Commentary

Cardiac Myosin and the TH1/TH2 Paradigm in Autoimmune Myocarditis

Madeleine W. Cunningham

*From the Department of Microbiology and Immunology,
University of Oklahoma Health Sciences Center, Oklahoma
City, Oklahoma*

Myocarditis and dilated cardiomyopathy may represent acute and chronic stages of a progressive organ-specific autoimmune disease of the myocardium.^{1–3} Myocarditis is an inflammatory disease of the heart that is characterized by a cellular infiltrate in the myocardium, and dilated cardiomyopathy is a chronic heart muscle disease characterized by ventricular hypertrophy.^{1–4} Dilated cardiomyopathy is a primary cause of severe heart failure with subsequent transplantation or death within several years after diagnosis.^{1,5} The direct result of cellular infiltration of the myocardium is necrosis and loss of myocytes leading to the development of contractile dysfunction and ventricular dilatation. The loss of myocytes and formation of scar tissue in the myocardium would lead to loss of contractile function and ventricular enlargement. In a small percentage of individuals, loss of myocyte function and development of dilated cardiomyopathy can result from mutations in or viral proteolytic digestion of dystrophin or dystrophin-associated glycoproteins that lead to cytoskeletal disruption and loss of overall contractile function in the heart.^{4,6–8}

However, the most common cause of myocarditis is viral infection. An exhaustive list of myocarditis-inducing agents including microbial pathogens and toxins is provided by Brown and O'Connell² in a review of myocarditis and dilated cardiomyopathy. Among the most common viral causes of human myocarditis are coxsackieviruses.^{2,9–11} Mouse models of coxsackievirus-induced myocarditis were studied by Woodruff and colleagues^{12–14} who demonstrated that lymphocytes from mouse models of myocarditis could destroy cardiomyocytes in culture. Gauntt and colleagues¹⁵ also investigated the role of virulent myocarditic *versus* nonvirulent amyocarditic coxsackieviruses in mouse models of myocarditis. In their studies, there appeared to be several stages of disease in their mouse model. The first early stage of viral replication and cell lysis in the heart showed no evidence of heart failure or cellular inflammation, however, with the onset of specific immune responses, cellular infiltration of the myocardium was observed in sus-

ceptible mice. The cellular infiltrates in the myocardium were minimal to severe and led to the loss of myocytes by necrosis. The development of dilated cardiomyopathy is thought to represent the third and more chronic stage of heart disease that develops after the cellular inflammation.¹⁶ It would follow that once the damage has been done to the cardiomyocyte function either by elimination of large numbers of myocytes because of inflammation, necrosis, and scarring or by disruption of the cytoskeleton so that the myocyte is dysfunctional, then anti-inflammatory therapy would not be effective. Anti-inflammatory therapies would only be efficacious during the time of inflammatory onset. In fact, immunosuppressive therapy was not effective in the Myocarditis Trial.² By the time myocarditis symptoms present, many cases may have already advanced to a stage in which necrosis and scarring have already led to ventricular dilatation and contractile dysfunction. For comparison, in rheumatic carditis, the valve is insidiously and permanently damaged by autoimmune attack after streptococcal pharyngitis. In rheumatic carditis, valve injury may not be evident until a heart murmur is perceived that reflects the scarred valve. In myocarditis, the end-stage of disease may represent a physical, structural defect as a result of autoimmune-mediated damage during the inflammatory stage of the disease.

Mechanisms in the Pathogenesis of Myocarditis

Cardiac Myosin and Infection: the Mimicking Autoantigen

Although infectious pathogens, including viruses,¹⁷ group A streptococci,¹⁸ or chlamydia,¹⁹ are important

Supported by grants HL35280 and HL56267 from the National Heart Lung and Blood Institute of the National Institutes of Health.

Accepted for publication May 2, 2001.

Address reprint requests to Madeleine W. Cunningham, Ph.D., George Lynn Cross Research Professor, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Biomedical Research Center—Room 217, 975 NE 10th St., Oklahoma City, OK 73162. E-mail: madeleine-cunningham@ouhsc.edu.

etiological agents of inflammatory heart disease, immune and specifically autoimmune mechanisms are the major effectors of pathogenic injury.² The autoantigen most often associated with both myocarditis and rheumatic carditis is cardiac myosin.^{18,20–27} In 1985, myosin was identified as an autoantigen involved in cross-reactivity between the group A streptococcus and heart.²⁸ Since this time, evidence has supported the molecular mimicry hypothesis that streptococcal M protein and the group A carbohydrate both induce anti-myosin responses that attack the heart.^{22,29–33} In fact, immunological mimicry was demonstrated between streptococcal M protein and myocarditic coxsackieviruses and was linked to cytotoxic antibody against heart cells as well as T lymphocyte responses.^{32,34–36} Interestingly, a myocarditic peptide of streptococcal M protein that mimics cardiac myosin tolerized and protected MRL/++ mice against autoimmune myocarditis after coxsackieviral infection.³⁷ Nevertheless, it is debated as to whether or not mimicry between coxsackieviruses and cardiac myosin plays a role in the pathogenesis of myocarditis.^{38,39}

