Commentary

Cardiomyopathy Is Linked to Complement Activation

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Cardiomyopathies are traditionally divided into dilated cardiomyopathy (DCM), restrictive cardiomyopathy, and hypertrophic cardiomyopathy. DCM is characterized by depressed systolic function and an increase in the size of the left ventricle usually involving dilatation of all four chambers.¹ DCM represents a common cause of congestive heart failure and frequently leads to cardiac transplantation.² Approximately 30 to 40% of patients with DCM have a familial form of the disease involving mutations in genes coding for cytoskeletal and sarcomeric proteins.^{2,3} The remaining cases are idiopathic, many of which are believed to be due to preceding myocarditis, particularly virus-induced. The detection of a cardiotropic virus, such as coxsackievirus and adenovirus, in endomyocardial biopsy specimens from patients with DCM supports this hypothesis.^{4,5} The presence of a virus in the myocardium has been shown to be associated with a poorer prognosis in DCM.^{6,7}

Animal Models

The ability of cardiotropic viruses to cause myocarditis and subsequently trigger an autoimmune response to autologous cardiac tissue has been demonstrated in animal models.^{8–10} In susceptible mouse strains such as BALB/c, coxsackievirus B3 (CB3) induces myocarditis which consists of an early, viral phase followed by a late, autoimmune phase.¹⁰ On the other hand, C57BL/6 mice develop only early, viral disease, suggesting the importance of the genetic constitution of the host in determining the progression from an infectious to an autoimmune process. In BALB/c mice, the autoimmune phase of myocarditis (which typically occurs 28–56 days after infection) is characterized by the absence of infectious virus in the heart; however, the viral genome may persist in a small proportion of cardiomyocytes.⁹ Similar disease can be induced in mice by murine cytomegalovirus¹¹ and encephalomyocarditis virus,¹² also leading to a late, autoimmune phase, in BALB/c but not in C57BL/6.

Many cases of myocarditis in humans have been associated with autoantibodies to cardiac myosin (CM).^{13,14} CM immunization of mice induces autoimmune myocarditis,¹⁵ which resembles both human autoimmune myocarditis and the autoimmune phase of murine virus-induced myocarditis. Interestingly, mice that are susceptible to CB3-induced myocarditis also exhibit susceptibility to CM-induced disease. Similarly, mice that are resistant to CB3-induced myocarditis demonstrate resistance in the CM model. The advantage of the CM-induced model is the ability to study the effects of different interventions on a purely autoimmune process without the associated complication of a viral infection. Moreover, CM immunization might provide a better model for those myocarditides that are not associated with viral infections, such as giant cell myocarditis.

Similar to humans, mice with myocarditis can progress to DCM and end-stage heart failure. Some mice, either infected with a cardiotropic virus or immunized with CM, develop enlarged hearts with dilated left and right ventricular cavities.^{12,16} Such morphological presentation of DCM is accompanied by impairment of cardiac function. Echocardiographic examination reveals increased left-ventricular chamber dimensions, decreased fractional shortening, and global left-ventricular wall hypokinesis, classic hallmarks of DCM.¹⁷ Pressure-volume relations obtained by means of left-ventricular catheterization further confirm the diagnosis of DCM in a subgroup of mice immunized with CM (Figure 1). These mice demonstrate increased left-ventricular volumes, decreased end-systolic pressure, decreased cardiac output, pronounced depression of systolic function manifested by reduced slope and rightward shift of the end-systolic pressure-volume relation (ESPVR), reduced maximal rate of pressure development (dp/dt_{max}), and reduced stroke work-end diastolic volume relation. Systolic dysfunction is accompanied by impairment of diastolic function with significantly increased passive stiffness (β), prolonged time constant of pressure relaxation (τ), decreased peak filling rate, and increased end-diastolic pressure

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Figure 1. DCM in murine model of experimental autoimmune myocarditis. Multiple pressure-volume loops were derived by acutely reducing end-diastolic volume through transient occlusion of the inferior vena cava. **Top:** Normal mouse heart and corresponding pressure-volume relations. **Bottom:** A heart and pressure-volume relations represent day 32 after immunization with CM. DCM is manifested by the enlarged cavity of the left ventricle. The pressure-volume relations demonstrate large volumes, reduced end-systolic pressure, depressed and rightward shifted end-systolic pressure-volume relations (ESPVR), and reduced stroke volume (the width of a loop). The line connecting the upper left corners of the loops represents ESPVR. The slope of this line represents a load-independent measure of systolic function.

