Heartache of Fc Receptors*

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Idiopathic dilated cardiomyopathy (DCM) is characterized by the progressive depression of cardiac function and left ventricular dilation in the absence of coronary artery disease. There is evidence to suggest that humoral immunity plays an important role in the pathogenesis of DCM. In particular, the role of autoantibodies in the pathogenesis of DCM has been emphasized (1). Sera from patients with DCM show a variety of autoantibodies, including those against membrane proteins, mitochondrial proteins, heat-shock proteins, and myocyte structural sarcolemmal proteins. Because all these antibodies are not present in each patient with DCM, it is apparent that all these autoantibodies are not pathogenic in DCM. To date, only 2 autoantibodies have satisfied the criteria of cause-and-effect relationship for DCM, and both these antibodies display stimulatory activities. The first comes from a report that program death receptor-1 (PD-1) mice develop autoantibodies against cardiac troponin 1 (cTpn 1) and dilated heart, and second from the finding that the injection of autoantibodies against cTpn-1 from these mice into normal mice induces dilation and dysfunction of the heart in recipient mice (2), therefore establishing a direct role of anti-cTpn-1 antibody in dilatation and dysfunction of heart resembling human DCM.

Because antibodies to cTpn-1 are present in approximately 15% of patients with DCM (3), it suggests that additional autoantibodies and/or mechanisms may be responsible for DCM. Second, autoantibody, which satisfies the cause-and-effect relationship, is an autoantibody against β1-adrenergic receptor. Mice immunized with a synthetic peptide corresponding to the sequences of the second extracellular loop of β1-adrenergic receptor developed both stimulatory β1-adrenergic receptor antibody and development of disease resembling DCM. Isogenic transfer of stimulatory anti–β1-adrenergic receptor antibodies from cardiomyopathic mice into healthy inbred mice reproduced the disease (4). Because β1-adrenergic receptors are ubiquitously expressed, why other tissues/organ are not affected remains an enigma. The role of autoantibodies in the pathogenesis of DCM is supported further by effectiveness of removal of autoantibodies by immunoadsorption on both cardiac functions and clinical improvement in patients with DCM (5,6).

How these autoantibodies induce changes characteristics of DCM remains unclear; antibody-mediated complement-dependent cytotoxicity and apoptosis of cardiomyocytes have been suggested (7). Because β1-adrenergic receptor antibody increases voltage-dependent calcium flux, an increase in free intracellular calcium may trigger apoptosis in cardiomyocytes via the endoplasmic reticulum-mitochondrial pathway (8,9). Because anion nucleotide transporter (ANT) is a component of mitochondrial transport pore, which plays a role in apoptosis, and ANT autoantibodies are present in patients with DCM, it would be of interest to determine whether anti–ANT antibodies are present in subgroup of patients with DCM who are positive for β1-adrenergic receptor antibodies. Antibody-induced apoptosis of cardiomyocytes may be one of the mechanisms for pathogenesis of DCM because there is very little inflammatory response in DCM, a characteristic of apoptosis.

In this issue of the Journal, Staudt et al. (10) have presented evidence to suggest that binding of autoantibody to both cardiac antigen and to the Fcγ receptor IIa (CD32a) plays an important role in the pathogenesis of DCM. In the first set of experiments, they demonstrated an involvement of F(ab')2 of immunoglobulin (Ig)G in inducing negative isotropic effect. They showed that intact IgG from sera of patients with DCM, and not from healthy controls, induced a negative inotropic effect in rat cardiomyocytes, whereas F(ab')2 fragments of patient IgG did not produce a negative inotropic effect, suggesting that F(ab')2 fragments alone were not sufficient to induce negative inotropic effects. However, an involvement of the F(ab')2 fragment from patient IgG was substantiated by experiments, which demonstrated that preincubation of cardiac myocytes with patient IgG F(ab')2 and not from control F(ab')2 blocked the negative inotropic effect of patient’s intact IgG.

In the second set of experiments, they demonstrated a role Fc of IgG in inducing negative inotropic effect. They demonstrated that preincubation of cardiomyocytes with Fc fragments of normal IgG blocked the negative inotropic effect of intact IgG of DCM, suggesting that the Fc portion of IgG antibodies is involved. To further demonstrate that both Fab and Fc portion of IgG were involved, authors “reconstituted” Fc by sequential incubation of cardiomyocytes with F(ab')2 of DCM IgG, followed by intact goat anti-human F(ab')2 IgG, and demonstrated that “reconstituted” Fc regain the negative inotropic effect of F(ab')2. In contrast, preincubation with control F(ab')2 IgG and then reconstitution with goat antihuman F(ab')2 IgG had no effect. These experiments do not exclude a possibility that

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the negative inotropic effects were caused by crosslinking of F(ab')2. F(ab')2 fragments of goat antihuman F(ab')2 IgG (devoid of Fc) and CD32a knock out by small interference RNA, would establish whether both binding to cardiac antigen (via Fab) and CD32a are required for autoantibody-induced negative inotropic effects.

