and/or pulmonary capillary wedge pressure ≥16 mm Hg. Coronary heart disease was excluded by angiography. Patients were excluded if they had had active infectious diseases, cancer, chronic alcoholism, or heart failure due to known origins (eg, primary valvular disease). In all patients, acute myocarditis was excluded according to Dallas criteria by endomyocardial biopsy. Biopsies, however, do not completely exclude acute myocarditis owing to patchy focal infiltrates. Disease duration for all patients was longer than 2 years, which argues for chronic myocardial processes. Viral persistence and myocardial inflammation were not analyzed in this first study on protein A in DCM. All patients had received stable oral medication for more than 3 months before the study (Table 1). Furthermore, all patients had been treated with a stable dose of a β-blocker for at least 6 months before the study.

Written consent was obtained from each patient, and the protocol was approved by the Ethics Committee. None of the patients had taken part in a previous immunoabsorption study.

Clinical Findings

Echocardiography was performed at baseline and after 3 months. The results were recorded, and a reader blinded to the treatment group performed offline assessment of LVEF and of left ventricular internal diameter in diastole. LVEF was measured according to the Simpson rule. Determination of β₁-receptor autoantibodies took place as described elsewhere.

Hemodynamics

We conducted right-heart catheterization with a Swan-Ganz thermodilution catheter. Hemodynamic measurements were performed 4 times per day. The interval between 2 measurements was at least 3 hours. Hemodynamic measurements were performed 1 day before and 1 day after the first course.

In both groups, immunoabsorption took place in 4 courses, at 1-month intervals until month 3, as described elsewhere. During the first course (course I), all patients underwent 1 immunoabsorption session daily on 3 consecutive days. After the final immunoabsorption session of each course, the patients received 0.5 g/kg polyclonal IgG (Venimun N) to restore IgG plasma levels. During the fourth course (course IV), hemodynamic monitoring again took place, 1 day before immunoabsorption and 1 day after immunoabsorption.

Bioactivity of the CE

During the first immunoabsorption session, CE containing the eliminated antibodies was collected and dialyzed as described elsewhere. The bioactivity of CE diluted with experimental buffer (1:5) on Ca²⁺ transient and systolic cell shortening was analyzed in isolated rat cardiomyocytes as described recently. Effects of CE from patients treated with protein A (n=6) and anti-IgG (n=6) were analyzed in comparison to CE obtained from 500 mL of blood from healthy age-matched blood donors treated with anti-IgG adsorption (n=6). Furthermore, the antibodies belonging to IgG-3 were removed from CE (total IgG 1.5±0.3 g/L) of the anti-IgG group by means of a specific column against IgG-3 (Biogenes). After removal of IgG-3, bioactivity of CE was analyzed. IgG-3 antibodies were also eluted from the specific column and superfused to cardiomyocytes.

Statistical Analysis

Results are expressed as mean±SEM. We applied the paired Wilcoxon test to detect changes within treatment groups. Differences between the 2 treatments were investigated by the Mann-Whitney U test. Changes in NYHA classification were analyzed by means of singly ordered 2×4 contingency tables using the exact Kruskal-Wallis test of identically distributed rows. Significance was assessed at the P<0.05 level.

Results

Characteristics of Patients at Baseline

In the protein A and anti-IgG groups, the following data were comparable: age, sex, disease duration, NYHA classification,
TABLE 2. Characteristics of Patients at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Protein A</th>
<th>Anti-IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>51.7±5</td>
<td>49.0±3</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>8/1</td>
<td>8/1</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>5.3±1</td>
<td>5.2±1</td>
</tr>
<tr>
<td>NYHA classification II/IV</td>
<td>8/1</td>
<td>8/1</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>76±3</td>
<td>72±2</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>19.8±2</td>
<td>21.5±2</td>
</tr>
<tr>
<td>β-receptor autoantibody level, relative units</td>
<td>4.8±0.5</td>
<td>4.8±0.3</td>
</tr>
</tbody>
</table>

LVIDd indicates left ventricular internal diameter in diastole.