(Figure 1).¹⁷ These functional changes are virtually identical to human DCM. Thus, experimental autoimmune myocarditis in the rodent provides an excellent model to study both immunological and hemodynamic aspects of inflammatory heart disease, including DCM.

Complement: An Overview

Complement (C), or alexin, was first described in the second half of the 19th century as a heat-labile component of blood with bactericidal and hemolytic properties.¹⁸ Currently, complement is known to represent a complex system consisting of over 30 proteins.¹⁹ These proteins interact with one another to initiate and propagate a cascade of enzymatic activation leading to the non-enzymatic assembly of the membrane attack complex on the surface of target cells causing cell lysis. Three modes of complement activation have been described: the classical, lectin, and alternative pathways. The classical pathway, the first to be described, is initiated by IgM or IgG antibody bound to its cognate antigen. It is now known that some other substances, including C-reactive

protein (CRP),²⁰ can also initiate classical pathway. The cascade starts with activation of the C1 complex. Antibody, or sometimes other substances, binds to C1q and induced its conformational change leading to subsequent activation of the two enzymes, C1r and C1s; the latter then sequentially cleaves C4 and C2. The lectin pathway, the last to be described, is initiated in an antibody-independent fashion by mannose and N-acetyl glucosamine residues present abundantly in bacterial cell walls.²¹ These residues are recognized by mannose binding lectin (MBL), which has a structure similar to that of C1q and which activates two associated serine proteases, MASP-1 and MASP-2. These proteases, which are homologous to C1r and C1s, cleave C4 and C2. Thus, MBL and the associated proteases represent a C1-like complex that initiates events similar to that of the classical pathway. Other lectins can trigger complement activation in a fashion similar to MBL.

Both the classical and lectin pathways result in cleavage and activation of C3 and, at this step, they converge with the alternative pathway. Antibody-independent alternative pathway does not involve C1, C2, or C4, but starts

with an activation of C3. This activation step is a result of spontaneous hydrolysis of the thioester in C3 producing C3b. C3b may deposit on host cells, but this deposition normally does not lead to further activation of the complement cascade because factor B does not bind to C3b and surface-bound C3b is degraded by factors I and H. However, C3b deposition on microorganisms or foreign cells favors binding of factor B and initiates subsequent events in the cascade leading to cleavage of factor B by factor D, formation of C3 convertase (C3bBb) and further cleavage of C3. The classical and lectin pathways involve C4b2a as C3 convertase, which acts like C3bBb. All three pathways result in cleavage and activation of C5 involving somewhat different C5 convertases. Cleavage of C3 and C5 produces the anaphylatoxins, C3a and C5a, which act as strong chemoattractants. C5a is the more potent chemoattractant, recruiting neutrophils, monocytes, basophils, and eosinophils, whereas C3a acts mainly on mast cells and eosinophils.¹⁸ Engagement of the C5a receptor on monocytes activates NF- κ B and triggers production of interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor (TNF)- α .^{19,22–25}

All pathways may eventually lead to the assembly of the membrane attack complex. C5b combines sequentially with terminal complement components, C6, C7, C8, and C9, resulting in a formation of a pore in the plasma membrane of a susceptible target cell. If sufficiently high densities of membrane attack complex are assembled on the cell surface, the cell is lysed. However, the terminal complement complex does not typically lyse allogeneic cells because of the presence of complement regulators, such as protectin (CD59) on the surface of these cells. In this case, sublytic amounts of membrane attack complex induce activation of the target cells.¹⁹ The liver represents the primary source of circulating complement components. Other sources of certain complement components include monocytes, macrophages, fibroblasts, endothelial cells, mucosal epithelial cells, and adipocytes.^{26,27} A simplified diagram of the three complement pathways is provided in Figure 2.

The Role of Complement in Heart Disease

Early Complement Components

The role of the adaptive immune system in the development of experimental autoimmune myocarditis has been extensively studied. It is now clear, however, that mediators generated during the innate immune response modulate the initiation of the subsequent adaptive immune reaction. Components of the complement system provide an important link between the innate and adaptive immune systems. Regardless of the pathway, activation of the complement cascade results in the formation of the active products of C3 cleavage. To better understand the role of complement system in the development of inflammatory heart disease, studies were performed addressing the effects of C3 on the autoimmune response to CM and subsequent development of disease in a murine model of CM-induced myocarditis.²⁸ C3 de-

Figure 2. Pathways of complement activation and effects of membrane attack complex on cardiomyocytes. Enzymatic cleavages and activation are indicated by **thick arrows**. fB, factor B; fD, factor D. C3 and C5 convertases are underlined.