There is strong evidence that cardiac myosin is a dominant autoantigen in autoimmune myocarditis²³ and viral-induced myocarditis.²⁵ Cardiac myosin-induced myocarditis histologically resembles the viral-induced disease. Investigators have demonstrated that myosin-induced myocarditis can be adoptively transferred by CD4⁺ T lymphocytes.²⁷ Although CD4⁺ lymphocytes can transfer disease, it has been shown that autoimmune myocarditis can occur in mice lacking CD4 or CD8 molecules,⁴⁰ and that myocardial infiltrates consisted of T cells that were double-negative T cells with $\alpha\beta$ TCR. In addition to T cells, passive administration of anti-myosin monoclonal antibody was found to induce myocarditis in DBA/2 but not BALB/c mice because of the presence of myosin or a myosin-like protein in the extracellular matrix of DBA/2 mice.⁴¹ Gauntt and colleagues^{42–44} investigated the relationship between coxsackievirus and myosin and suggested that molecular mimicry between myosin and coxsackieviruses may play a role in myocarditis. Anti-coxsackieviral-neutralizing antibody produced myocardial inflammation in mice.⁴² Therefore, both antibody and T cells may contribute to the pathogenesis of inflammatory myocardial lesions. Susceptibility to anti-myosin antibody-induced myocarditis was dependent on the strain of mice. For example, different pathogenic mechanisms have been reported in DBA/2 (antibody-mediated disease) and BALB/c (T-cell-mediated) mouse strains.^{41,45} Susceptibility may be related to genetic factors including target organ sensitivity or influences such as infection and polarizing TH1/TH2 cytokines. Antibodies against myosin were elevated after coxsackieviral infection of susceptible mouse strains.²⁵ In rheumatic carditis, infection plays a major role as well as anti-myosin antibody^{46,47} that has been shown to be cytotoxic for heart cells in culture and to recognize cell surface cross-reactive antigen laminin on the valve and myocyte cell surface.^{29,30} In rheumatic carditis, antibodies deposit in myocardium as well as valvular endothelium^{30,48,49} and T cells infiltrate the valve.⁵⁰ There have been a number of studies that have demonstrated anti-cardiac myosin or

anti-heart antibodies in acute and chronic myocarditis^{51–54} and soluble interleukin-2 levels were correlated with disease severity and cardiac autoantibodies.⁵⁵ Thus, in inflammatory heart diseases, myocarditis and rheumatic carditis, both antibody and T cells are implicated in the disease.

Susceptibility to Myocarditis in Rodent Models

Using rodent models, the role of cardiac myosin as an autoantigen in the pathogenesis of autoimmune myocarditis has been well established^{23,24,26,27,56–58}. Myocarditis can be induced by cardiac myosin in A/J mice,^{23,27} BALB/c mice,^{56,59} and in Lewis rats.^{24,58} However, C57BL/6 mice are resistant to myosin-induced myocarditis. Induction of coxsackievirus-induced myocarditis is seen in a similar group of mouse strains including A/J and A.SW,^{25,60} C3H,¹⁵ and BALB/c and DBA/2.⁴⁵ BALB/c mice that have disruption of the gene for the negative immunoregulatory receptor PD-1 develop dilated cardiomyopathy with diffuse deposition of IgG throughout the heart and on the surface of cardiomyocytes. Autoantibodies in the disease model reacted with an unidentified 33-kd heart-specific protein.⁶¹

Exposure of cardiac myosin in the heart may be an important event leading to the onset of disease in the susceptible host.⁶² Evidence has shown that in normal myocardium myosin-class II major histocompatibility antigen complexes are present before the induction of autoimmune myocarditis.⁶³ Induction of myocarditis is seen only with cardiac myosin and not skeletal myosin²³ or other α -helical coiled-coil proteins such as tropomyosin (Galvin and Cunningham, unpublished data). Streptococcal M protein mimics cardiac myosin sequences or epitopes and immunization with the M protein or peptides leads to myocardial and valvular inflammatory heart lesions in BALB/c mice and Lewis rats.^{22,31} Therefore, it would seem that the α -helical structure is not the critical factor in breaking self tolerance, but that unique and/or cryptic epitopes present in cardiac myosin are the decisive factor. In fact, unique epitopes within cardiac myosin have been described to produce myocarditis. Myocarditis was induced by amino acid residues 334 to 352, located in the S1 region of A/J mouse cardiac myosin,²⁶ residues 736 to 1032 in BALB/c cardiac myosin,⁵⁹ acetylated residues 614 to 643 of rat cardiac myosin produced disease in BALB/c mice,⁵⁶ residues 1070 to 1165 of porcine cardiac myosin induced disease in Lewis rats,⁵⁷ residues 1107 to 1186 in the Lewis rat,⁶⁴ and acetylated Lewis rat LMM region residues 1539 to 1555.⁵⁸ Our studies in Lewis rats (Galvin et al, manuscript in preparation) suggest that epitopes within the LMM region produce valvulitis whereas the most severe myocarditis is produced by an epitope within the S2 region of cardiac myosin (Cunningham and Li, unpublished data). A diagram of the myosin molecule and its fragments is shown in Figure 1. It has been proposed that only self epitopes would induce autoimmune myocarditis, however, it was reported that both murine and porcine cardiac myosins produced the same disease immunologically (IgG sub-

Diagram of the myosin molecule

Figure 1. Diagram of the myosin molecule illustrating the proteolytic fragments of myosin. S1 contains the globular portion of the molecule and the ATPase activity, whereas the α -helical coiled-coil rod region is comprised of two fragments, S2 and light meromyosin (LMM). S2 is considered to be the hinge region and the LMM the rigid tail region of the molecule.

class antibody, cytokines, and cell adhesion molecules) and histopathologically in genetically susceptible mice.⁶⁵ The high degree of amino acid sequence similarity among the cardiac myosins would seem to be the reason that both porcine and mouse molecules produce a similar disease in the mouse. Cross-reactive epitopes of cardiac myosin that are recognized by TCR in the thymus with moderate affinity may allow self-reactive T cells to escape deletion. T cells that respond to self epitopes must then be controlled in the periphery.⁶⁶ Removal or inactivation of regulatory T cells can lead to the breakdown of self tolerance. Both regulatory T cells that are identified by cell surface markers and T cells of the TH1/TH2 subsets secreting distinct cytokine patterns that demonstrate effector functions and cross inhibition play important roles in immunoregulation and development of autoimmune disease.⁶⁶ The role of epitopes in cytokine production and TH1 and TH2 lymphocyte selection is debatable. Studies are investigating whether cytokines mediate the suppression of regulatory T cells or influence the development of regulatory T cells.⁶⁶ Investigation of $\gamma\delta$ T cell subsets in coxsackieviral myocarditis has shown that $V\gamma 1+$ T cells suppress myocardial inflammation whereas the $V\gamma 4+$ subset promoted myocarditis.⁶⁷ The $V\gamma 4$ subset was found to produce interferon (IFN)- γ and promote TH1 responses.

