Nonischemic dilated cardiomyopathy (DCM) is an uncommon cause of heart failure but has widespread importance because it is the cause of 45% of heart transplantations. Multiple experimental and clinical lines of evidence have implicated altered immunity in the pathogenesis of DCM. However, advances in the understanding of the mechanisms of altered immunity have not affected the diagnosis or treatment of DCM. In recognition of this problem, the National Institutes of Health sponsored an expert workshop with 2 aims: to review the current understanding of inflammation and immunity as they relate to DCM and to identify the most promising areas for future clinical research efforts in the field. This report summarizes the scientific opportunities, perceived needs and barriers, and workshop recommendations on research directions in DCM. The major recommendations from the members of the workshop are organized according to the following themes: cardiotropic viruses, innate and acquired immune responses, environmental factors, novel diagnostics, and novel therapeutics.


DCM = dilated cardiomyopathy; GPx = glutathione peroxidase; MRI = magnetic resonance imaging; NIH = National Institutes of Health; qPCR = quantitative polymerase chain reaction; qRT-PCR = quantitative reverse transcriptase-PCR; TE = trace element

Dilated cardiomyopathy (DCM) is an uncommon cause of heart failure but has widespread importance because it is the cause of 45% of heart transplantations. The last National Institutes of Health (NIH)–sponsored expert workshop on this subject was held in 1989 and identified the need for investigation into the immunologic basis of the disease. Since then, exceptional strides have been made in our understanding of the immunology and pathophysiology of viral and autoimmune myocarditis. Most of these advances have been made through the use of inbred and transgenic rodent models. Advances in clinical treatment and diagnosis of myocarditis and DCM have lagged behind the advances in our understanding of disease mechanisms.

The workshop was organized to address the need to translate advances at the bench into bedside medicine. Experts from a range of relevant specialties, including virology, biochemistry, immunology, pathology, and clinical cardiology, met with representatives of the NIH on May 21 and 22, 2004. The conference was organized into themes consisting of cardiotropic viruses, innate and acquired immunity, environmental factors, novel diagnostics, and novel therapeutics. Within each theme, the participants identified perceived challenges and opportunities and made recommendations for support of future research. This report summarizes the discussions and recommendations from that conference.

CARDIOTROPIC VIRUSES

Viral myocarditis is pathogenically determined by at least 3 factors: (1) the virus and its receptor, (2) the host immune system, and (3) cardiac remodeling. In the past 18 years, we have seen important advances in our ability to detect cardiotropic viruses from fresh and frozen heart tissue using in situ hybridization, quantitative polymerase chain reaction (qPCR), nested qPCR, and most recently quantitative reverse transcriptase–PCR (qRT-PCR). The latest technologies can detect 1 to 10 gene copies present in myocardium. These rapid advances in technology have changed the sensitivity of molecular techniques for viral pathogens and have provided both challenges and opportunities.

New viral pathogens have emerged in the past decade, including adenovirus, Epstein-Barr virus, cytomegalovirus, hepatitis C, and most recently parvovirus B19. Enterovirus serotypes have been identified as different from the 64 serotypes recognized previously. Given the high rate of enterovirus recombination, the current diagnostic method of qRT-PCR of conserved regions of the 5′ nontranslated region cannot discriminate among the currently recognized enterovirus serotypes. Portions of the enterovirus genome other than the 5′ nontranslated region could be explored for unique and conserved sequences. Such studies may allow for a molecular identification of the known serotypes or
even an ability to classify viruses according to genotype. Such newly identified genotypes may show stronger correlations with myocarditis and DCM than seen among the current serotypes. Of note, there are currently no standard methods for validating the detection of these new viruses. Furthermore, studies that show a direct pathogenic effect of persistent viral genome in human DCM are lacking. A few antiviral agents, including interferon beta, interferon alfa, immunoglobulin, and pleconaril, have been used to treat enteroviral DCM, but none have been studied in randomized trials.

