Innovative Drug Treatments for Viral and Autoimmune Myocarditis

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Myocarditis has been shown to be a common cause of cardiomyopathy and is believed to account for 25% of all cases in human beings. Unfortunately, the disease is difficult to detect before a myopathic process ensues. Treatment of myocarditis-induced heart failure includes the standard regimen of diuretics, digoxin, angiotensin-converting enzyme inhibitors, and currently, β-adrenergic blockers. Treatment of myocarditis itself is dependent on the etiology of the illness. Treatments under investigation include immunosuppressants, nonsteroidal antiinflammatory agents, immunoglobulins, immunomodulation, antiadrenergics, calcium-channel blockers, angiotensin-converting enzyme inhibitors, nitric oxide inhibition (e.g., aminoguanidine), and antiviral agents. Despite advances in treatment, more work needs to be done in the early detection of myocarditis. Additionally, better means need to be established for distinguishing between viral and autoimmune forms of the disease, so that appropriate treatment can be instituted.

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For more than two centuries, the treatment of myocarditis has been a source of confusion and controversy. Since the early nineteenth century, therapies have included everything from lemon juice and diuretics to a variety of antitoxins.[1] Unfortunately, none of these strategies have been proven to alter the course of the illness. To this day, treatment is almost entirely supportive; bed rest remains the only therapy that has been consistently shown to improve outcomes.[1–4]

Much of our difficulty in defining treatment lies in our inability to accurately diagnose myocarditis, and subsequently establish its etiology. Currently, there is no gold standard for diagnosing this illness. Even endomyocardial biopsy, which is considered the definitive means of establishing the diagnosis, lacks sensitivity. Because of focal myocardial involvement of the disease, endomyocardial biopsy may underdiagnose myocarditis by sampling error.[5] Moreover, the Dallas Criteria, created in 1987 to standardize the diagnosis, rely on histologic evidence of myocardial degeneration. Thus, mild cases may be overlooked.[6] Distinguishing between viral and autoimmune forms of the illness has proven to be an additional problem.

To establish successful treatment regimens, it will be essential to define exactly what we are treating.

Despite these obstacles, the past several years have generated a large quantity of data regarding the pathogenesis and treatment of myocarditis. The following article will review the recent literature on myocarditis, focusing heavily on treatment. Although there have been a number of clinical trials, most current work has been in murine models of viral and autoimmune myocarditis. A variety of potential therapeutic options have emerged, ranging from antiviral agents and nitric oxide inhibitors to immunosuppressants and immunomodulators. These treatment strategies will be looked at in detail later. However, to best understand these therapeutic options, it is necessary to first look at the etiology and pathogenesis of myocarditis.

ETIOLOGY AND PATHOGENESIS OF MYOCARDITIS

There are a large number of agents, infectious and noninfectious, that can cause myocarditis (Table I). Although most patients have no identifiable source of the illness, viruses are believed to be the most common cause of lymphocytic myocarditis.[5,7] Of these, the enteroviruses (particularly Coxsackievirus) are the most frequent etiologic agents,[8,9] along with adenovirus and herpesvirus.[6,7] Indeed, enteroviruses have been detected by molecular hybridization techniques in the myocardium of 10% to 53% of patients with active myocarditis or idiopathic di-

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Table I Agents Associated with Myocarditis

Infectious Agents

Viral	Adenovirus	Influenza (A,B)
	Arbovirus (Dengue, yellow fever)	Junin
	Arenavirus (lymphocytic choriomeningitis)	Mumps
	Coronavirus	Polio
	Coxsackievirus (A,B)	Rabies
	Cytomegalovirus	Respiratory syncytial
	Echovirus	Rubella
	Encephalomyocarditis	Rubeola
	Epstein Barr virus	Vaccinia
	Hepatitis B	Varicella-Zoster
	Herpes simplex	Variola
	Human immunodeficiency virus	