Influence of Cytokines on Myocarditis and TH1/TH2 Immune Responses

Infection is a strong inducer of either TH1 or TH2 cytokines (Table 1), depending on the type of infectious pathogen, such as intracellular microorganisms,^{68–71} viruses,¹⁷ extracellular bacteria and superantigens,^{72,73} or parasitic microbes.^{74–81} Infection as well as genetic predisposition may play an important role in the cytokine phenotype expressed and the subsequent onset of autoimmune myocarditis.

Pathogenic mechanisms in myocarditis may be related to specific cytokine production in a particular animal strain or individual and participate in onset and further development of myocarditis. Cytokines are important in controlling T cells responsive to self antigens and are critical in shifting the immune response toward a TH1 or a TH2 pattern.^{82,83} Recent evidence suggests that both B and T cells are involved in polarized cytokine production⁷¹ and $CD4^+$ and $CD8^+$ T cells as well as natural killer and dendritic cells may also be involved in production of polarizing cytokines. The TH1 response shifts the cytokine profile toward delayed hypersensitivity, macrophage activation, and a proinflammatory T cell response associated with IFN- γ and interleukin (IL)-12, whereas the TH2 response is associated with B cell activation and humoral immunity, and IL-4, -5, -10, and IgE production. IL-12 has been shown to induce the differentiation of TH1 autoreactive T cells and to enhance autoimmune disease in certain animal models.⁸⁴ TH1 T cells secrete IL-2 and IFN- γ that suppresses TH2 responses, whereas TH2 T cells secrete IL-4 and IL-10 that inhibit TH1 responses.⁸⁵ Previous studies in the Lewis rat model of cardiac myosin-induced myocarditis have shown that cardiac myosin-sensitized T lymphocytes could transfer myocarditis to naïve recipient rats more effectively when cultured *in vitro* with IL-12, whereas IL-2 was less effective,⁸⁶ and IL-12 induced IFN- γ production in lymph node cells. The $CD4^+$ T cells were found to be required for the transfer and infiltration of the myocardium, whereas $CD8^+$ T cells were not required to cause myocarditis in the Lewis rat model of myosin-induced myocarditis.⁸⁶ In addition, the study found that IL-12 (p40) mRNA was expressed by macrophages infiltrating the heart.⁸⁶ In addition, administration of IL-12 enhanced myocarditis in the rat. Thus, autoimmune myocarditis in the Lewis rat model was promoted by a TH1 response.⁸⁶

Table 1. Table Illustrating the TH1/TH2 Paradigm Indicating the Characteristics of TH1 and TH2 T Cell Subsets

TH1	TH2
Activate macrophages (delayed-type hypersensitivity) IL-12 and IFN- γ Induces TH1 Subset Secrete IFN- γ and IL-2 that inhibit TH2 IgG 2a (mice), IgG1, IgG3 (humans)	Activate B cells (humoral immunity) IL-4 induces TH2 subset Secrete IL-4 and IL-10 which inhibit TH1 IgE, IgG1 (mice), IgG4 (humans)

Recent evidence suggests that both B and T cells are involved in polarized cytokine production⁷¹ and $CD4^+$ and $CD8^+$ T cells as well as natural killer and dendritic cells may also be involved in production of polarizing cytokines.

However, it is important to understand that pathogenic mechanisms among the susceptible animal models of myocarditis may not all be identical. In the A/J and BALB/c strains studied by Afanasyeva and colleagues⁸⁷ in this issue of *The American Journal of Pathology*, the TH2 response is favored. In BALB/c mice, IL-4 renders T cells unresponsive to IL-12.⁸⁸ The study by Afanasyeva and colleagues in this issue of the *American Journal of Pathology* describes the role of TH2 cytokines, IL-4 in particular, in the development of myocarditis. The importance of TH1/TH2 immune responses is the power of specific cytokines to drive the immune response in an individual or particular animal model toward or away from disease. In the myocarditis model described in A/J mice, anti-IL-4 treatment markedly reduced myocarditis presumably by switching the cytokine production from a TH2 to TH1 profile that would be a switch in cytokine production from IL-4 to up-regulation of IFN- γ . The TH1 phenotype is associated with production of IFN- γ and TH1 T cell development, activation of macrophages, induction of delayed-type hypersensitivity, and production of IgG2a subclass in mice (IgG1 and IgG3 in humans).

However, in the TH2 phenotype, IL-4 is produced with activation of B cells, allergic reactions, and the production of IgG1 in mice and IgG4 in humans and IgE in both. The work describes A/J mice (and BALB/c mice as well), which are susceptible to cardiac myosin-induced myocarditis, to have eosinophils in the cellular infiltrate in the myocardium as well as giant cells within the heart lesions. The presence of eosinophils and giant cells were presented as suggestive histopathological evidence for a TH2 response in the myocarditis in A/J mice. Eosinophils are well known to be associated with production of cytokines IL-4, IL-5, IL-10, and IgE.⁸⁹⁻⁹¹ In 1988, a report demonstrated that IL-4 induced formation of giant multinucleated cells in culture.⁹² However, it is well known that giant multinucleated cells are associated with granulomatous lesions formed in response to intracellular bacteria such as *Mycobacterium tuberculosis*,⁹² and it may be possible that giant cell formation occurs under either TH1 or TH2 conditions. Granulomatous lesions contain macrophages/monocytes from which the multinucleated giant cells form.⁹² In the pattern of granulomatous lesions, lymphocytes are recruited and surround the macrophages/monocytes. Granulomas containing activated macrophages are the immune response against intracellular bacteria and part of the response to TH1 cytokines such as IFN- γ . Organ-specific autoimmune diseases have been attributed to TH1 mechanisms and tissue damage that could be down-regulated by switching the autoimmune disease model to TH2 cytokine production.^{82,83} The role of IFN- γ in progressive autoimmune disease may be related to its effect on increasing uptake and presentation of self epitopes in target organs.⁹³ As described by Falcone and Sarvetnick,⁸³ the classical inflammatory pathway is characterized by production of the cytokines IL-1, tumor necrosis factor (TNF), and free radicals that are induced in activated macrophages by IFN- γ and inhibited by IL-4. An alternative nonclassical inflammatory cytokine pathway is exhibited by IL-4 that can induce macrophage activation and enhance phago-

cytosis as well as expression of MHC class II molecules.^{83,94} In addition, the timing of cytokine production may enhance or abrogate autoimmune disease. Large amounts of IL-12 or TNF produced early in disease may lead to progression of autoimmunity whereas their production later in disease could institute terminal differentiation and death of T cells and abrogation of disease.⁸³ Therefore, IFN- γ can be protective against autoimmune disease in certain instances.⁸³ Regulatory T cell subsets may require IFN- γ to maintain their down-regulatory mechanisms.^{95,96}