In the last set of experiments, authors examined the expression of different Fc receptors and observed the presence on CD32a and none other receptors. Therefore, they concluded that CD32a plays an important role in autoantibodies-mediated pathogenesis of DCM.

A role of Fc receptors in autoimmunity has been supported by knock out and transgenic experiments (11). Eight Fcγ receptors have been defined in humans, including 3 high-affinity and 5 low-affinity receptors (12). All these Fcγ receptors, with the exception of Fcγ receptor IIb (CD32b), have intracytoplasmic immunotyrosine activation motif (stimulatory receptors); CD32b containing immunotyrosine inhibitory motif (inhibitory receptor). Although monomeric IgG may bind to CD32a, it does not induce any signal, and only immune complexes when interacting with CD32a induce activating signals. However, in the study by Staudt et al. (10), no immune complexes were used to induce signals via CD32a. They also have reported that in DCM autoantibodies of IgG3 subclass induces negative inotropic effects (13). Because the IgG3 subclass has a greater tendency to aggregate (simulate immune complexes), a possibility of aggregation of intact IgG and Fc fragments from DCM should be ruled out. It is intriguing how CD32a with an ITAM motif provides a negative signal. Both anti-cTpn and anti-β1-adrenergic antibodies induce positive signals. Staudt et al. (10) have demonstrated the presence of CD32a in cardiomyocytes from DCM and the lack of CD32b, which mediates inhibitory signal. However, there are certain limitations to their studies of CD32. First, CD32 antibodies used are raised against extracellular domain of CD32, which has a high degree of homology between CD32 subtypes and, therefore, does not distinguish between CD32a and CD32b. Second, because no studies were performed with cardiomyocytes from a healthy heart, it is unclear whether the density of CD32b is reduced in cardiomyocytes from DCM and, thus, not detected by a relatively insensitive technique of immunofluorescence microscopy. Therefore, studies should be performed with cardiomyocytes from a healthy heart and from DCM with antibodies that distinguish CD32a from CD32b. In addition, the use of more sensitive techniques, including flow cytometry and real time PCR, should be used.

Several studies have reported beneficial effects of high-dose intravenous immunoglobulin (IVIG) on cardiac functions in patients with dilated cardiomyopathy (14–16). However, underlying mechanisms remain unclear. Larsson et al. (15) reported that the beneficial effect of IVIG on cardiac functions in DCM is not caused by neutralization of anti-β1 adrenergic receptor antibody (anti-idiotypic effect). Gullestad et al. (16) observed an induction of anti-inflammatory mediators interleukin–10, interleukin–1 receptor antagonist, and soluble tumor necrosis factor receptors in the IVIG-treated group and none in the placebo group in patients with DCM. Because one of the major mechanisms of beneficial effects of IVIG in autoimmune diseases is via stimulation of CD32b (12), it is crucial that the expression of CD32b in cardiomyocytes be investigated in detail.

In summary, certain autoantibodies appear to play a pathogenic role in patients with DCM. Several mechanisms of autoantibody-mediated dysfunction/damage to cardiac myocytes, including complement-mediated cytotoxicity and apoptosis, have been reported (7). Staudt et al. (10) have provided data in support of a role of Fcγ receptors in DCM. Although there are limitations with their investigation, the study of the role of Fc receptors by Staudt et al. (10) should encourage investigators to explore this novel pathway to understand the pathogenesis of DCM and other autoimmune cardiac diseases. It is important that the patients with DCM be subclassified according to the presence of pathogenic antibodies and that these pathogenic antibodies should be used in the study of the mechanisms of DCM and as a target for removal by immunoadsorption using specific columns. It is critical that the entire protein, rather than a peptide, be used in immunoadsorption columns because the epitopes recognized by autoantibodies may be different in different patients. Furthermore, multicenter double-blind control-placebo trials of high-dose IVIG should be initiated and perhaps compared with that of immunoadsorption. Because the disease can be transferred by lymphocytes (17), anti-CD20 antibody treatment (to remove autoreactive B cells), similar to other autoimmune diseases, may be effective in treating DCM and other autoimmune myocarditis. It is likely that immunoadsorption/IVIG/anti-CD20 monoclonal antibodies therapy would replace cardiac transplantation, at least in a subset of patients with DCM, where autoantibodies are demonstrated to be pathogenic.

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