Bacterial, Protozoal, Rickettsial, and Spirochetal		
	Balantidium coli	Mycobacteria (*tuberculosis, Avium-intracellulare, leprae*)
	Borrelia (*burgdorferi, recurrentis*)	*Mycoplasma pneumoniae*
	Brucella species	*Neisseria (meningitides, gonorrhea)*
	Campylobacter jejuni	*Rickettsia rickettsii*
	Chlamydia (*trachomatis, psittaci*)	*Salmonella typhi*
	Clostridia	*Shigella enteritis*
	Coxiella burnetti (Q fever)	Staphylococci
	Diphtheria	Streptococci
	Entamoeba histolytica	*Toxoplasma gondi*
	Franciscella tularensis	*Treponema pallidum*
	Legionella pneumophila	*Tropheryma whippelii*
	Leishmania species	*Trypanosoma cruzi*
	Leptospira interrogans	*Yersinia enterocolitica*
	Listeria monocytogenes	

Fungal	Actinomyces	Cryptococcus
	Aspergillus	Histoplasma
	Blastomyces	Mucormyces
	Candida	Nocardia
	Coccidioides	Sporothrix

Helminths	Ascaris	*Taenia solium*
	Echinococcus granulosus	Trichinella
	Paragonimus westermani	*Visceral larva migrans*
	Schistosoma	*Wucheria bancrofti*

(continues)

lated cardiomyopathy.[7] During the past few years, human immunodeficiency virus (HIV) has fast become the most common cause of myocarditis observed at necropsy.[10] However, the disease is rarely diagnosed during a patient's life, as cardiac deterioration is usually overshadowed by a multitude of noncardiac symptoms.

The pathophysiology of lymphocytic myocarditis in humans is not entirely understood. It has been shown that mice inoculated with Coxsackievirus B3 develop myocardial changes resembling human lymphocytic myocarditis.[7] In this experimental model, virus-induced cell lysis takes place during days 1 through 3 of infection. Nonetheless, most myocardial damage is immune mediated, a result of the lysis of uninfected cells by cytotoxic T-lymphocytes. By day 15, most animals have cleared the virus. However, in certain genetically predisposed mice, antibodies are produced against one of the several myocytic components: the mitochondrial ADP/ATP carrier protein,[11] myosin or laminin heavy chains,[12] β-adrenoceptors[13] or cardiac sarcoplasmic reticulum calcium ATPase.[14] Thus, a chronic inflammatory process is established in the absence of viral material. This may eventually culminate in idiopathic dilated cardiomyopathy.

Table I (continued) Agents Associated with Myocarditis

Immune-Mediated Myocarditis		
Drug Allergens	Acetazolamide	Methyldopa
	Amitryptiline	Penicillins
	Colchicine	Phenytoin
	Furosemide	Reserpine
	Isoniazid	Streptomycin
	Lidocaine	Tetracycline
Serum sickness		
Tetanus toxoid		
Alloantigens	Organ transplants	
'Autoantigens	Inflammatory bowel disease	Sarcoidosis
	Giant cell myocarditis	Systemic lupus erythematosus
	Kawasaki disease	Wegener granulomatosis
	Myasthenia gravis	
Toxic Agents		
Drugs	Amphetamines	5-Flourouracil
	Anthracyclines	Hemetine
	Catecholamines	Interleukin-2
	Cocaine	Lithium
	Cyclophosphamide	
Heavy metals	Copper	Lead
	Iron	Phosphorus
Miscellaneous	Arsenic	Carbon monoxide
	Azide	Scorpion, snake and spider bites
	Bee and wasp stings	
Physical agents	Electric shock	
	Hyperpyrexia	
	Ionizing radiation	

These pathogenic mechanisms are common to other viruses and infectious agents as well.[2] It is believed that these mechanisms also apply to myocarditis in humans. In fact, in human models of myocarditis, the adenine nucleotide translocator, calcium channel, β-receptor, myosin heavy chain, extracellular matrix proteins (laminin and collagen), cardiac conduction system, and vascular endothelium have been shown to be involved in the humoral immune response leading to inflammatory heart muscle disease.[3]

Although lymphocytic (viral) myocarditis is most common, there are various other forms of myocarditis based on histopathologic classification. Autoimmune lymphocytic or giant cell myocarditis is characterized by diffuse myocardial necrosis with multinucleated giant cells.[15] It occurs in genetically predisposed mice after immunization with cardiac myosin in the absence of viral antigen.[16] In humans,

the disease is similar to that in murine models, and circulating autoantibodies to myosin have been detected in a significant proportion of patients.[17] The disease is rare and is often associated with other autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, Wegener granulomatosis, etc.[18] The disease has a rapidly progressive course and produces severe hemodynamic instability, arrhythmias, heart block, and congestive heart failure. Unfortunately, these patients are not good candidates for transplantation, as the disease has a high level of recurrence in the transplanted heart.[19]