In the A/J mouse, a TH2 pathogenic mechanism accounted for severe myocarditis that correlated with eosinophilic infiltrates, giant cell formation, and IgG1 and IgE antibody responses against myosin. Abrogation of myocarditis was observed when anti-IL4 was administered suggesting that TH2 immune responses were very important in development of myocarditis in the A/J mouse model of myosin-induced myocarditis. Administration of anti-IFN- γ exacerbated disease demonstrating a protective effect by IFN- γ . A/J mice that are known to produce strong TH2 responses are partially deficient in IL-12 and develop asthma. Myocarditis in humans is less well understood compared to animal models, but could result from either a TH1 or a TH2 response. The A/J mouse model of TH2-mediated disease is an excellent example of how IL-4 can mediate myocarditis and IFN- γ can protect against disease. Therefore, modulation of the TH1/TH2 cytokines as a therapy for myocarditis will require careful evaluation for it to be safe and efficacious in humans. It is possible that the different stages of myocarditis may be associated with different cytokine profiles.

In previous studies of coxsackievirus-induced myocarditis, IL-1 and TNF- α have been shown to promote myocarditis in myocarditis-resistant C57BL6 congenic mice, indicating that the immune system in resistant mouse strains can be provoked to generate an autoimmune response against the heart.⁹⁷ In A/J mice, monoclonal antibody neutralization of TNF- α reduced the severity of myocarditis whereas anti-IFN- γ increased the severity of disease.⁹⁸ TNF- α is believed to be produced by heart-infiltrating macrophages in the rat model of myocarditis and to be important in cardiovascular diseases.⁹⁹ It has been reported that the lack of TNF- α receptor (TNF-R) p55 gene expression could interfere with either lymphocyte activation or target organ susceptibility.¹⁰⁰ In fact, A/J mice lacking TNF-R p55 are susceptible to *Listeria* infections,¹⁰¹ but were protected from cardiac myosin-induced myocarditis.¹⁰⁰ TNF- α when administered to animals induces MHC class II molecule expression.¹⁰² In animals lacking the TNF-R p55, no MHC class II expression was observed after cardiac myosin injection as compared with wild-type animals expressing the TNF-R and MHC class II molecules.¹⁰⁰ These data link the TNF-R with myocarditis and MHC class II expression in the myocardium.

Recently, a study of a transgenic mouse model of IFN- γ expression in the pancreas indicated that the IFN- γ protected the mice against lethal coxsackie virus infection and subsequent myocarditis.³⁹ In this model, IFN- γ

expression was limited to the pancreas and was not expressed in the heart.³⁹ The protective mechanism proposed was that viral infection in the heart was subdued, and therefore, myosin was not released from the lysis of infected myocytes and induction of autoimmune heart disease would not occur. Protection was attributed to the antiviral effect of IFN- γ . In the study of the transgenic animals, viremia was reduced in the heart.³⁹ Other factors besides reduction of viremia may play a role in protection against viral-induced autoimmune myocarditis. In the study reported herein, in the absence of viral infection but in the presence of immunization with cardiac myosin, IFN- γ protected against myocarditis presumably by controlling the expression of IL-4 by T cells. The myocardial cytokine environment and the release of myosin in the heart may be two very important factors in generation or prevention of myocardial inflammatory disease. Cardiac myosin epitopes presented in the heart may lead to a loss of tolerance against cardiac-specific cryptic epitopes in the presence of threshold concentrations of cytokines. In the myocarditis models, it will be important to evaluate the local cytokine environment in the heart during the different stages of myocarditis and compare it with circulating and inducible cytokines in the spleen.

Genes in Myocarditis

To understand the multiple factors involved in the onset and progression of myocarditis, studies are beginning to determine some of the genes that are involved in causing the inflammatory and autoimmune disease state in myocardium. It is clear that structural genes are involved in hereditary forms of cardiomyopathy and have been documented in a review by Towbin and colleagues.⁴ Mutations in genes encoding sarcomeric proteins including cardiac myosin heavy and light chains, cardiac tropomyosin, cardiac troponins, and myosin-binding protein C have been associated with familial hypertrophic cardiomyopathy.⁴ Overexpression of calcineurin, a calcium-regulated phosphatase, can in transgenic mice lead to cardiac hypertrophy and dilated cardiomyopathy.¹⁰³ Mutations in dystrophin, dystrophin-associated glycoproteins, and actin lead to dilated cardiomyopathy.⁴ The role of the coxsackieviral 2A protease in cleavage of dystrophin leads to dilated cardiomyopathy.^{6,7} Abnormalities of the cytoskeletal proteins lead to dilated cardiomyopathy.

Obviously, expression of cytokine genes in transgenic mice lead to either enhancement or protection against myocarditis. Other genes that have been reported to be involved in inflammatory myocarditis include Fas ligand when expressed in the heart led to a mild inflammatory infiltrate¹⁰⁴ and apolipoprotein J/clusterin that was found to limit the severity of murine autoimmune myocarditis presumably by binding immunoglobulin and complement and protecting cardiomyocytes from injury.¹⁰⁵ Disruption of the gene encoding the negative immunoregulatory receptor PD-1 led to dilated cardiomyopathy with sudden death and congestive heart failure in mice.⁶¹ Hearts from PD-1-deficient mice exhibited diffuse deposition of IgG on the surface of myocytes and antibody in the disease

recognized a 33-kd protein specific to heart tissues. PD-1 may contribute to protection against autoimmune myocarditis. Studies of DBA/2 $\times$ CbyD2F1 mice susceptible to myocarditis revealed a locus on chromosome 12 that was strongly linked with myocardial inflammation. DBA/2 mice are susceptible to anti-myosin antibody mediated myocarditis whereas BALB/c are resistant. The investigation of genes involved in myocarditis indicates that they are diverse and are related to several different mechanisms of pathogenesis.