The workshop participants identified major challenges to the translation of scientific advances. The greatest challenge is a lack of human tissue, which is required for the diagnosis. It is probable that endomyocardial biopsy sampling error plays a major role in reports of different rates of viral heart disease. Because endomyocardial biopsy may not be performed frequently in the routine diagnosis of cardiomyopathies, extra effort should be made to obtain specimens from other sources, such as cardiac tissue explants and autopsy specimens. Indirect methods of detecting virus infection, such as serology, virus-specific T-cell activation, cytokine/chemokine levels, and expression profiling, should also be developed and pursued since cardiac pathology may not occur until well after immune clearance of virus. The members recommend a study to correlate the rate of viral-positive genomes on sham biopsies with explanted heart and/or autopsy tissue.

A second barrier to standard assessment of viruses in cardiac disease is a lack of standard methods of tissue collection, tissue handling, and virus detection. Genome target signals can degrade if tissues are fixed in formalin, or the sample may be contaminated if the tissue is not protected from exposure. The members recommend that standard operating procedures for tissue acquisition, handling, storage, and transportation be adopted. Quantitative PCR and qRT-PCR techniques should be used to assess the sensitivity of detecting viruses in tissue samples obtained by core viral laboratories involved in cardiomyopathy research. Standardization of methodology and determination of sensitivity are critical to assess patient samples in different clinical studies. Although this workshop did not define the methods for standardization, such methods need to be proposed, discussed, and agreed on for the collaboration of cardiologists and virologists.

The types of viruses that affect different ethnic populations are also unknown. Epidemiological studies that are designed to address the rates of viral cardiomyopathy in DCM should be performed before large clinical studies of antiviral therapy are considered. The role of host genetics in the susceptibility to viral infection should receive attention as a means of stratifying risk, including studies of viral receptors, immune mediators, and the cellular cytoarchitecture.

INNATE AND ACQUIRED IMMUNITY

During the past decade, inbred and transgenic rodent models of myocarditis and DCM have yielded profound insights into the pathogenesis of DCM. The role of specific cytokines in inflammation was found to vary between different models that used different viruses and genetic backgrounds. The pathogenic role of complement C5a receptor 4 and decay accelerating factor has been identified as important in viral pathogenesis. The ubiquitin and proteasome pathways can be used by Coxsackie B virus to cause cell arrest and promote viral replication.

Murine models of coxsackievirus-induced myocarditis have been developed, and viral-induced myocarditis has been associated with immune responses against cardiac myosin, sarcomeral Na,K-ATPase, G protein-linked receptors, and mitochondrial antigens in humans with myocarditis and cardiomyopathy. Protection against myocarditis by immunization with myosin-like or cardiac myosin peptides has been shown to protect against viral or myosin-induced myocarditis, and neutralization of tumor necrosis factor α also ameliorates disease in mouse models. There is evidence of autoantibody responses against cardiac myosin, sarcomeral Na,K-ATPase, G protein-linked receptors, and mitochondrial antigens in humans with myocarditis and cardiomyopathy. Protection against myocarditis by immunization with myosin-like or cardiac myosin peptides has been shown to protect against viral or myosin-induced myocarditis, and neutralization of tumor necrosis factor α also ameliorates disease in mouse models. Therefore, in animal models, immune responses against a variety of cardiac antigens induce an inflammatory state in the heart, and case-control studies in humans show an association between antineutrophil antibodies and DCM. Further studies are needed to determine the pathogenic role of immune responses against cardiac proteins in human myocarditis and DCM.

A major challenge is that the relevance of these models in human disease is unknown, and the hypotheses that have been generated in animal models need to be tested in human tissue. Available human tissue linked to well-characterized disease phenotypes is lacking. A recommendation of the workshop is to form a multicenter human tissue registry consisting of biopsy, surgical, explanted heart, and autopsy tissues with standardized tissue acquisition, han-
ENVIRONMENTAL FACTORS

Deficiencies of selenium or high levels of cobalt, both trace elements (TEs), can induce clinically progressive cardiac dilation and dysfunction that is histologically indistinguishable from DCM. Myocardial TE accumulation or deficiency may result from several factors, including environmental factors, abnormal dietary intake, intestinal malabsorption, or an acquired viral myopathy. Selenium deficiency is one of the most clinically relevant TE disorders.\(^41\)\(^42\) Selenium is an unusual TE in that it has its own messenger RNA codon that specifies its insertion into proteins as selenocysteine. Selenocysteine, the 21st physiologically essential amino acid, is present at the active site of all known selenium-dependent enzymes where its unique redox potential facilitates their biochemical functions in support of human health.\(^41\)\(^42\) More than 25 mammalian selenium-dependent proteins are currently recognized, each with distinct patterns of tissue and subcellular distribution and occurrence.\(^41\)\(^42\) Normal nutritional selenium status is necessary to maintain an adequate immune system, and there is increasing evidence that augmented selenium status is associated with diminished cancer risk.\(^43\)