There are two other forms of myocarditis worth mentioning briefly. In South America, *Trypanosoma cruzi* is a frequent cause of Chagas myocarditis. Because of the neurotoxic properties of *T. cruzi*, Chagas myocarditis is marked by frequent conduction disturbances, tachyarrhythmia, heart block, and the im-

pairment of autonomic control.[4] Interestingly, the disease is more prevalent in younger individuals.[20] Lastly, eosinophilic myocarditis is fairly rare and develops in response to certain drugs (Table I). Histopathologically, it presents with perivascular infiltrates involving a predominance of eosinophils, lymphocytes, and histiocytes. It is believed to be either a toxic, dose-dependent phenomenon, or an allergic (hypersensitivity) reaction. The patient will frequently present with rash and fever; complete blood count will often reveal peripheral eosinophilia.[6]

TREATMENT OF MYOCARDITIS

The past several years have generated a large quantity of data regarding the treatment of myocarditis. Animal studies and clinical trials have focused on a variety of therapeutic options, including immunosuppressants, immunomodulators, antiviral agents, calcium-channel blockers, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, nitric oxide inhibitors, α-blockers, nonsteroidal antiinflammatory agents, immunoglobulin, and transplantation (Table II). Each of these will be discussed briefly.

The one treatment strategy that is universally advocated for myocarditis is bed rest. Results of both animal studies and clinical trials support this theory. In guinea pigs infected with myocarditis, higher mortality and more extensive histologic disease was observed in animals that were exercised compared with unexercised controls.[21] In terms of clinical trials, one metaanalysis of data available on a mixed cohort of subjects (with biopsy and with no biopsy) revealed that 57% improved with standard therapy and restricted physical activity alone.[3]

Thus, all patients suspected of having acute myocarditis should be admitted to a hospital for observation and a regimen of modified bed rest designed to decrease myocardial damage and promote healing. A noninvasive assessment of myocardial function can be assessed by means of physical examination, Holter monitoring, echocardiography, and radionuclide ventriculography. A duration of convalescence can then be recommended to the patient.[4]

Conventional medical management (Table III) of the patient with myocarditis includes the following: general measures designed to decrease cardiac work; specific treatment of any underlying infection; and control of complications such as congestive heart failure, cardiogenic shock, heart block, arrhythmias, and thromboembolism.[4] Additionally, patients should avoid alcohol and cigarettes. A passive physical activity program with a very low level of caloric expenditure is appropriate and will help prevent venous stasis and skeletal muscle atrophy. Other supportive measures include supplemental oxygen for patients who are tachycardic, hypoxemic, or have

Table II Susceptibility Factors in Viral and Autoimmune Experimental Myocarditis

Factor	Viral	Autoimmune
Host genes and age	Variable	variable
Exercise and malnutrition	Increases	unknown
Sex (androgens)	Increases	increases
Sex (estrogens)	Decreases	decreases
NSAID		
Ibuprofen	Increases	unknown
Indomethacin, aspirin	Increases (early), No effect (late)	no effect
Immunosuppressive Agents		
Corticosteroids	Increases (early), No effect (late)	no effect/ decreases
Cyclosporine	Increases	decreases
Cyclophosphamide	Increases	unknown
15-deoxyspergualin	Unknown	decreases
anti-TCR antibody	Unknown	decreases
Immunomodulatory Agents		
Human immunoglobulin	Decreases	decreases
Interleukin-1	Increases	increases
Interleukin-2	Decreases/increases	increases
TNF	Increases	increases
anti-TNFα/β antibody	No effect	decreases
anti-IFN-gamma antibody	Unknown	increases
Vesnarinone	Decreases	unknown
Levamisole	Increases	unknown
Cuinoline-3-carboxamide	Decreases	unknown
Anti-Viral Agents		
BICLF-70	Decreases	unknown
IFN-α/β	Decreases	unknown
Ribavirin	Decreases	unknown
WIN 54954	Decreases/no effect	unknown
Conventional Heart Failure Drugs		
Captopril	Decreases	unknown
TCV-116	Decreases	unknown
Metoprolol	Increases	unknown
Heparin	Decreases	unknown

Adapted with permission from Caforio and McKenna.[7] NSAIDs, nonsteroidal antiinflammatory agents; TCR, T cell receptor; TNF, tumor necrosis factor; IFN, interferon.

low cardiac output. Anemia should be corrected so as to improve tissue oxygenation.