Perspectives

The study on TH2 cytokines in myocarditis in A/J mice may be an important guide for understanding human myocarditis as well as other myocarditis animal models in mice and rats. Further studies of human myocarditis will be informative as well as investigation of the cytokine profiles in the heart, spleen, and circulation of animal models of carditis. Not enough is known about cytokines or the TH1/TH2 paradigm in our animal models or in the human disease. In humans with rheumatic carditis following streptococcal infection, IgG1 and IgG3 antibody subclasses were elevated against streptococcal M protein as well as cytotoxic antibody suggesting a TH1 response after development of acute rheumatic heart disease.¹⁰⁶ The investigation of the A/J mouse model of myocarditis will no doubt lead to studies in other models and humans to determine the TH1/TH2 phenotype.

Because development of the different stages or types of myocarditis is dependent on several factors, it is difficult to ascribe the disease to a single entity. However, cytokines are a prominent influence on the disease as can be seen in the investigation of the A/J model. As the human genome is investigated further and genes controlling autoimmune disease as well as inflammation in the heart are revealed, we will learn more about how myocarditis develops with its different stages or phenotypes and the relationship of the protective or enhancement genes found in different animal models to human disease.

References

1. Caforio ALP, Goldman JH, Haven AJ, Baig KM, McKenna WJ: Evidence for autoimmunity to myosin and other heart-specific autoantigens in patients with dilated cardiomyopathy and their relatives. *Int J Cardiol* 1996, 54:157-163
2. Brown CA, O'Connell JB: Myocarditis and idiopathic dilated cardiomyopathy. *Am J Med* 1995, 99:309-314
3. Felker GM, Hu W, Hare JM, Hruban RH, Baughman KI, Kasper EK: The spectrum of dilated cardiomyopathy. The Johns Hopkins experience with 1,278 patients. *Medicine* 1999, 78:270-283
4. Towbin JA, Bowles KR, Bowles NE: Etiologies of cardiomyopathy and heart failure. *Nat Med* 1999, 5:766-767
5. Fuster V, Gersh BJ, Giuliani ER, Tajik AJ, Brandenburg AO, Frye RL: The natural history of idiopathic dilated cardiomyopathy. *Am J Cardiol* 1981, 47:525-531
6. Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE, Knowlton KU: Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med* 1999, 5:320-326
7. Badorff C, Berkely N, Mehrotra S, Talhouk JW, Rhoads RE, Knowlton