β-receptor autoantibody, LVEF, left ventricular internal diameter in diastole, and hemodynamic parameters (Table 2). In both groups, stable medication and dosage did not differ significantly during the study (Table 1). In particular, the dosage of β-blockers was comparable between the 2 groups, an important matter because it has been shown that β-blockers induce a dose-related increase in LVEF after 6 months.14

Clinical Findings

All patients tolerated immunoadsorption and subsequent IgG substitution well. No major complications occurred. Neither clinical examination nor laboratory data revealed any signs of infection in either group.

During the first immunoadsorption course, IgG levels decreased in the protein A group from 10.4±0.6 to 1.8±0.3 g/L (−83±3%) and in the anti-IgG group from 11.3±0.4 to 1.7±0.3 g/L (−85±3%). Comparable reductions were obtained during subsequent courses (eg, last course: protein A −82±3%; anti-IgG −83±3%).

The levels of IgG-1, -2, and -4 and IgM were not differently influenced by protein A or anti-IgG columns (Figure 1A). The reduction of IgG-3, however, was differently modified, during the first course in the protein A group by −37±4% (from 0.71±0.05 to 0.44±0.04 g/L) and in the anti-IgG group by −89±3% (from 0.79±0.05 to 0.09±0.03 g/L; P<0.001 versus protein A; Figure 1A). During subsequent courses, comparable reductions of IgG-3 were obtained (eg, last course: protein A −36±4%; anti-IgG −93±2%).

During the first course, β-receptor autoantibody activity decreased in the protein A group from 4.8±0.5 to 3.7±0.4 relative units and in the anti-IgG group from 4.8±0.3 to 1.2±0.3 relative units (P<0.01 versus baseline, P<0.01 versus protein A; Figure 1B). Furthermore, and in contrast to the anti-IgG group, β-receptor autoantibody activity was higher in the protein A group after 3 months of immunoadsorption (protein A 3.4±0.4 relative units; anti-IgG 1.1±0.3 relative units; P<0.01 versus protein A).

During the first immunoadsorption course, hemodynamics in the protein A group did not change significantly (Figure 2). No significant changes were determined for CI, stroke volume index (SVI), or SVR.

In the anti-IgG group, in contrast, CI increased from 2.3±0.1 to 3.0±0.1 L·min⁻¹·m⁻² (P<0.01 versus baseline, P<0.01 versus protein A) during the first course (Figure 2A).

Because heart rate remained stable, SVI also increased significantly (Figure 2B). SVR decreased from 1305±48 to 925±55 dyne·s⁻¹·cm⁻² (P<0.01 versus baseline, P<0.05 versus protein A; Figure 2C). Mean arterial blood pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and right atrial pressure did not change significantly in either group.

The prolonged benefits for both groups were also different. After 3 months, before the last immunoadsorption course in the protein A group, none of the measured hemodynamic parameters differed from those at baseline (Figure 2). In contrast, hemodynamic improvement persisted after anti-IgG immunoadsorption; after 3 months, before the final immunoadsorption course, CI was 3.0±0.2 L·min⁻¹·m⁻² in the anti-IgG group (P<0.01 versus baseline, P<0.01 versus protein A; Figure 2A). SVI and SVR were likewise significantly different compared with baseline (Figure 2, B and C).

The final immunoadsorption course induced moderate improvement of CI, SVI, and SVR in both groups. In contrast to the protein A group, however, these parameters were significantly different from baseline in the anti-IgG group (Figure 2). After the final course, CI was 2.5±0.2 L·min⁻¹·m⁻² in the protein A group and 3.2±0.2 L·min⁻¹·m⁻² in the anti-IgG group (P<0.01 versus baseline, P<0.05 versus protein A; Figure 2A).

After 3 months, moreover, LVEF increased only moderately in the protein A group, from 19.8±2% to 22.6±3%
In the anti-IgG group, improvement in hemodynamic parameters was paralleled by a comparable increase in LVEF from 21.5 ± 2% to 31.3 ± 2% (P < 0.01 versus baseline, P < 0.05 versus protein A; Figure 3).

