Autoimmune Mechanisms Underlying Dilated Cardiomyopathy

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Autoimmune abnormalities, as well as viral infection and genetic abnormalities, appear to be major predisposing factors for dilated cardiomyopathy (DCM). Abnormalities of cell-mediated immunity are mainly involved in the onset of cardiomyopathy secondary to myocarditis. However, various antimyocardial antibodies are detected in the serum of patients with DCM. The appearance of these antibodies was considered to be an epiphenomenon associated with myocyte injury resulting from myocarditis, but recent findings have suggested that at least some of them are directly related to the pathophysiology of DCM. In particular, an autoantibody targeting the \( \beta_1 \)-adrenergic receptor that exhibits an agonist-like effect is related to the persistent myocardial damage resulting in DCM and provides substrates for fatal ventricular arrhythmias. In addition, an antibody for the muscarinic M2 receptor is related to atrial fibrillation, an antibody targeting Na-K-ATPase is closely related to sudden cardiac death as a result of fatal ventricular arrhythmias, and an autoantibody for troponin I increases the L-type calcium current and is related to the myocardial damage. Based on these findings, immunoabsorption therapy was developed to remove such autoantibodies in patients with refractory heart failure as a result of DCM. (Circ J 2009; 73: 602 – 607)

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Cardiomyopathy used to be a chronic disease of unknown etiology, and its clinical outcome was extremely poor. According to data compiled by the Idiopathic Cardiomyopathy Survey and Research Group in 1982, the 5-year and 10-year survival rate of dilated cardiomyopathy (DCM) patients with marked contractile dysfunction was as low as 54% and 36%, respectively. According to the epidemiological survey done in 2002, the estimated number of patients with DCM, hypertrophic cardiomyopathy, and restrictive cardiomyopathy in Japan was 17,700, 21,900, and 300, respectively. According to a recent outcome survey, the 5-year survival rate of DCM patients was 78.6%, which was higher than in the previous survey. This change was mostly ascribable to advances in pharmacotherapy, such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and \( \beta \)-blockers, but the beneficial effect of increased awareness (namely “early detection/early treatment”) cannot be overlooked. In recent years, progress in studies on the etiology of DCM has shown that viral infection, genetic abnormalities and autoimmune mechanisms are 3 major causes of this disease. As a result, the term “idiopathic” was deleted from the ISFC/WHO Diagnostic Criteria in 1995. This fact still clearly remains in our mind. According to the classification proposed by the American Heart Association Committee in 2006, cardiomyopathy is divided into 3 categories, which are “hereditary,” “mixed,” and “acquired.” DCM is classified as “mixed.” The present article focuses on autoimmune abnormalities as one of the 3 major causes of DCM, and provides an outline of their involvement in the pathophysiology of DCM.

Abnormalities of Cell-Mediated Immunity

In acute myocarditis, viral antigens are presented on major histocompatibility antigen (MHC) class I molecules, and infected cells are damaged by perforin that is produced by CD8+ T cells. Various peptides of the damaged target cells are presented by MHC class II molecules, and then prolonged immune response is induced via CD4+ T cells. Cytokines that play an important role in this process are classified as Th1 cytokines, including interleukin-2, interferon-\( \gamma \), and tumor necrosis factor-\( \alpha \), and Th2 cytokines that include interleukin-4, -5, -6, and -13. Some auxiliary signals via costimulatory molecules are also involved along with these immune responses. In studies of myosin-sensitized autoimmune myocarditis models, myocarditis could be transferred to severe combined immunodeficiency mice by T cells, but not by the antimyosin antibody. Furthermore, myocarditis could not be prevented by depletion of CD8+ T cells, but was prevented by depletion of CD4+ T cells. It was also possible to prevent myocarditis with MHC class II molecule inhibitors. From these results, it might be concluded that the autoimmune mechanism underlying myocarditis involves cellular immune responses, with MHC class II-CD4+ T cell pathway playing a pivotal role, whereas humoral immunity is not important. In addition to antigen-presenting cells, programmed death (PD)-1 receptors are present in cardiac endothelial cells, among which PD-L1 suppresses the cell-mediated immune response in acute myocarditis? Abnormalities of cell-mediated immunity are also observed in DCM. It has been reported that suppressor T cells are decreased and helper T cells are increased in DCM patients, whereas increased MHC class II expression has been noted in studies of myocardial biopsy specimens.
Abnormalities of Humoral Immunity