- KU: Enteroviral protease 2A directly cleaves dystrophin and is inhibited by a dystrophin-based substrate analogue. *J Biol Chem* 2000, 275:11191–11197
8. Lee GH, Badorff C, Knowlton KU: Dissociation of sarcoglycans and the dystrophin carboxyl terminus from the sarcolemma in enteroviral cardiomyopathy. *Circ Res* 2000, 87:489–495
9. Woodruff JF: Viral myocarditis—a review. *Am J Pathol* 1980, 101:425–466
10. Martino TA, Liu P, Sole MJ: Viral infection and the pathogenesis of dilated cardiomyopathy. *Circ Res* 1994, 74:182–188
11. Baboonian C, Davies MJ, Booth JC, McKenna WJ: Coxsackie B viruses and human heart disease. *Curr Top Microbiol Immunol* 1997, 223:31–52
12. Wong CY, Woodruff JJ, Woodruff JF: Generation of cytotoxic T lymphocytes during coxsackievirus B-3 infection. II. Characterization of effector cells and demonstration of cytotoxicity against viral-infected myofibers. *J Immunol* 1977, 118:1165–1169
13. Wong CY, Woodruff JF, Woodruff JJ: Generation of cytotoxic lymphocytes in the pathogenesis of coxsackievirus B3 heart disease. *J Immunol* 1977, 118:1159–1174
14. Huber SA, Job LP, Woodruff JF: Lysis of infected myofibers by coxsackievirus B3 immune lymphocytes. *Am J Pathol* 1980, 98:681–694
15. Gauntt C, Tracy S, Chapman N, Wood H, Kolbeck P, Karaganis A, Winfrey C, Cunningham M: Coxsackievirus-induced chronic myocarditis in murine models. *Eur Heart J* 1995, 16(Suppl O):S56–S58
16. Reyes MP, Ho KL, Smith F, Woodruff JF: A mouse model of dilated type cardiomyopathy due to coxsackie B3 heart disease. *J Infect Dis* 1981, 144:232–236
17. Huber SA, Gauntt CJ, Sakkinen P: Enteroviruses and myocarditis: viral pathogenesis through replication, cytokine induction, and immunopathogenicity. *Adv Virus Res* 1998, 51:35–80
18. Cunningham MW: Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* 2000, 13:470–511
19. Bachmaier K, Neu N, de la Maza L, Pal S, Hessel A, Penninger JM: Chlamydia infections and heart disease linked through antigenic mimicry. *Science* 1999, 283:1335–1339
20. Cunningham MW, Hall NK, Krisher KK, Spanier AM: A study of monoclonal antibodies against streptococci and myosin. *J Immunol* 1985, 136:293–298
21. Cunningham MW, Swerlick RA: Polyspecificity of antistreptococcal murine monoclonal antibodies and their implications in autoimmunity. *J Exp Med* 1986, 164:998–1012
22. Cunningham MW, Antone SM, Smart M, Liu R, Kosanke S: Molecular analysis of human cardiac myosin-cross-reactive B- and T-cell epitopes of the group A streptococcal M5 protein. *Infect Immun* 1997, 65:3913–3923
23. Neu N, Rose NR, Beisel KW, Herskowitz A, Gurri-Glass G, Craig SW: Cardiac myosin induces myocarditis in genetically predisposed mice. *J Immunol* 1987, 139:3630–3636
24. Kodama M, Matsumoto Y, Fujiwara M, Massani M, Izumi T, Shibota A: A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin Immunol Immunopathol* 1991, 57:250–262
25. Neu N, Beisel KW, Traystman MD, Rose NR, Craig SW: Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to coxsackievirus B3 induced myocarditis. *J Immunol* 1987, 138:2488–2492
26. Donermeyer DL, Beisel KW, Allen PM, Smith SC: Myocarditis-inducing epitope of myosin binds constitutively and stably to I-A^K on antigen presenting cells in the heart. *J Exp Med* 1995, 182:1291–1300
27. Smith SC, Allen PM: Myosin-induced acute myocarditis is a T cell mediated disease. *J Immunol* 1991, 147:2141–2147
28. Krisher K, Cunningham MW: Myosin: a link between streptococci and heart. *Science* 1985, 227:413–415
29. Adderson EE, Shikhman AR, Ward KE, Cunningham MW: Molecular analysis of polyreactive monoclonal antibodies from rheumatic carditis: human anti-N-acetyl-glucosamine/anti-myosin antibody V region genes. *J Immunol* 1998, 161:2020–2031
30. Galvin JE, Hemric ME, Ward K, Cunningham MW: Cytotoxic monoclonal antibody from rheumatic carditis reacts with human endothelium: implications in rheumatic heart disease. *J Clin Invest* 2000, 106:217–224
31. Quinn A, Kosanke S, Fischetti VA, Factor SM, Cunningham MW: Induction of autoimmune valvular heart disease by recombinant streptococcal M protein. *Infect Immun* 2001, 69:4072–4078
32. Shikhman AR, Greenspan NS, Cunningham MW: A subset of mouse monoclonal antibodies cross-reactive with cytoskeletal proteins and group A streptococcal M proteins recognizes N-acetyl-beta-D-glucosamine. *J Immunol* 1993, 151:3902–3913
33. Malkiel S, Liao L, Cunningham MW, Diamond B: T-cell-dependent antibody response to the dominant epitope of streptococcal polysaccharide, N-acetyl-glucosamine, is cross-reactive with cardiac myosin. *Infect Immun* 2000, 68:5803–5808
34. Cunningham MW, Antone SM, Gulizia JM, McManus BM, Fischetti VA, Gauntt CJ: Cytotoxic and viral neutralizing antibodies crossreact with streptococcal M protein, enteroviruses, and human cardiac myosin. *Proc Natl Acad Sci USA* 1992, 89:1320–1324
35. Huber SA, Moraska A, Cunningham M: Alterations in major histocompatibility complex association of myocarditis induced by coxsackievirus B3 mutants selected with monoclonal antibodies to group A streptococci. *Proc Natl Acad Sci USA* 1994, 91:5543–5547
36. Huber S, Polgar J, Moraska A, Cunningham M, Schwimmbeck P, Schultheiss P: T lymphocyte responses in CVB3-induced murine myocarditis. *Scand J Infect Dis* 1993, 88(Suppl):S67–S78
37. Huber SA, Cunningham MW: Streptococcal M protein peptide with similarity to myosin induces CD4+ T cell-dependent myocarditis in MRL/++ mice and induces partial tolerance against coxsackieviral myocarditis. *J Immunol* 1996, 156:3528–3534
38. Rose NR: Viral damage or 'molecular mimicry'—placing the blame in myocarditis. *Nat Med* 2000, 6:631–632
39. Horwitz MS, La Cava A, Fine C, Rodriguez E, Illic A: Pancreatic expression of interferon-gamma protects mice from lethal coxsackievirus B3 infection and subsequent myocarditis. *Nat Med* 2000, 6:693–697
40. Penninger JM, Neu N, Timms E, Wallace VA, Koh D-R, Kishihara K, Plummer C, Mak TW: Induction of experimental autoimmune myocarditis in mice lacking CD4 or CD8 molecules. *J Exp Med* 1993, 178:1837–1842
41. Liao L, Sindhwani R, Rojkind M, Factor S, Leinwand L, Diamond B: Antibody-mediated autoimmune myocarditis depends on genetically determined target organ sensitivity. *J Exp Med* 1995, 187:1123–1131
42. Gauntt C, Arizpe H, Higdon A, Bowers D, Rozek M, Crawley R: Molecular mimicry, anti-coxsackievirus B2 neutralizing monoclonal antibodies and myocarditis. *J Immunol* 1995, 154:2983–2995
43. Gauntt CJ, Arizpe HM, Higdon AL, Rozek MM, Crawley R, Cunningham MW: Anti-coxsackievirus B3 neutralizing antibodies with pathological potential. *Eur Heart J* 1991, 12:124–129
44. Gauntt CJ, Higdon AL, Arizpe HM, Tamayo MR, Crawley R, Henkel RD, Pereira ME, Tracy SM, Cunningham MW: Epitopes shared between coxsackievirus B3 (CVB3) and normal heart tissue contribute to CVB3-induced murine myocarditis. *Clin Immunol Immunopathol* 1993, 68:129–134
45. Huber SA, Lodge PA: Coxsackievirus B-3 myocarditis: identification of different pathogenic mechanisms in DBA/2 and BALB/c mice. *Am J Pathol* 1986, 122:284–291
46. Cunningham MW, McCormack JM, Talaber LR, Harley JB, Ayoub EM, Muneer RS, Chun LT, Reddy DV: Human monoclonal antibodies reactive with antigens of the group A Streptococcus and human heart. *J Immunol* 1988, 141:2760–2766
47. Cunningham MW, McCormack JM, Fenderson PG, Ho MK, Beachey EH, Dale JB: Human and murine antibodies cross-reactive with streptococcal M protein and myosin recognize the sequence GLN-LYS-SER-LYS-GLN in M protein. *J Immunol* 1989, 143:2677–2683
48. Kaplan MH, Bolande R, Ratika L, Blair J: Presence of bound immunoglobulins and complement in the myocardium in acute rheumatic fever. *N Engl J Med* 1964, 271:637–645
49. Gulizia JM, Cunningham MW, McManus BM: Immunoreactivity of anti-streptococcal monoclonal antibodies to human heart valves. Evidence for multiple cross-reactive epitopes. *Am J Pathol* 1991, 138:285–301
50. Guilherme L, Cunha-Neto E, Coelho V, Snitcowsky R, Pomerantzeff PMA, Assis RV, Pedra F, Neumann J, Goldberg A, Patarroyo ME, Pileggi F, Kalil J: Human heart-filtrating T cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. *Circulation* 1995, 92:415–420