After 3 months, examination based on NYHA classification revealed improvement in the anti-IgG group (P < 0.05 versus protein A). In contrast, patients in the protein A group obtained no significant relief from symptoms.

**Bioactivity of the CE**

Compared with the protein A group, CE from patients treated with anti-IgG induced a more pronounced reduction in Ca^{2+} transients and in cell shortening of the cardiomyocytes (P < 0.01 versus protein A; Figure 4). After removal of IgG-3 by a specific column, CE from anti-IgG patients did not induce a cardiodepressant effect. On the other hand, when the IgG-3 antibodies were eluted from the specific column and superfused to cardiomyocytes, the cardiodepressant effect on Ca^{2+} transients and cell shortening was again detectable (Figure 4).

**Discussion**

An association between myocarditis and DCM has been hypothesized for a subset of DCM patients. Abnormalities of the cellular and humoral immune systems are present in these patients. A number of different autoantibodies have been identified in DCM. Elimination of antibodies by anti-IgG immunoadsorption improves myocardial function and alleviates the myocardial inflammation observed in DCM. This improvement of cardiac performance is simultaneously associated with a reduction in oxidative stress. Recent findings from in vitro analysis have demonstrated that the removal of negative inotropic autoantibodies from plasma may contribute to the acute hemodynamic effects of immunoadsorption in DCM.

Cardiac antibodies belong to the IgG fraction. IgG subclasses differ from one another immunologically and functionally. The antibodies that trigger effector functions and that are most likely to be involved in immunoregulatory activity are IgG-3 and IgG-1. Complement activation is most effective with IgG-3 and, to a lesser extent, with IgG-1. Because IgG-3 is the most active complement-fixing IgG subclass, antibodies belonging to IgG-3 may have severe
proinflammatory effects. Furthermore, IgG-3 antibodies are more efficient than IgG-1 as mediators of viral neutralization and of antibody-dependent cellular cytotoxicity. As shown for rheumatoid arthritis, IgG-3 antibodies may play an important role in autoimmune mechanisms of injury that entail inflammatory events and tissue damage.17 Furthermore, findings have disclosed the potential pathogenetic role of IgG-3 autoimmune antibodies in various connective-tissue diseases (eg, anti-neutrophil cytoplasmic antibody in Wegener’s granulomatosis).18 A recent study of DCM patients disclosed elevated levels of IgG-3 antibodies against α- and β-myosin heavy chains. The level of these antibodies correlates with the degree of left ventricular dysfunction.19 The plasma concentration of IgG-3 is low, and its half-time is shorter than that of any of the other IgG-subclasses. The pathophysiological role played by IgG-3 antibodies in DCM is still unclear. In the present study, anti-IgG immunoadsorption almost completely eliminated IgG-3 from plasma. In contrast, protein A was less effective in the reduction of the IgG-3 plasma level. Because IgG-3 levels were differentially modulated by anti-IgG and protein A immunoadsorption, we were able to ascertain the potential contribution of these antibodies to cardiac dysfunction in DCM.

The present study clearly demonstrates that the removal of antibodies belonging to IgG-1, IgG-2, IgG-4, and IgM did not significantly influence hemodynamics or LVEF. On the other hand, removal of total IgG, including IgG-3, induced acute and prolonged hemodynamic improvement, as well as an increase in LVEF. Furthermore, compared with the CE received from patients treated with protein A, CE from patients treated with anti-IgG induced a more pronounced reduction in Ca2+ transients and in cell shortening of cardiomyocytes. Additional experiments indicated that the negative inotropic effect of CE obtained from patients treated with anti-IgG was primarily mediated by IgG-3 antibodies (Figure 4). The present study therefore indicates that antibodies belonging to IgG-3 may play an important role in cardiac dysfunction.