If the patient develops clinical signs of heart failure, medical therapy should be initiated. As recent investigations have revealed, the activation of neurohumoral mechanisms with subsequent left ventricular impairment can occur even in asymptomatic patients.[13] Thus, ACE inhibitors are recommended at a very early stage of heart failure to prevent myocardial remodeling. Although effective at reducing symptoms and improving exercise tolerance, diuretics further stimulate neurohumoral systems.[13] Thus, diuretics should only be used in combination with ACE inhibitors, and only if necessary.

Table III Medical Management of Myocarditis-
Induced Heart Failure

Bed rest
Salt and fluid restriction
Digitalis
Angiotensin-converting enzyme inhibitors
α- and β-adrenergic blockers/β-adrenergic blockers
Diuretics
Anticoagulants
Antiarrhythmics
Correction of anemia or infection

With progressive heart failure, combination therapy with ACE inhibitors, diuretics, and cardiac glycosides should be initiated. With diuretic use, serum potassium levels should be monitored, as hypokalemia may provoke arrhythmias and myocardial abnormalities. Arrhythmias should be detected and treated before they become life-threatening. Since most antiarrhythmic agents produce some depression of myocardial contractility, they should be used with caution.

In patients with New York Heart Association (NYHA) class II or III heart failure and dilated cardiomyopathy, the addition of a β-blocker may be beneficial.[13] A low-dose regimen of carvedilol (3.25 mg orally twice daily) can be started and the dose increased slowly. If the patient shows no improvement or reveals evidence of decompensation, a positive inotrope (e.g., intravenous dobutamine) can be used intermittently.

In general, the prognosis of myocarditis-related new onset heart failure is as follows: half of patients attain major improvement in left ventricular function, one quarter stabilize at low function, and one quarter deteriorate so rapidly as to require urgent transplantation.[22]

TREATMENT STRATEGIES UNDER INVESTIGATION

Immunosuppression

The major difficulty in defining a specific therapy for myocarditis lies in characterizing each patient's illness as either viral or autoimmune. Multiple studies have shown that immunosuppressive therapy should be avoided in patients with active viral myocarditis[23–27] because it promotes viral spread in the heart and hampers antiviral immune reactions, thus allowing unhindered viral cytolysis. Unfortunately, distinguishing viral from autoimmune forms of myocarditis has proven to be difficult. Techniques designed to distinguish autoimmune from viral myocarditis (e.g., viral hybridization) vary greatly

between institutions. Even the Dallas criteria, which were created to standardize the histopathologic diagnosis of myocarditis, allow considerable interobserver variability.[28]

This difficulty with histopathologic classification partially explains the inconclusive results of immunosuppressive trials in myocarditis/dilated cardiomyopathy. Apart from the ongoing European Study of Epidemiology and Treatment of Cardiac Inflammatory Disease (ESETCID) trial,[29] the recent Myocarditis Treatment Trial (MTT)[30] is the only double-blind, randomized trial of immunosuppressive therapy to have ever been done. Patients in this trial were classified with active myocarditis based on the Dallas criteria and received either azathioprine and prednisone or cyclosporine and prednisone. The results were inconclusive: there appeared to be no hemodynamic or prognostic benefit to immunosuppression after 6 months of treatment.[30]

There are several reasons why the MTT may have failed to demonstrate any benefit of immunosuppression.[3] One is that the trial relied on light microscopy to screen patients for myocarditis. Light microscopy is no longer considered the best method for histologic classification, as there are currently far more sensitive methods for detecting immunohistochemical changes. Thus, it is possible that many of the patients treated with immunosuppressive therapy may have harbored viral material that was not detected by endomyocardial biopsy and light microscopy. It is also likely that the duration of treatment (6 months) was too short to reveal improvement. Moreover, the trial failed to distinguish between the different immunosuppresive agents, some of which are more beneficial than others. Mason et al[31] and Maisch et al[32] reported decreased myocardial inflammation using a regimen of prednisone and azathioprine. The combination of cyclosporine and prednisone, however, has not been shown to be beneficial, as demonstrated by the MTT.[30]