pletion through activation by cobra venom factor resulted in impaired IgG antibody responses to CM and prevention of myocarditis. It was critical to deplete C3 at the time of initiation of the immune response since multiple injections of cobra venom factor between days 1 and 9 after immunization, but not between days 10 and 18, were effective in preventing myocarditis. Blockade of the C3 receptors, complement receptor 1 (CR1), or CD35, and complement receptor 2 (CR2), or CD21, with a monoclonal antibody (mAb) that binds to the extracellular domain shared by the two receptors, led to the abrogation of disease and dramatically reduced the production of CM-specific IgG. CM immunization of mice genetically deficient in both CR1 and CR2 further confirmed that these complement receptors are required for the development of autoimmune myocarditis. These studies involved A/J mice, which harbor a spontaneous deletion in the gene encoding C5 rendering them deficient in the functional C5 protein.²⁹ Due to this deficiency, A/J mice enable the separation of the effects of early complement components from those exerted by the membrane attack complex, C5b-9. Binding of the products of C3 cleavage, C3d in particular, to CR1 and CR2 has been shown to be important for antigen presentation to B cells and subsequent B cell activation and antibody production.³⁰ Additionally, it was demonstrated that these receptors are present on a subset of activated/memory

CD44^{high}CD62L^{low} T cells and their engagement triggers T cell responses.²⁸ T cell expression of CR1/CR2 was required for optimal up-regulation of CD69 or CD25 in response to *in vitro* stimulation with antigen in the presence of either macrophages or B cells.²⁸ Recently, Pratt and colleagues³¹ further confirmed the observation that murine T cells up-regulate CR1/CR2 on activation and that these receptors are important for effective T cell responses. They found that CR1/CR2-expressing T cells belong to CD4⁺ population, whereas CD8⁺ T cells do not express these receptors.

In addition to being involved in exacerbation of myocarditis, complement mediates myocardial damage associated with other heart diseases, such as myocardial infarction. It has been shown that complement deposits on injured cardiomyocytes within the infarcted area.³² The pathogenic role of complement in mediating ischemic injury and increasing infarct size has been demonstrated in a number of experimental studies.^{33–38} CRP, which activates complement through initiation of the classical cascade by binding to C1q,¹⁹ has been shown to colocalize with complement in infarcted myocardium.³² Griselli and colleagues³⁹ have demonstrated that CRP-induced complement activation contributed to myocardial damage in a rat model of acute myocardial infarction. Furthermore, complement has been implicated as a mediator of reperfusion injury.^{40,41}

Membrane Attack Complex C5b-9

In this issue of *The American Journal of Pathology*, Zwaka and colleagues⁴² explore the role of the terminal membrane attack complex of complement, C5b-9, in the development of DCM. They report that myocardial biopsy specimens from patients with DCM stained positively for IgG, C5b-9, and TNF- α by immunohistochemistry. The diagnosis of DCM was based on clinical and echocardiographic features of heart failure unrelated to coronary artery disease, valvular or congenic defects, and supported by histological evidence of myocardial fibrosis, infiltration, hypertrophy, and myocyte degeneration. Control specimens, obtained from patients with similar clinical and echocardiographic manifestations but no histological evidence of myocardial disease, were negative for IgG, C5b-9, or TNF- α . The authors further demonstrated that the amount of C5b-9 found in the myocardium correlated with TNF- α production. They suggest that sublytic amounts of C5b-9 attached to the surface of cardiomyocytes trigger intracellular signaling which leads to TNF- α production thereby promoting the development of cardiomyopathy and heart failure. To test this hypothesis, the authors undertook a series of *in vitro* experiments using rat cardiomyocytes and found that C5b-9 induces both mRNA and protein synthesis of TNF- α . Using the electrophoretic mobility shift assay, Zwaka and colleagues also observed that C5b-9 induces NF- κ B activation, an important mediator of TNF- α gene expression.

The importance of TNF- α in heart disease has been demonstrated in a number of animal models as well as in clinical studies.^{43,44} Being a potent proinflammatory cy-

