Immunohistological Changes in Dilated Cardiomyopathy Induced by Immunoadsorption Therapy and Subsequent Immunoglobulin Substitution

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Background—Immunoadsorption (IA) and subsequent immunoglobulin (Ig) G substitution represent an additional therapeutic approach in dilated cardiomyopathy (DCM). It remains to be elucidated whether this treatment modulates myocardial inflammation, which is possibly a causal factor of ventricular dysfunction.

Methods and Results—From 25 DCM patients (EF <30%), 12 patients were randomized for IA therapy and subsequent IgG substitution at 1-month intervals until month 3. Before (<7 days) and after IA therapy, right ventricular biopsies were obtained from all patients. Biopsies were also obtained at intervals of 3 months from 13 patients without IA/IgG treatment (controls). IA/IgG treatment induced improvement in left ventricular ejection fraction from 21.3±1.7% (SEM) to 27.0±1.3% (P<0.01 versus baseline/controls) and reduction of the β-receptor autoantibody serum levels (P<0.01 versus baseline/controls). The number of CD3 cells decreased from 5.7±0.8 to 2.9±0.5 cells/mm² (P<0.01 versus baseline/controls). This decline was paralleled by a decrease in CD4 (P<0.01 versus baseline/controls) and CD8 (P<0.05 versus baseline/controls) lymphocytes. The number of leukocyte common antigen–positive cells (leukocytes) was reduced from 20.0±3.2 to 9.9±2.8 cells/mm² (P<0.01 versus baseline/P<0.05 versus controls). HLA class II expression decreased from 2.1±0.7% to 1.1±0.4% (P<0.05 versus controls/baseline). The number of immunopositive cells and the expression of HLA class II in controls remained stable. In both groups, the degree of fibrosis remained unchanged.

Conclusions—IA and subsequent IgG substitution mitigate myocardial inflammation in DCM. (Circulation. 2001;103:2681-2686.)

Key Words: cardiomyopathy ■ immunology

Dilated cardiomyopathy (DCM) is a myocardial disease characterized by progressive depression of myocardial contractile function and by ventricular dilatation.1 Experimental and clinical data suggest a causal relationship between myocarditis and DCM. Inflammatory processes may be involved in the pathogenesis of DCM and may represent an important factor causing progression of ventricular dysfunction. Immunohistological methods have been successfully introduced for diagnosis of myocardial inflammation, allowing identification and quantification of inflammatory cells.2-3 Infiltration with lymphocytes and mononuclear cells, as well as increased HLA-antigen expression, are frequent phenomena in DCM. These findings support the hypothesis that the immune process is still active.

Disturbances in both humoral and cellular immunity have been described in myocarditis and DCM patients.4,5 Subsequent myocardial necrosis induced by these immune reactions can result in fibrosis as a common phenomenon in DCM. The relative contributions of cellular and humoral immune disturbances to the pathogenesis and progression of DCM have not been determined. According to an experimental model of myocarditis induced by myosin, myocardial damage is mediated by T lymphocytes.6 The role of T lymphocytes in DCM is still unclear. Indices of T-cell activation have been described in a substantial proportion of DCM.4,7 A number of antibodies against cardiac cell proteins have been identified in DCM, eg, antibodies against mitochondrial proteins, contractile proteins, cardiac β₁-receptors, and muscarinic receptors.8-12 The functional role of cardiac autoantibodies is still unclear. They may reflect an inflammatory response to myocyte necrosis, thereby representing an epiphenomenon. Cardiac autoantibodies may also play an active role in the persistence of myocardial inflammation in DCM.
role in the pathogenesis of DCM, however, by initiating the disease process or contributing to the progression of myocardial contractile malfunction. If cardiac autoantibodies contribute to cardiac malfunction in DCM, their removal would be expected to improve myocardial function. Cardiac antibodies are extractable by immunoadsorption (IA)\(^\text{13-15}\). Intravenous administration of immunoglobulin (Ig) also influences the cellular and humoral immune system by different mechanisms.\(^\text{16}\)

An initial uncontrolled pilot study and a randomized study were performed to ascertain the short-term and prolonged hemodynamic effects of IA and subsequent IgG substitution in DCM patients.\(^\text{13,14}\) The cardiac index rose immediately, and systemic vascular resistance simultaneously fell. IA and IgG substitution were repeated at monthly intervals until month 3. Acute hemodynamic improvement persisted over this period.\(^\text{14}\) In contrast, the hemodynamic situation did not improve among control patients. It remains to be elucidated whether IA and subsequent IgG substitution not only improve hemodynamics but also modulate myocardial inflammation in DCM. The present study accordingly investigated immunohistological changes induced by IA therapy and subsequent IgG substitution in patients with DCM compared with controls without immunomodulatory therapy.