Various antimyocardial antibodies can be detected in the serum of DCM patients. A combination of the indirect immunofluorescent antibody, immunoblotting, and enzyme-linked immunosorbent assay (ELISA) techniques can detect some type of autoantibody in sera from approximately 85% of DCM patients. It has been suggested that these autoantibodies are associated with myocyte injury resulting from myocarditis, with no causal association with the pathogenesis. On the contrary, there is some evidence suggesting that these aberrations in humoral immunity play a role in mediating pathophysiology of DCM. One of them is the role of the presence of antymyosin antibody in patients with chronic myocarditis. The left ventricular ejection fraction (LVEF) was found to improve spontaneously after 6 months in patients without this antibody, whereas cardiac dysfunction persists in those who were positive for the antibody. Another piece of evidence is the finding that detection of antimyocardial antibody by the indirect fluorescence technique is useful for predicting the progression to DCM in asymptomatic relatives of DCM patients. Antimyocardial antibody was detected in 188 (32%) out of 592 asymptomatic relatives of DCM patients, and the detection rate was higher in the group with a positive family history. During a 5-year follow-up period, progression to DCM or related conditions was noted in 26 out of 311 relatives. The antimyocardial antibody-positive rate was higher in the group showing progression to DCM than in the group without progression (69% vs 37%). Cox proportional hazards analysis has also confirmed that positivity for this autoantibody is a useful predictor of progression to DCM. These findings suggest that detection of the autoantibody reflects the process of persistent myocardial damage associated with DCM, but still do not confirm direct involvement of autoantibodies in the pathogenesis of DCM. However, various basic experiments have shown that at least a couple of autoantibodies exhibit biological activity in vivo and in vitro. Therefore, the possibility has been suggested that at least some antimyocardial antibodies play a role in the development of DCM. Furthermore, antimyocardial antibody is also detected in patients with hypertrophic cardiomyopathy.

Antibodies Targeting the β1-Adrenergic Receptor

In 1989, Limas et al reported that a substance inhibiting the binding of radioactive ligands to membrane β-adrenergic receptors on rat myocardial cells was detected in the serum of DCM patients. Because its effects were inhibited by antihuman IgG serum, this substance was considered to be an autoantibody. Magnusson et al demonstrated that an autoantibody targeting the second extracellular loop of the β1-adrenergic receptor affected ligand binding to C6 glioma cells and itself bound to the membranes of cardiomyocytes, as shown by immunohistological staining of human myocardial tissue. They also showed that this autoantibody increases the beating rate of neonatal rat cardiomyocytes, but unlike the response to isoproterenol, this positive chronotropic effect shows little evidence of desensitization. However, prolonged exposure to this autoantibody for 72 h was able to decrease the β1-adrenergic receptor protein and mRNA content.

Cardiac dysfunction mimicking cardiomyopathy can be recapitulated by repeated immunization with peptides corresponding to the second extracellular loop of this receptor. After 6 months of immunization, we observed concentric hypertrophy accompanied by diastolic dysfunction and uncoupling of the β-adrenergic receptor. When Lewis rats were immunized with the peptides for 12 months, cardiomyopathy mimicking DCM was elicited. In studies using SWXJ mice, the acute effect of this autoantibody on β-adrenergic receptor signaling was assessed in more detail. It was found that this autoantibody increases the number of high-affinity binding sites for the β-receptor. When it was added to isolated cardiomyocytes, intracellular calcium transients increased and contraction was enhanced. The activity of protein kinase activated by cyclic AMP (PKA) was also increased. Apoptosis of myocardial cells was also noted. This apoptosis was inhibited by β-adrenergic receptor blockers, cyclic AMP inhibitors, PKA inhibitors, and L-type calcium channel blockers, but it was not prevented by calcium calmodulin kinase II inhibitors. After adding this autoantibody, caspase-3 activity was increased, and apoptosis was inhibited by Z-VAD-FMK (a caspase inhibitor). These results indicate that the autoantibody targeting the β1-adrenergic receptor induces apoptosis via β1-adrenergic receptor activation.

Experiments on antibody transfer to healthy animals have been performed to assess the in vivo influence of this autoantibody. However, because healthy animals show a normal immune response, it was found that only non-specific immune reactions were caused by autoantibody transfer. Therefore, congenital severe combined immunodeficiency mice or recombination activating gene 2 (Rag2) knockout mice were used for such purposes. Inherently, these mice cannot produce mature T cells and B cells. When the IgG fraction purified from immunized rabbits with the peptides corresponding to the second extracellular loop of the β1-adrenergic receptor was transferred to Rag2 knockout mice, the fractional shortening of the left ventricular internal dimensions was decreased after 3 months. The left ventricular end-diastolic pressure was increased, whereas dp/dt under dopamine challenge was lower than in the control group. Apoptosis was also noted in the cardiac tissue of autoantibody-treated mice. This phenomenon was accompanied by activation of caspases 3, 9, and 12, as well as enhancement of endoplasmic reticulum stress markers, including an increase of 78kDa glucose regulated protein and C/EBP-homologous protein.