Because the anti-IgG columns were generally oriented to total IgG, no conclusion is possible on whether the observed beneficial effects were in fact due to extraction of a specific antibody (eg, β1-receptor autoantibody). The β1-receptor autoantibody levels diminished in parallel with the reduction of IgG-3. The relative contribution of the β1-receptor antibody to cardiac dysfunction remains to be elucidated. Various findings indicate that autoimmuneologic reactions to the β1-receptor may play a pathogenic role in DCM.20 The incidence of the β1-receptor autoantibody is higher in patients with poorer left ventricular function.21 Immunization of rabbits with a peptide that corresponds to the second extracellular loop of the β1-receptor induces histopathological changes in the hearts of the immunized animals, with phenomena comparable to those found in DCM.5 Analysis of β1-receptor autoantibody measured by a bioassay system was used as a marker for autoimmuneologic reactions, which occur in a majority of DCM patients.13 and in turn, as a means of evaluating the efficacy of immunoadsorption. Additional analysis of other autoantibodies by ELISA techniques is limited, because these antibodies are detectable only in a small subgroup of patients.19,22 A large-scale study is thus necessary to clarify the role played by various autoantibodies belonging to the IgG-3 subclass in the short- and long-term hemodynamic effects of immunoadsorption.

After IgG depletion induced by immunoadsorption, IgG was substituted in both groups for safety reasons, because the risk of acute infection increases when IgG level falls below 5 g/dL.23 In addition to immunoadsorption, IgG treatment influences the immune system through various mechanisms.24 Autoantibodies are neutralized by the anti-idiotypic properties of IgG. Binding of immunoglobulins to B-cell Fc receptors decreases the production of autoantibodies. IgG induces a blockade of the reticulendothelial system. Polyclonal IgG may directly affect an infectious agent by transfer of antiviral antibodies. IgG modulates cellular immunity.24 IgG also demonstrates anti-inflammatory activities generally mediated by Fc domains.25 IgG can inhibit inflammatory cytokine production in experimental myocarditis.26 Gullestad et al27 demonstrated that IgG treatment may have beneficial effects in patients with heart failure; the improvement of LVEF correlated with changes in inflammatory and anti-inflammatory mediators. In contrast, McNamara et al28 showed that patients with acute cardiomyopathy who received IgG therapy obtained no further relief of symptoms compared with placebo. In our patients treated with protein A and subsequent IgG substitution, hemodynamic parameters did not change significantly. The data of the present study may therefore indicate that IgG substitution performed after immunoadsorption does not influence the hemodynamic parameters measured. On the other hand, IgG substitution may have contributed to the observed hemodynamic improvement after effective removal of IgG-3.

The results of the present study have revealed that protein A columns do not effectively eliminate IgG-3 when they are used in the manner described here. It is possible, however, that the efficacy of this intervention may be equivalent to that obtained by anti-IgG immunoadsorption if protein A columns are applied in a different treatment regimen (eg, longer treatment periods). Elimination of IgG-3 depends on total IgG level.11 Elimination of IgG-3 by protein A is consequently more efficient in the presence of low total IgG. During longer immunoadsorption by protein A columns, it should therefore be possible to reduce total IgG to a level at which it would likewise be feasible to effectively eliminate the IgG-3 subclass.

The present study has disclosed that IgG-3 antibodies may play a significant role in cardiac dysfunction in DCM. The precise mechanisms of preferential subclass switching are not entirely known. Cytokines are the major regulatory factors in the production of subclass-specific immunoglobulins.29 Altered levels of circulating cytokines have been described in patients with heart failure.30

Conclusions

In DCM patients, cardiac antibodies belonging to the IgG-3 subclass may play a potential role in cardiac dysfunction. We therefore hypothesize that removal of antibodies of the IgG-3 subclass may accordingly represent an essential mechanism in immunoadsorption therapy in DCM.
Acknowledgment
This study was supported in part by grant NBL3 from the German Ministry of Education and Research (BMBF).

References
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_Circulation_. 2002;106:2448-2453
doi: 10.1161/01.CIR.0000036746.49449.64
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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