51. Lauer B, Padberg K, Schultheiss HP, Strauer BE: Autoantibodies against human ventricular myosin in sera of patients with acute and chronic myocarditis. *J Am Coll Cardiol* 1994, 23:146–153
52. Neumann DA, Burek CL, Baughman KL, Rose NR, Herskowitz A: Circulating heart reactive antibodies in patients with myocarditis or cardiomyopathy. *J Am Coll Cardiol* 1990, 16:839–846
53. Maisch B, Drude L, Hengstenberg C, Herzum M, Hufnagel G, Kochsiek K, Schmaltz A, Schonian U, Schwab MD: Are antisarcolemmal (ASAs) and antimyolemmal antibodies (AMLAs) "natural" antibodies? *Basic Res Cardiol* 1991, 86 (Suppl 3):101–114
54. Neumann DA, Rose NR, Ansari AA, Herskowitz AH: Induction of multiple heart autoantibodies in mice with coxsackievirus B3- and cardiac myosin-induced autoimmune myocarditis. *J Immunol* 1993, 152:343–350
55. Limas CJ, Goldenberg IF, Limas C: Soluble interleukin-2 levels in patients with dilated cardiomyopathy. Correlation with disease severity and cardiac autoantibodies. *Circulation* 1995, 91:631–634
56. Pummerer CL, Luze K, Grassl G, Bachmaier K, Offner F, Lenz DM, Zamborelli TJ, Penninger JM, Neu N: Identification of cardiac myosin peptides capable of inducing autoimmune myocarditis in BALB/c mice. *J Clin Invest* 1996, 97:2057–2062
57. Inomata T, Hanawa T, Mihanishi E, Yajima S, Nakayama T, Maita M, Kodama T, Izumi A, Shibata A, Abo T: Localization of porcine cardiac myosin epitopes that induce experimental autoimmune myocarditis. *Circ Res* 1995, 76:726–733
58. Wegmann KW, Zhao W, Griffin AC, Hickey WF: Identification of myocarditogenic peptides derived from cardiac myosin capable of inducing experimental allergic myocarditis in the Lewis rat. *J Immunol* 1994, 153:892–900
59. Liao L, Sindhiani R, Leinwand L, Diamond B, Factor S: Cardiac α -myosin heavy chains differ in their induction of myocarditis: identification of pathogenic epitopes. *J Clin Invest* 1993, 92:2877–2882
60. Wolfgram LJ, Beisel KW, Herskowitz A, Rose NR: Variations in the susceptibility to coxsackievirus B3-induced myocarditis among different strains of mice. *J Immunol* 1986, 136:1846–1852
61. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi AH, Minato N, Honjo T: Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001, 291:319–322
62. McManus BM, Chow LH, Wilson JE, Anderson DR, Gulizia JM, Gauntt CJ, Klingel KE, Beisel KW, Kandolf R: Direct myocardial injury by enterovirus: a central role in the evolution of murine myocarditis. *Clin Immunol Immunopathol* 1993, 68:159–169
63. Smith SC, Allen PM: Expression of myosin-class II major histocompatibility complexes in the normal myocardium occurs before induction of autoimmune myocarditis. *Proc Natl Acad Sci USA* 1992, 89:9131–9135
64. Kohno K, Takagaki Y, Nakajima Y, Izumi T: Advantage of recombinant technology for the identification of cardiac myosin epitope of severe autoimmune myocarditis in Lewis rats. *Jpn Heart J* 2000, 41:67–77
65. Wang Y, Afanasyeva M, Hill SL, Rose NR: Characterization of murine autoimmune myocarditis induced by self and foreign cardiac myosin. *Autoimmunity* 1999, 31:151–162
66. Sakaguchi S: Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 2000, 101:455–458
67. Huber SA, Graveline D, Newell MK, Born WK, O'Brien RL: V γ 1+ T cells suppress and V γ 4+ T cells promote susceptibility to coxsackievirus B3-induced myocarditis in mice. *J Immunol* 2000, 165:4174–4181
68. Modlin RL: Th1-Th2 paradigm: insights from leprosy. *J Invest Dermatol* 1994, 102:828–832
69. Yamamura M, Wang X-H, Ohman JD, Uyemura K, Rea TH, Bloom BR, Modlin RL: Cytokine patterns of immunologically mediated tissue damage. *J Immunol* 1992, 149:1470–1475
70. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, Bloom BR: Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 1991, 254:279–282
71. Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, Johnson LL, Swain SL, Lund FE: Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol* 2000, 1:475–482
72. Marrack PC, Kappler JW: The staphylococcal enterotoxins and their relatives. *Science* 1990, 248:705–708
73. Kotb M: Role of superantigens in the pathogenesis of infectious diseases and their sequelae. *Curr Opin Infect Dis* 1992, 5:364–374
74. Locksley RM, Pingel S, Lacy D, Wakil AE, Bix M, Fowell DJ: Susceptibility to infectious diseases: leishmania as a paradigm. *J Infect Dis* 1999, 179(Suppl 2):S305–S308
75. Locksley RM, Scott P: Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function. *Immunol Today* 1991, 12:A58–A61
76. Heinzel FP, Sadick MD, Mutha SS, Locksley RM: Production of interferon gamma, interleukin 2, interleukin 4, and interleukin 10 by CD4+ lymphocytes in vivo during healing and progressive murine leishmaniasis. *Proc Natl Acad Sci USA* 1991, 88:7011–7015
77. Sadick MD, Street N, Mosmann TR, Locksley RM: Cytokine regulation of murine leishmaniasis: interleukin 4 is not sufficient to mediate progressive disease in resistant C57BL/6 mice. *Infect Immun* 1991, 59:4710–4714
78. Amiri P, Locksley RM, Parslow TG, Sadick M, Rector E, Ritter D, McKerrow JH: Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature* 1992, 356:604–607
79. Locksley RM: Interleukin 12 in host defense against microbial pathogens. *Proc Natl Acad Sci USA* 1993, 90:5879–5880
80. Locksley RM: Th2 cells: help for helminths. *J Exp Med* 1994, 179:1405–1407
81. Wakil AE, Wang ZE, Locksley RM: Leishmania major: targeting IL-4 in successful immunomodulation of murine infection. *Exp Parasitol* 1996, 84:214–222
82. Liblau RS, Singer SM, McDevitt HO: TH1 and TH2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995, 16:34–38
83. Falcone M, Sarvetnick N: Cytokines that regulate autoimmune responses. *Curr Opin Immunol* 1999, 11:670–676
84. Seder R, Gazzinelli AP, Sher A, Paul W: Interleukin-12 acts directly on CD4+ T cells to enhance priming for IFN- γ production and diminishes interleukin-4 inhibition of such priming. *Proc Natl Acad Sci USA* 1993, 90:10188–10192
85. Paul WE, Seder RA: Lymphocyte responses and cytokines. *Cell* 1994, 76:241–251
86. Okura Y, Takeda K, Honda S, Hanawa H, Watanabe H, Kodama M, Izumi T, Aizawa Y, Seki S, Abo T: Recombinant murine interleukin-12 facilitates induction of cardiac myosin-specific type 1 helper T cells in rats. *Circ Res* 1998, 82:1035–1042
87. Afanasyeva M, Wang Y, Kaya Z, Park S, Zilliox MJ, Schofield BH, Hill SL, Rose NR: Experimental autoimmune myocarditis in A/J mice is an interleukin-4-dependent disease with a Th2 phenotype. *Am J Pathol* 2001, 159:193–203
88. Himmelrich H, Launois R, Maillard I, Biedermann T, Tacchini-Cottier F, Locksley RM, Rocken M, Louis JA: In BALB/c mice, IL-4 production during the initial phase of infection with Leishmania major is necessary and sufficient to instruct Th2 cell development resulting in progressive disease. *J Immunol* 2000, 164:4819–4825
89. Finkelman FD, Holmes J, Katona IM, Urban Jr JF, Beckman MP, Park LS, Schooley KS, Coffman RL, Mosman TR, Paul WE: Lymphokine control of in vivo immunoglobulin isotype selection. *Ann Rev Immunol* 1990, 8:303–333
90. Mosman TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986, 136:2348–2357
91. Mosman T, Coffman T: Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989, 7:145–173
92. McInnes A, Rennick DM: Interleukin 4 induces cultured monocytes/macrophages to form giant multinucleated cells. *J Exp Med* 1988, 167:598–611
93. Campbell IL, Oxbrow L, Koulmanda M, Harrison LC: IFN- γ induces islet cell MHC antigens and enhances autoimmune streptozotocin-induced diabetes in the mouse. *J Immunol* 1988, 140:1111–1116
94. Goerdt S, Orfanos CE: Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity* 1999, 10:137–142
95. Kumar V, Sercarz E: Induction or protection from experimental autoimmune encephalomyelitis depends on the cytokine secretion profile of TCR-peptide specific regulatory CD4 T cells. *J Immunol* 1998, 161:6585–6591