Because the autoantibody targeting the second extracellular loop of the β1-adrenergic receptor exhibits an acute agonist-like effect, as mentioned above, a method of autoantibody detection utilizing this effect has been reported. With this method, the autoantibody is detected from the change of fluorescence intensity after adding the target IgG fraction to HEK293 cells expressing the cyclic AMP fluorescence sensor-transfected human β1-receptor. Autoantibodies detected by this system are classified as showing high and low cyclic AMP signals. When their neutralizing effect was investigated using peptides corresponding different portions of the β1-adrenergic receptor, it was confirmed that the epitope was the second extracellular loop for high cyclic AMP signal and the first extracellular loop for the latter autoantibody, respectively.

What is the clinical significance of these autoantibodies in DCM patients? This autoantibody was detected in 26% of DCM patients with advanced heart failure, and the incidence of its detection was related to the severity of cardiac failure. Both the LVEF and the cardiac output were lower in
the autoantibody-positive group than in the autoantibody-negative group. Cox proportional hazards analysis showed that positivity for this autoantibody was useful in predicting the overall death rate and cardiovascular death rate in DCM patients, in addition to the pulmonary wedge pressure and cardiac index, which are well-known conventional predictors of death. This autoantibody was detected in 13% of patients with heart failure of ischemic etiology, but was not useful as a predictor of death. Unfortunately, analysis of the cause of death was not performed in this study. We detected this autoantibody in 38% of 104 mildly symptomatic DCM patients, including those in New York Heart Association class I. There was no difference of cardiac function between the autoantibody-positive and autoantibody-negative groups, but the frequency of non-sustained ventricular tachycardia was higher in the positive group. Cox proportional hazards analysis showed that the presence of this autoantibody was an independent predictor of sudden death.

Several reports have been published that indicate a role of this autoantibody in the onset of ventricular arrhythmias. When the β1-adrenergic receptor antibody purified from DCM patients was added to neonatal rat ventricular myocytes, the repolarization phase of the action potential was prolonged. This change was accompanied by an increase of the L-type calcium current and was inhibited by β-blocking effect of bisoprolol. In rabbits that were chronically immunized with peptides corresponding to the second extracellular loop of the β1-adrenergic receptor, the repolarization phase of the action potential was also prolonged and the frequency of ventricular tachycardia increased, whereas the transient outward potassium current and delayed rectifier potassium current were decreased. Remodeling of the ion channels expressed by myocytes was thought to occur in the chronically immunized model, resulting in modification of the action potential, especially the potassium current. Thus, it has been shown that autoantibody-mediated activation of the β-adrenergic receptor not only induces persistent myocardial damage following myocarditis but also provides substrate for fatal ventricular arrhythmias resulting in sudden cardiac death.

Antibody Targeting the Muscarinic M2 Receptor

The presence of this autoantibody modifies the binding of radioactive ligands to the muscarinic M2-acetylcholine receptor, and the ligand binding is inhibited by atropine. In guinea pig myocardial cells, this autoantibody inhibited the increase of the L-type calcium current and prevented action potential prolongation induced by isoproterenol. We detected this autoantibody in 40% of 104 DCM patients. Incidence of atrial fibrillation was higher in the autoantibody-positive group than in the autoantibody-negative group. Multivariate analysis showed that the presence of this autoantibody was a predictor of atrial fibrillation. After adding the IgG fraction purified from autoantibody-positive patients to fertile chicken eggs, there was an occurrence of the premature supraventricular contractions. These results suggest that autoantibodies targeting the muscarinic M2 receptor can trigger atrial fibrillation in DCM patients. This autoantibody is also detected in patients with sick sinus syndrome.

Antibody for Na-K-ATPase

We detected an autoantibody targeting Na-K-ATPase during the survey for autoantibodies using Western blot analysis of the serum from DCM patients. Autoantibody detected by this method at approximately 100kDa corresponded with a polyclonal antibody for the α-subunit of Na-K-ATPase. In addition, the band at this region observed in the presence of autoantibody-positive sera from DCM patients was absorbed by pretreatment with Na-K-ATPase. In the presence of this autoantibody, porcine cerebral Na-K-ATPase activity was reduced and 3H-ouabain binding affinity was reduced. Therefore, it was presumed that this autoantibody binds to the α-subunit (the catalytic subunit of Na-K-ATPase) and decreases Na-K-ATPase activity. By inhibiting Na-K-ATPase activity, long-term exposure to such an autoantibody might cause chronic intracellular calcium overload. When rabbits were immunized repeat-
edly with this peptide, cardiac hypertrophy was induced as was observed in the $\beta$-adrenergic receptor autoimmunization models. Although inhibitory guanine nucleotide binding protein expression was increased in both types of immunization models, a decrease of the cardiac-specific isoform $\alpha$3-subunit of myocardial Na-K-ATPase was noted in the Na-K-ATPase autoimmunization model, but not in the $\beta$-adrenergic receptor autoantibody model, suggesting that cardiac phenotype induced by chronic immunization does not appear to be a non-specific change during the process of cardiac hypertrophy. When 104 DCM patients were screened for this autoantibody by ELISA, it was detected in 26% of them. The incidence of ventricular premature contractions and non-sustained ventricular tachycardia was higher in the group positive for this autoantibody than in the negative group. Cox proportional hazards analysis showed that the presence of this autoantibody was the most powerful predictor of sudden death among variables including a decrease of the LVEF.