Other than the MTT, there are several other clinical trials—controlled and uncontrolled—that have looked at the effect of prednisone, azathioprine and/or cyclosporine treatment in myocarditis. All controlled studies of azathioprine have looked at the drug in combination with prednisone.[32–35] Of these studies, only that of Maisch et al[32] demonstrated significant improvement in the patients given azathioprine and prednisone compared with control subjects. The only controlled clinical trial with cyclosporine is the MTT, which looked at the drug in combination with prednisone. The MTT revealed neither benefits of nor harm from cyclosporine treatment.[30]

There has been much more research on corticosteroids than either cyclosporine or azathioprine. Unfortunately, results of animal studies of corticoste-

Table IV Placebo-Controlled Trials with Immunosuppressives in Myocarditis: A Metaanalysis

Study (ref.)	Tx	No. of Patients, Tx/Co	Tx		Co	
			Improvement	Unchanged/ Deteriorated	Improvement	Unchanged/ Deteriorated
Edwards (43)	P	5/5	3 (60%)	2 (40%)	3 (60%)	2 (40%)
Fenoglio (37)	P, A + P	18/4	7 (39%)	11 (61%)	2 (50%)	2 (50%)
Dec (38)	A + P	9/18	4 (44%)	5 (56%)	6 (33%)	12 (67%)
Anderson (39)	A + P	7/10	2 (29%)	4/1 (57%/14%)	3 (30%)	5/2 (50%/20%)
Maisch (3)	A + P, P	16/18	9 (56%)	7 (44%)	7 (39%)	8/3 (44%/17%)
Maisch (36)	A + P	21/21	10 (47%)	65 (29%, 24%)	3 (14%)	12/6 (57%/29%)
All control trials	P, A + P	76/76	35 (46%)	41 (54%)	24 (32%)	52 (68%)
MTT (30)	P + Cy	64/47	49 (76%)	15 (24%)	25 (53%)	14/8 (30%/17%)

Adapted with permission from Maisch et al.[3] Values are given as number (%). Tx, treatment group; Co, control group; A, azathioprine; P, prednisone; Cy, cyclosporine.

roids have not been promising. Using a murine model of Coxsackievirus B3 myocarditis, Kilbourne et al[36] showed an increase in mortality and myocardial damage with corticosteroid treatment early in the course of infection. Matsumori et al[26] demonstrated similar results in mice infected with encephalomyocarditis virus. They also demonstrated that mice treated later in the course of infection (more than 14 days after inoculation) did not experience an increase in mortality with corticosteroid treatment. In terms of clinical trials, several small, uncontrolled trials have shown a benefit with prednisone treatment in acute myocarditis.[33,37–39] However, the two controlled clinical trials[33,39] have shown no advantage to prednisone therapy.

Maisch et al[3] did a metaanalysis of all trials of immunosuppressive agents (cyclosporine, azathioprine, and/or prednisone) and noted that 61% of all subjects showed improvement with immunosuppressive therapy. No change or deterioration was seen in 39%. When the controlled trials were selected from the metaanalysis, there was little difference between treated and control subjects with regard to improvement, deterioration, or clinical and hemodynamic parameters (Table IV).

In murine models of myocarditis, the response to immunosuppressive treatment has been largely predictable based on the etiology of the myocarditis. In autoimmune giant cell myocarditis, cyclosporine and FK 506 but not prednisolone have been shown to improve or prevent myocardial damage.[40,41] In enteroviral myocarditis, however, treatment with cyclosporine and corticosteroids has been shown to worsen or not change outcome.[26,42]

Thus, at present, immunosuppression cannot be recommended as routine treatment for myocarditis until further clinical trials are carried out in patients who are identified as having either viral or autoimmune involvement. If conventional management

fails and the patient has severely disturbed left ventricular function, immunosuppression may be considered. However, viral diagnosis either by in situ hybridization or polymerase chain reaction is essential. Strauer et al[13] suggest that the following immunohistologic criteria be met before immunosuppression is instituted: 1) lymphocyte infiltrate; 2) increased HLA class I and II expression; 3) increased expression of adhesion molecules on the endothelium and in the interstitium; and 4) increased finding of IgA, IgG, and IgM. Additionally, there should be no evidence of myocytolysis or persistent viral genome. Preliminary results of prednisone therapy in patients who have met the above criteria are positive.[38,40]