Methods

Study Protocol
Twenty-five DCM patients were admitted. Patients with positive evidence of the \(\beta\)-receptor autoantibody, with borderline as well as apparent signs of myocardial inflammation (lymphocytes \(>2.4\) cells/mm\(^3\)), were included. This limit was chosen because, in patients without inflammatory heart disease (coronary heart disease, valvular heart disease, and hypertrophic cardiomyopathy), the number of CD3-positive cells was \(<2.4\) lymphocytes/mm\(^3\).\(^\text{2,2}\) Twelve patients were randomized (closed-label) for treatment with IA and subsequent IgG substitution (IA/IgG group) at monthly intervals until month 3. In 13 patients (controls), conventional therapy was continued without IA/IgG treatment. Biopsies (n=5 to 8) were obtained from all patients from the interventricular septum of the right ventricle at baseline, before the study began (<7 days), and after 3 months. Right ventricular biopsies from the septum interventriculare were used instead of left ventricular biopsies to avoid risk of cerebral embolization. All patients demonstrated left ventricular dysfunction (left ventricular ejection fraction, LVEF, \(<30\%\) as assessed by 2D echocardiography), as well as symptoms of severe chronic heart failure (NYHA functional class III to IV). Coronary heart disease was excluded by angiography. Patients were excluded if they had suffered from active infectious diseases, cancer, chronic alcoholism, or heart failure of known origin (eg, primary valvular disease). All patients were treated with ACE inhibitors, digitalis, and diuretics. Twelve patients received nitrates (IA/IgG group, n=7; controls, n=5). Sixteen patients were treated with a \(\beta\)-blocker (IA/IgG group, n=8; controls, n=8). In these cases, doses of \(\beta\)-blockers had been stable for \(\geq 6\) months before the present study. All patients had received stable oral medication for \(\geq 3\) months before the study. Medication and dosage for the treatment group did not differ significantly from those for controls.

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

Immunoadsorption
Ig extraction from the plasma took place with Ig-Therasorb (Baxter), an immunoadsorber for Ig as described recently.\(^\text{13}\) In the IA/IgG group, IA was performed in 4 courses at 1-month intervals until month 3. After every final IA session, the patients received 0.5 g/kg polyclonal IgG (Venimun-N) to restore IgG plasma levels.

Clinical Findings
Echocardiographic parameters were determined in both groups by 2D echocardiography performed at baseline and after 3 months. The readings were recorded, and a reader blinded to the treatment group performed offline assessment of LVEF and left ventricular internal diameter in systole (LVIDs) and in diastole (LVIDd). LVEF was measured according to the Simpson rule. Determination of \(\beta\)-receptor autoantibodies took place as described elsewhere.\(^\text{13,17,18}\)

Histological Examination
Biopsies were fixed in 5% formaldehyde and embedded in paraffin. Slices 2 \(\mu\)m thick were cut and investigated by light microscopy in series of 8 to 10 pieces after staining with hematoxylin-eosin and for connective tissue (elastica–van Gieson). This procedure was intended to exclude myocarditis according to the Dallas criteria and to determine the degree of fibrosis, the latter by means of computer-assisted image analysis. Immunohistological stainings were done by the labeled streptavidin-biotin method. Primary antibodies were anti-CD3, -CD4, -CD8, -leukocyte common antigen (LCA), and HLA class II antigens (DP, DQ, DR). Manufacturers were Dako and Novacasta. Antibodies against LCA labeled all leukocytes, and antibodies against CD3, CD8, and CD4 labeled T lymphocytes. Immunopositive cells were counted under high-power magnification (\(\times 400\)) by 2 independent observers in blinded mode. The density of HLA class II antigen expression and the degree of fibrosis were ascertained by computer-assisted image analysis (Contron). For this purpose, the microscopic image was depicted on a 17-in screen, stored in a frame grabber, and analyzed pixel by pixel. Every pixel with color values within previously defined ranges was set as “1,” and every other pixel was “0.” The total number of “1” pixels provided exact measurement of the immunopositive/elastica–van Gieson–positive area. This area was calculated as percentage area of the biopsy plane. Each biopsy was investigated stepwise, with \(\geq 10\) steps per biopsy.

Statistics
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. For study of the lymphocytes CD3, CD4, and CD8 versus treatment groups, we performed a 2-factor ANOVA, followed by Bonferroni-Holm–adjusted post hoc analyses. Changes in NYHA classification after treatment were analyzed by singly ordered 2×4 contingency tables using the exact Kruskal-Wallis test of identically distributed rows (for treatments). Significance was assessed at the \(P<0.05\) level.