Selenium deficiency may derive from its deprivation in the soil of some geographic areas (Keshan disease)\(^41\)\(^42\)\(^44\) or from intestinal malabsorption or TE loss caused by an intestinal bypass aimed to treat severe obesity. Selenium deficiency exerts its pathogenic effect by reducing the antioxidant properties of the heart, causing an impairment of the immune system and increasing viral genome virulence, thus making the heart more susceptible to viral infections.\(^35\)\(^46\) Recent work has shown that an amyocarditic strain of Coxsackie B3 virus (CVB3/0) was converted to virulent in selenium-deficient mice. This conversion was accompanied by changes in the genetic structure of the virus to resemble the genome of other virulent Coxsackie B3 virus strains. Similar increases in virulence accompanied by multiple changes in the viral genome by passage through selenium-deficient hosts have been shown in influenza virus A/Bangkok/1/79.\(^47\)

Another possible link between selenium and viral infections could be represented by the evidence that several viral pathogens possess a highly conserved gene sequence capable of encoding for a truncated glutathione peroxidase (GPx). Such viruses include coxsackievirus, human immunodeficiency virus 1, human immunodeficiency virus 2, hepatitis C, hepatitis B, measles, polio, and Ebola. Although the target tissues for these viruses vary widely, it is possible that the viruses have some common functional effects. Specifically, diversion of selenium into the selenocysteines of virally encoded GPx homopeptides would deplete the host cells of selenium that would otherwise be available for normal selenoprotein synthesis.\(^48\) Since selenophosphate synthetase, one of the cellular proteins required for selenoprotein synthesis, is itself a selenoprotein, viral diversion of cellular selenium stores into viral GPx homopeptides could result in irrecoverable elimination of selenoenzyme metabolic processes within cells that have been virally infected. Infected cells would not be able to reinitiate selenoenzyme synthesis, resulting in permanent impairment of free radical detoxification and other selenium-dependent cellular processes. To establish whether this mechanism contributes to DCM, selenium-dependent messenger RNA levels could be measured by in situ hybridization with selenoenzyme functional assays in DCM and healthy myocardium.

Cobalt accumulation has been associated with excessive alcohol intake, and mercury intoxication with intake of contaminated fish or abnormal handling of dental amalgams.\(^49\) Cobalt and mercury accumulation in myocytes is accompanied by ultrastructural changes of mitochondria and other intracellular organelles; however, the mechanism of cobalt toxicity is unknown. Pronounced accumulation of heavy metals (mercury and antimony) has been detected by neutron activation analysis in endomyocardial biopsy specimens from patients with DCM, independent of systemic disorders or environmental exposure.\(^50\) At this time, it is not known whether administration of a deficient element or elimination of a toxic source may lead to functional and clinical improvement in DCM.

A major obstacle to research is the lack of systematically collected tissue linked to well-characterized clinical data. The interaction among viral heart disease, excessive alcohol intake, and heavy metals has been suggested but not investigated substantially. In addition, recent population-based studies suggest an increased risk of DCM in black vs white Americans\(^51\) and in indigenous vs nonindigenous Australian children.\(^52\) Both the genetic and the environmental basis for these differences need to be pursued in future studies. Specific recommendations include (1) studies in family members of affected individuals to define host factors responsible for the increased risk of DCM and (2) a clinical study designed to confirm the role of TE and heavy metals in DCM and to better characterize the interaction among environmental factors, cardiotropic virus infection, and host genetics.