tokine, TNF- α is involved in the development of inflammatory processes in the heart leading to myocarditis and its sequelae, such as DCM. Using a murine model of CM-induced myocarditis, Smith and colleagues⁴⁵ demonstrated that anti-TNF mAb reduced the severity of myocarditis and Bachmaier and colleagues⁴⁶ showed that mice genetically deficient in the TNF receptor subunit p55 are resistant to the induction of the disease. The ability of splenocytes from immunized mice to produce TNF- α on *in vitro* stimulation correlates with the severity of CM-induced myocarditis.⁴⁷ The pathogenic role of TNF in the development of cardiac inflammation has also been confirmed in CB3-induced murine myocarditis.^{48,49} In addition to its proinflammatory effects, TNF- α exerts direct negative inotropic effects and promotes cardiomyopathy.^{50–52} Patients with congestive heart failure have increased levels of circulating TNF- α and greater expression of this cytokine in the myocardium.⁵³ Increased myocardial expression of TNF- α has been observed in response to increases in wall stress caused by either volume or pressure overload.^{54,55} Chronic volume unloading by means of a left ventricular assist device in patients with congestive heart failure significantly reduced TNF- α levels in the myocardium as assessed by immunostaining of myocardial samples.⁵⁶ Similarly, decreasing the intraventricular pressure gradient with septal reduction therapy in patients with obstructive hypertrophic cardiomyopathy resulted in a significant decrease in TNF- α levels in the myocardium.⁵⁷ Reducing TNF- α levels with either a nonspecific agent, pentoxifylline, or the recombinant TNF- α receptor fusion protein, etanercept, has been successful in improving symptoms in patients with heart failure.^{58–60} Thus, the finding by Zwaka and colleagues⁴² that C5b-9 induces TNF- α production in cardiomyocytes provides an important link between complement activation and cardiac dysfunction in DCM.

The role of sublytic C5b-9 in producing acute metabolic and proinflammatory changes in target cells has also been studied in disorders other than heart muscle disease. The proinflammatory effects of the sublytic C5b-9 have been implicated in the pathogenesis of atherosclerosis and transplant rejection.^{19,27,61–64} C5b-9 has been shown to promote vascular injury and inflammation by activating vascular smooth muscle and endothelial cells. In response to C5b-9, endothelial cells release von Willebrand factor (vWF), P-selectin, and CD63 from the Weibel-Palade granules, produce IL-8, monocyte chemoattractant protein-1, fibroblast growth factor, and platelet-derived growth factor.^{19,65,66} Additionally, the terminal complement complex potentiates TNF- α -induced up-regulation of E-selectin and ICAM-1 on the surface of endothelial cells.⁶¹ These events lead to leukocyte adhesion, platelet aggregation, and endothelial cell injury, thereby facilitating the formation of atherosclerotic lesions and allograft rejection. Sublytic amounts of C5b-9 also induce proliferation of vascular smooth muscle cells resulting in neointimal thickening and vascular remodeling, characteristic of both atherosclerotic lesions and accelerated graft arteriosclerosis.⁶² The importance of the late complement components in mediating both

acute and chronic allograft rejection has been demonstrated using C6-deficient rats.^{27,64} Viedt and colleagues⁶³ demonstrated that C5b-9 induces IL-6 production in vascular smooth muscle cells. Consistent with the observation by Zwaka and colleagues⁴² that C5b-9 induces NF- κ B in cardiomyocytes, the authors also found that C5b-9 signaling triggers activation of NF- κ B. Viedt and colleagues⁶³ further found that NF- κ B activation was required for IL-6 production, since blocking NF- κ B activity either with its pharmacological inhibitor, pyrrolidine dithiocarbamate (PDTCT), or with decoy oligonucleotides completely inhibited IL-6 release. NF- κ B is a dimeric transcription factor formed by hetero- or homodimerization of protein members of Rel family, which include p65, RelB, cRel, p50, and p52.⁶⁷ The authors demonstrated that p65 and p50 subunits participated in DNA binding on induction by C5b-9. Furthermore, C5b-9 has been shown to activate NF- κ B in endothelial cells leading to production of IL-8 and monocyte chemoattractant protein-1.⁶⁶ These findings suggest that C5b-9-induced NF- κ B activation represents a general phenomenon independent of the target cells.

Concluding Remarks

Evidence has been rapidly emerging that inflammation and its mediators are involved in the pathogenesis of a number of heart diseases and facilitate the deterioration of heart function. Inflammatory mediators are capable of altering cardiomyocyte homeostasis leading to the activation of multiple signaling cascades, ultimately affecting myocardial function. The role of complement and C5b-9 in particular has been extensively studied in models of atherosclerosis and transplant rejection. Very little data, however, existed about the role of the terminal complement complex in inflammatory heart disease. The findings described by Zwaka and colleagues⁴² in this issue are the first demonstration of the ability of C5b-9 to induce NF- κ B activation and TNF- α production in cardiomyocytes. These findings draw our attention to the importance of complement in DCM and provide a basis for new therapies targeting components of the complement system that may one day be included as a part of the usual regimen in the fight against the devastating and usually fatal course of heart failure.

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