96. Falcone M, Yeung B, Tucker L, Rodriguez E, Sarvetnick N: A defect in IL-12 induced activation and IFN- γ secretion of peripheral natural killer T cells in nonobese diabetic mice suggests new pathogenic mechanisms for insulin-dependent diabetes mellitus (IDDM). *J Exp Med* 1999, 190:963–972
97. Lane JR, Neumann DA, Lafond-Walker A, Herskowitz A, Rose NR: Interleukin 1 or tumor necrosis factor can promote coxsackie B3-induced myocarditis in resistant B10.A mice. *J Exp Med* 1992, 175:1123–1129
98. Smith SC, Allen PM: Neutralization of endogenous tumor necrosis factor ameliorates the severity of myosin-induced myocarditis. *Circ Res* 1992, 70:856–863
99. Okura Y, Yamamoto T, Goto S, Inomata T, Hirono S, Hanawa H, Feng L, Wilson CB, Kihara I, Izumi T, Shibata A, Aizawa Y, Seki S, Abo T: Characterization of cytokine and iNos mRNA expression in situ during the course of experimental autoimmune myocarditis in rats. *J Mol Cell Cardiol* 1997, 29:491–502
100. Bachmaier K, Pummerer C, Kozieradzki I, Pfeffer K, Mak TW, Neu N, Penninger JM: Low-molecular weight tumor necrosis factor receptor p55 control induction of autoimmune heart disease. *Circulation* 1997, 95:655–661
101. Pfeffer K, Matsuyama T, Kuendig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Kroenke M, Mak TW: Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *Listeria monocytogenes* infection. *Cell* 1993, 73:457–467
102. Glimcher LH, Kara CJ: Sequences and factors: a guide to MHC class-II transcription. *Annu Rev Immunol* 1992, 10:13–49
103. Leiden JM: The genetics of dilated cardiomyopathy—emerging clues to the puzzle. *N Engl J Med* 1997, 337:1080–1081
104. Nelson DP, Setser E, Hall DG, Schwartz SM, Hewitt T, Klevitsky R, Osinka H, Bellgrau H, Duke RC, Robbins J: Proinflammatory consequences of transgenic Fas ligand expression in the heart. *J Clin Invest* 2000, 105:1199–1208
105. McLaughlin L, Shu G, Mistry M, Ley-Ebert C, Stuart WD, Florio CJ, Groen PA, Witt SA, Kimball TR, Witte DP, Harmony JAK, Aronow BJ: Apolipoprotein J/clusterin limits the severity of murine autoimmune myocarditis. *J Clin Invest* 2000, 106:1105–1113
106. Cunningham MW, Beeker A, Heuser JS, Hill H, Veasy G, Augustine N, Adderson EE: Insights into the immunology of acute rheumatic fever: a study of cytotoxicity and the TH1/TH2 paradigm. Abstracts of the 101th Annual Meeting of the American Society for Microbiology, 2001