As mentioned previously, the use of immunosuppressive therapy in patients with viral myocarditis may promote viral spread and lead to clinical deterioration. However, in instances where the underlying disease is clearly immune-related (such as systemic lupus erythematosus) or believed to be immune-related (such as sarcoidosis), a trial of immunosuppressants may be helpful. In patients with giant cell myocarditis who fail to respond to conventional management, immunosuppression with a combination of cyclosporine, azathioprine, and corticosteroids may be considered as well.[43] However, more data need to be obtained before recommendations can be made.

The ongoing ESETCID[29] trial attempts to address these treatment issues by dividing patients into three subgroups based on the etiology of their disease: autoimmune, enteroviral, or cytomegalovirus-induced disease. Enterovirus-positive patients will be treated with interferon-α; cytomegalovirus patients with hyperimmunoglobulin; and autoimmune (viral negative) patients with immunosuppression. Hopefully this trial will better define the role of immunosuppressive agents in the treatment of myocarditis.

Nonsteroidal Antiinflammatory Agents

Although no prospective controlled studies have been conducted, nonsteroidal antiinflammatory agents have been studied in animal models of viral and autoimmune myocarditis. Indeed, a number of studies have shown that salicylates, indomethacin, and ibuprofen, given early in the course of myocarditis, lead to exacerbation of the disease with more severe histologic damage.

Costanzo-Nordin et al[44] found that ibuprofen (15 mg kg intraperitoneally) aggravated myocardial inflammation and necrosis when given to Balb/c mice with acute Coxsackievirus B3 myocarditis. In a similar model of myocarditis, CD-1 mice infected with Coxsackievirus received salicylates, indomethacin, or saline solution daily for 10 days.[45] When the mice were compared, mortality and virus titers were higher, interferon levels were lower, and pathologic changes were worse in the animals treated with nonsteroidal antiinflammatory drugs. When indomethacin was administered during the late phase of Coxsackievirus myocarditis (10–20 days after infection), it did not have a significant effect on mortality or histopathologic grade.[46] Thus, it is safe to administer indomethacin only after the acute phase of myocarditis when viral replication has been completed.

Zhang et al[41] investigated the effect of aspirin in Lewis rats with autoimmune myocarditis. After immunization with cardiac myosin, the rats were given either phosphate-buffered saline or aspirin (15 mg/kg) intraperitoneally for 21 days. Compared with phosphate-buffered saline, the aspirin had neither beneficial nor detrimental effects.

Thus, preliminary animal data offer no role for nonsteroidal antiinflammatory agents in the management of both viral and autoimmune myocarditis. Currently, nonsteroidal antiinflammatory agents are being commonly used in the treatment of pericarditis. However, the unfavorable data mentioned above should lead clinicians to question the use of such drugs in patients with combined myocarditis and pericarditis.

Immunoglobulins

Recently, an increasing amount of data have revealed a beneficial role for immunoglobulins in the treatment of viral myocarditis and dilated cardiomyopathy. The efficacy of high-dose immunoglobulins in the treatment of respiratory syncytial virus,[47] idiopathic thrombocytopenic purpura,[48] Kawasaki disease,[49] and other inflammatory diseases has been shown repeatedly over the past decade. In 1994, Drucker et al[50] reported improvement in survival and left ventricular function in children with acute Kawasaki myocarditis who were treated with high-dose intravenous immunoglobulins. Since then there have been several other studies with similarly positive results.

An initial in vitro study by Takada et al[8] revealed that immunoglobulin suppressed Coxsackievirus B3 in a dose-dependent manner. Immunoglobulin (1 g/kg/daily intraperitoneally) was administered to Coxsackievirus B3-infected mice for 2 weeks, beginning simultaneously with virus inoculation in experiment 1 and on day 14 after inoculation in experiment 2. In experiment 1, immunoglobulin administration completely suppressed the development of myocarditis. In both experiments, survival was higher in treated than in untreated mice; at the time of death, inflammatory cell infiltration, necrosis, and calcification were reduced as well.