NOVEL DIAGNOSTICS

Hematoxylin-eosin stained cardiac tissue from endomyocardial biopsy specimens interpreted by the Dallas criteria...
has been the gold standard for the antemortem diagnosis of myocarditis for more than 20 years.\textsuperscript{53} Major problems with this standard include sampling error and lack of expert consensus on myocyte necrosis and inflammation criteria. Furthermore, diagnosis of myocarditis based on the Dallas criteria is not prognostically useful in acute DCM.\textsuperscript{54} Therefore, heart biopsy is currently performed infrequently because it rarely changes prognosis or treatment in DCM.\textsuperscript{55} Uncommon but important exceptions to this general rule include giant cell myocarditis,\textsuperscript{56} cardiac sarcoidosis, eosinophilic myocarditis (including hypersensitivity myocarditis),\textsuperscript{57} and severe cases of myocarditis after small pox vaccine (vaccinia) myocarditis.\textsuperscript{58} The workshop members recommend investigation of the prognostic usefulness of novel immunohistologic markers and viral genomes coupled with standardized tissue acquisition.

Cardiac magnetic resonance imaging (MRI) has emerged as a promising, noninvasive imaging technique for the diagnosis of DCM and myocarditis. Recent studies have correlated the presence of myocarditis with gadolinium uptake on cardiac MRI.\textsuperscript{59} In these studies, the pattern of inflammation is usually distinct from ischemic damage. Therefore, MRI may be useful for identifying regional abnormalities that occur early in the course of clinical myocarditis. Future studies need to address the prognostic value of MRI in addition to established clinical variables.

Novel blood and serologic markers of prognosis were discussed by the workshop members, including soluble Fas (CD95/Apo1, a tumor necrosis factor receptor),\textsuperscript{60} soluble Fas ligand,\textsuperscript{61} interleukin 10, and genomic studies from peripheral blood mononuclear cells. All these hold promise for diagnosis and prognosis in DCM. The workshop strongly supported further investigation into these areas.

### NOVEL THERAPEUTICS

The workshop members addressed the potential for future randomized treatment trials for acute or chronic DCM. Because of the rarity of acute DCM and the large improvements in the placebo groups of the Myocarditis Treatment Trial\textsuperscript{62} and the Intervention in Myocarditis and Acute Myocardopathy trial,\textsuperscript{63} an adequately powered treatment trial of patients with acute DCM of less than 6 months’ duration was considered infeasible. In contrast, the workshop members noted several positive treatment trials of immunomodulatory and antiviral therapy for chronic DCM. These included interventions with azathioprine and prednisone,\textsuperscript{64,65} intravenous immunoglobulin,\textsuperscript{66} and interferon beta.\textsuperscript{67} Obstacles to an adequately powered antiviral or immunomodulatory treatment trial of chronic DCM include the need for a multicenter design, consensus on the optimal diagnostic criteria, and incorporation of current state-of-the-art qPCR methods for the detection of cardiotropic viruses. The workshop members agreed that such trials should have sufficiently large sample sizes and lengthy follow-up times to detect significant treatment effects on quality of life, functional capacity, or composite end points that may include death, cardiovascular death, and heart transplantation.

Immunoadsorption to remove anticardiac autoantibodies in patients with evidence of autoimmune cardiomyopathy is a promising new technology.\textsuperscript{67,68} Current limitations include the lack of an adequate animal model and a clear mechanistic rationale for persistent treatment benefit. Specifically, it is unclear why cardiac autoantibodies do not rebound after several weeks or months to pretreatment levels, and the mechanism of long-term benefit is unclear. Limitations of current data include the lack of adequately controlled studies and the use of different immunoadsorption columns and different treatment protocols in various reports. The workshop participants agreed that immunoadsorption is an appealing new technology that deserves further investigation.

### SUMMARY OF RECOMMENDATIONS

On the basis of the presentations and discussions of the workshop sections, the participants recommended 6 areas that would benefit from increased support from the NIH during the next 5 to 10 years:

1. Studies to determine the sensitivity and specificity of qRT-PCR to detect cardiotropic viruses in a broad population of heart failure patients with DCM
2. Determination of the prognostic value of viral genome detection in acute and chronic DCM—the study should be designed to detect added prognostic value beyond that available from clinical and noninvasive variables
3. Studies to determine the role of innate and adaptive immunity in human DCM—these studies should take advantage of registries of human tissue
4. Familial case-control studies to determine the genetic basis of DCM and the interaction of genetic predisposition with environmental variables
5. Studies designed to determine the prognostic usefulness of MRI and novel blood and serologic markers for DCM and myocarditis
6. Studies of immunoabsorption for the treatment of chronic DCM

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