**Antibody for Troponin I**

BALB/c mice develop cardiomyopathy mimicking DCM in the absence of the PD-1 receptor, which inhibits proliferation of T and B cells and downregulates the immune system. An autoantibody targeting troponin I was detected in the sera of these mice. When the antibody for troponin I was added to isolated myocardial cells, the L-type calcium current was increased. When this antibody was administered repeatedly to healthy mice for 12 weeks, DCM-like lesions were reproduced. After 270 days of immunization of A/J mice with troponin I and an adjuvant, DCM-like changes were also induced along with troponin I autoantibody production. On the contrary, repeated immunization with troponin T also induced antibody production, but not myocardial lesions. One of the reasons for this difference is that troponin T is located exclusively in the cytoplasm, whereas troponin I is also expressed on the cell membrane surface.

**Autoantibody Removal by Immunoadsorption Therapy**

Based on these results of basic and clinical studies related to antimyocardial autoantibodies in DCM patients, we set out to attempt the clinical application of immunoadsorption therapy for severe heart failure due to DCM in January 2006. The principle of this therapy involves the separation of plasma using a plasma separation device, after which a harmful substance in the blood is removed by a column containing an antibody to the target substance (Figure 1). This therapy has already been successfully applied for familial hypercholesterolemia, Guillain-Barré syndrome, myasthenia gravis, and systemic lupus erythematosus, etc. In 1996, Wallukat et al in Germany applied this therapy to patients with DCM for the first time, treating 8 DCM patients by removal of an autoantibody to the $\beta$1-adrenergic receptor. As the antibody titer decreased, symptoms and cardiac function both improved. Subsequently,
this therapy has been applied to approximately 200 patients, mainly using columns containing sheep anti-IgG antibody and protein A. Sheep anti-IgG column has safety problems as a result of antigenicity. Although IgG subclass 3 plays a key role in autoantibody-mediated cardiotoxicity (Figure 2), the effect of protein A column to remove IgG is subclass-non-specific. In Japan, high-profile tryptophan column, which has low antigenicity and high specificity for IgG-3, is available for clinical application.

We have performed this therapy on 10 patients with refractory heart failure as a result of DCM in our institution (Table). In the first 2 patients, it was done once a week for a total of 3 sessions. Once safety was confirmed, it was done 3 times a week for a total of 5 sessions from the third patient onward. Although evaluation of the chronic effect of this therapy on cardiac function has not been established, the plasma BNP concentration was reduced by 50% after treatment (Figure 3). In one patient that was followed up for 3 months after completion of this therapy, the LVEF increased from 10% to 24%, and an effect was still noted at 6 months after treatment. The plasma BNP concentration was 608 pg/ml before immunoadsorption therapy, whereas it was approximately 100 pg/ml after treatment. A young male patient recovered from prolonged administration of intravenous drugs for refractory heart failure after receiving this therapy. We used a tryptophan column for relatively selective removal of the important autoantibody-containing IgG3 fraction (Figure 4). Therefore, at the completion of treatment, immunoglobulin supplementation is not required. Long-term effects on cardiac function, morbidity and mortality await further investigation. More importantly, it is imperative for us to develop some effective screening methods to identify responders.

**Conclusion**

Although significant progress has been achieved in the treatment of heart failure as a result of cardiomyopathy, it is a still matter of fact that there are many patients with refractory heart failure who do not respond despite every available treatment. It is important to develop some alternative therapies for such patients in our country where there are severe limitations of heart transplantation because of donor supply shortage. Certain autoantibodies, including those targeting β1-adrenergic receptor, muscarinic M2-acetylcholine receptor, Na-K-ATPase, and troponin I, play a pivotal role in mediating pathophysiology of DCM. We expect further progress and the development of treatments...
targeting autoimmune mechanisms that might lead to a breakthrough in the present situation for patients with refractory heart failure because of DCM.

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