Characteristics of Patients at Baseline
The Table lists clinical characteristics, echocardiographic parameters, immunohistological findings, and \(\beta\)-receptor autoantibody levels at baseline. In the control group and IA/IgG group, the following data were comparable: age, sex, disease duration, NYHA classification, \(\beta\)-receptor autoantibody level, and immunohistochromatographic findings. LVEF, LVIDs, and LVIDd were also similar in both groups.

Clinical Findings
All patients tolerated IA and subsequent IgG substitution well. No major complications such as infection, major bleeding, or worsening of renal function occurred.
After 3 months’ evaluation of echocardiographic parameters, β-receptor autoantibody levels of the control group were comparable to those at baseline when the study began (Figure 1).

In the IA/IgG group, the β-receptor autoantibody level decreased from 4.3±0.3 to 1.1±0.4 relative units (P<0.01 versus baseline/controls). Simultaneously, LVEF increased significantly in the IA/IgG group, from 21.3±1.7% (range 15% to 29%) to 27.0±1.3% (22% to 36%) (P<0.01 versus baseline/controls) (Figure 1). LVIDs fell from 59.0±2.0 mm (50 to 72 mm) to 55.0±2.1 mm (40 to 68 mm), and LVIDd decreased from 70.1±1.9 mm (60 to 78 mm) to 67.5±1.7 mm (56 to 78 mm) (P<0.05 versus baseline). In the IA/IgG group, the mean peripheral arterial blood pressure did not change significantly during follow-up. After 3 months, examination for NYHA heart failure classification revealed improvement in the IA/IgG group (P<0.05 versus baseline/controls). In contrast, control patients obtained no relief from symptoms.

**Histological Findings**

Among control patients, the number of lymphocytes (CD3, CD4, and CD8) and of LCA-positive cells in the myocardium decreased (Figure 2A) from 5.7±0.8 to 5.4±1.2 cells/mm² (P<0.01 versus baseline/controls). The number of CD4-positive lymphocytes also decreased, from 2.3±0.4 to 2.2±0.6 cells/mm² (P<0.01 versus baseline/controls). The number of CD8-positive lymphocytes decreased from 2.8±0.5 to 2.8±0.5 cells/mm² (P=0.8 versus controls). In the IA/IgG group, the mean peripheral arterial blood pressure did not change significantly during follow-up. After 3 months, examination for NYHA heart failure classification revealed improvement in the IA/IgG group (P<0.05 versus baseline/controls). In contrast, control patients obtained no relief from symptoms.

**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. Immunological findings support the hypothesis that the immune process is still active in DCM patients. Immunoadsorption treatment induced a reduction of cardiotropic β-receptor autoantibodies, as shown by follow-up of the β-receptor autoantibody level. β-Receptor autoantibodies modulate the inotro-
pic responsiveness of β-agonists. These antibodies are capable of attenuating the positive stimulatory effect induced by agonists. In the absence of agonists, however, antibodies induce a positive chronotropic effect in neonatal cardiomyocytes. In the present study, analysis of β-receptor autoantibody was used as a marker for autoimmunological reaction, which occurs in 70% to 90% of DCM patients, and in turn, as a means of evaluating the efficacy of IA. Because the IA columns used in the present study were oriented to IgG in general, no conclusion is possible on whether the observed beneficial effects were due to extraction of a specific antibody (eg, β-receptor autoantibody).

A number of different cardiac autoantibodies have been identified in patients with myocarditis and DCM. It is unclear, however, whether these antibodies are directly pathogenic or whether they merely represent humoral markers of autoimmunity. In vitro data indicate a negative effect on cardiac performance for certain antibodies. A DCM model was created by immunizing rabbits with peptides of either β1-adrenoceptors or M2 muscarinic receptors. High titers of anti-peptide antibodies were found in the sera. Both groups of immunized rabbits demonstrated heart alterations similar to those found in human DCM. If cardiac autoantibodies in fact play a role as causal agents in initiating or triggering the disease process, their elimination or blockade would be expected not only to improve myocardial function but also to alleviate the myocardial inflammation observed in DCM.

After plasma IgG depletion induced by IA, IgG was substituted for safety reasons, because the risk of acute infection increases when the IgG level falls below 5 g/dL. In addition to IA, IgG treatment also influences the immune system through various mechanisms. Autoantibodies are neutralized by anti-idiotypic properties of intravenous IgG. Furthermore, binding of anti-idiotypic immunoglobulins to B-cell Fc receptors decreases the production of autoantibodies in B cells and thereby prevents a possible rebound phenomenon after IA. Finally, IgG modulates cellular immunity and cytokine metabolism. IgG treatment and IA have been used successfully for treatment of various autoimmune diseases. In addition, IgG substitution after IgG depletion induced by different methods (eg, plasmapheresis) represents a therapeutic option for various autoimmune diseases.

Treatment with IA/IgG has induced improvement in cardiac function, as revealed in the follow-up in LVEF, and may therefore represent an additional beneficial therapeutic approach in patients with DCM. The present study also demonstrates that IA/IgG therapy induces humoral and cellular alterations of the inflammatory process in the myocardium of DCM patients. Large-scale studies are necessary to clarify the immunohistochemical markers that may predict the efficacy of IA/IgG treatment.

IA/IgG therapy may have influenced cytokine metabolism. Cytokines are recognized as essential markers of immune responses in heart failure. Tumor necrosis factor-α (TNF-α) is able to depress myocardial contractility. As previously shown during IA and subsequent IgG therapy, there were no significant alterations in blood serum levels of different proinflammatory cytokines. This result concurs with a previous report on the relevance of TNF-α and TNF-α I and II receptors in decompensated heart failure and on the effects of clinical interventions on short-term elaboration of this cytokine. Despite clinical improvement, the cited report noted no alteration of high peripheral blood levels of TNF-α. IA/IgG therapy, however, may have influenced the concentration of cytokines in myocardial tissue. We were not able, however, to perform measurements of tissue concentration of cytokines because of the limited amount of myocardial tissue obtained by biopsies.

The present study successfully excluded the possibility that a decrease in inflammatory cells reflects the natural course of disease, because patients of the control group with similar clinical characteristics demonstrated no change in inflamma-
tion. It likewise proved possible to exclude an influence of medical treatment as a cause of these immunohistological alterations, because the patients in the medical-treatment group were stable during the study and because both groups were comparable in this regard. The study disclosed that various histological changes in long-lasting DCM are reversible alterations, eg, infiltration by lymphocytes or increased expression of HLA class II antigen. The reduction in autoantibody level may contribute to the histological alterations observed.

In the present study, a decrease in T lymphocytes was observed in myocardial tissue, a consequence of reduction in CD4 and CD8 cells. Referenced to findings before IA/IgG therapy, the number of leukocytes also decreased. In myocardial tissue of DCM patients, an increased number of leukocytes is associated with altered activation of heart-tissue T cells. This finding suggests a direct role of infiltrating leukocytes in the pathogenesis of DCM.\(^9\) Experimental data likewise suggest that inflammation is primarily involved in the disease process of autoimmune myocarditis and DCM: in autoimmune myocarditis induced by myosin, myocardial damage is mediated by T lymphocytes and is strictly dependent on class II antigens of the major histocompatibility complex.\(^6\) In addition, the impairment of left ventricular function in patients suffering from myocarditis can be transferred to mice by transfer of blood leukocytes.\(^30\)

In addition to the reduction of inflammatory cells, IA/IgG treatment induced a decline in expression of HLA class II antigens. Expression of class II antigens of the major histocompatibility complex plays an important role in regulation of immune responses, because HLA class II antigens can present peptides to T cells. Furthermore, susceptibility to development of DCM is associated with specific HLA alleles.\(^31\) Kühl and Schulteiss\(^32\) also demonstrated that treatment with 6-methylprednisolone induces reduction of lymphocytic infiltration and HLA class II antigen expression in the myocardium of DCM patients with increased CD3 cell count.

Reduction of fibrosis was observed in patients with end-stage heart failure who were treated with an assist device.\(^33\) The degree of fibrosis remained stable during the present study. In addition, changes have been described in collagen type I/III ratio in biopsies from patients suffering from DCM.\(^34\) Our study did not investigate various collagen contents, because biopsies are frequently too small to allow investigation more extended than that involving the parameters analyzed.

**Study Limitations**

The present study discloses immunohistological changes during IA/IgG therapy in patients suffering from a long-lasting DCM. The number of patients included was too small to assess the important question of whether IA/IgG therapy influences viral elimination. Alterations of a possible virus infection during follow-up were not analyzed because enteroviruses are typically detected in only 26% of DCM cases.\(^35\) Furthermore, large-scale studies are necessary to evaluate the relative contributions of IA and IgG treatment to the observed effects.

**Conclusions**

In DCM patients, myocardial inflammation involving both the cellular and the humoral immune systems can be influenced by IA and subsequent IgG substitution. This therapeutic approach significantly ameliorates the inflammatory process in myocardial tissue and stabilizes myocardial function.

**References**


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