In histologically proven cytomegaloviral myocarditis in humans, Maisch et al[3] demonstrated that treatment with cytomegaloviral hyperimmunoglobulin led to the improvement of hemodynamic function and the eradication of virus and lymphocytes from the myocardium. McNamara et al[51] examined the role of high-dose immunoglobulins in the treatment of 10 patients with new-onset dilated cardiomyopathy. Three of these patients revealed evidence of myocardial inflammation and all were hospitalized with NYHA class III/IV heart failure. In addition to conventional therapy for heart failure (ACE inhibitors, digoxin, and diuretics), each patient received 2 g/kg of intravenous immunoglobulin after their initial evaluation. After a median of 18 months of therapy (range 14–24 months), all patients demonstrated an improvement in left ventricular ejection fraction ranging from 6 to 28 points. Left ventricular ejection fraction for the entire group improved from 24% to 42%.

Thus, recent data suggest a potentially therapeutic role for immunoglobulin in viral myocarditis and its unfortunate sequelae, dilated cardiomyopathy. Takada et al[8] suggest two potential mechanisms for this effect. In the acute viremic stage it is possible that polyclonal immunoglobulin may directly transfer antiviral antibody. In the later aviremic stage of myocardial inflammation, immunoglobulin may alter the immune response (by reticuloendothelial system blockade or induction of idiotype-antiidiotype networks), thus leading to a decrease in cardiac inflammation.

Compared with steroids, cyclosporine, and other immunosuppressive agents, immunoglobulin is much better tolerated and has fewer adverse effects. Indeed, the most common side effects of therapy are mild flu-like symptoms or headache, observed in 5% to 10% of patients.[51] Consequently, a large, randomized, placebo-controlled clinical trial of immunoglobulin therapy in patients with myocarditis is much needed. The ESETCID fulfills part of this need

by examining the effect of hyperimmunoglobulin in cytomegalovirus myocarditis.[29] However, the studies by McNamara et al[51] and Drucker et al[50] suggest that this may be an effective therapy for other viral forms of myocarditis as well.

Cytokines and Immunomodulation

As previously mentioned, the process by which viral myocarditis progresses to dilated cardiomyopathy has two stages. Initially, a protective immune response is induced by infiltrating macrophages, natural killer cells, and antiviral antibodies. This is followed by a destructive immune response to cardiac tissue.[52] A growing body of evidence suggests that cytokines play an important role in this process. In murine models of myocarditis and heart failure, cytokines were shown to be expressed along with segments of the viral genome.[53] Further, studies have proven that patients with acute myocarditis have elevations of interleukin (IL)-1_α, IL-1_β, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF), and macrophage colony-stimulating factor (M-CSF). Patients with dilated cardiomyopathy have elevations of TNF-α as well.[54] Given these findings, it is hardly surprising that many researchers are attempting to modulate cytokine expression in viral myocarditis. Most of this work has focused on the modulation of IL-2, TNF, and interferon-α (IFN-α).

Interleukin-2

Released from activated T cells, IL-2 induces the differentiation and proliferation of T and B cells. It is also responsible for the induction of antigen-specific killer cells and the proliferation of natural killer cells. Although IL-2 has been reported to exert an antiviral effect in other diseases, its role in the treatment of myocarditis remains tenuous. Huber et al[55] reported a worsening of myocarditis with administration of IL-1 and IL-2 to mice infected with Coxsackievirus. On the other hand, Kishimoto et al[56] noted that the administration of IL-2 during the acute viremic stage of myocarditis increased survival and decreased myocardial damage. However, administration of the same amount of IL-2 in the subacute aviremic stage reduced survival and exacerbated the severity of disease. This seems to imply that cytokines aid in the induction of a beneficial immune response in the acute stage of viral myocarditis. In later stages, however, these cytokines may promote a destructive response against myocardial tissue.

Interferon-α

Produced primarily by leukocytes, IFN-α appears 4 to 6 hours after viral stimulation and is believed to reduce viral replication. In 1988, Kishimoto et al[57] demonstrated that IFN-α was capable of inhibiting viral replication and reducing myocardial inflammation when given to mice before infection with Coxsackievirus B3. When Coxsackievirus B3-infected mice were treated with M-CSF from days 0 to 14 of infection, cardiac disease was noted to be significantly reduced. The reduction in myocardial pathologic changes and viral titers was correlated directly with elevations in IFN-α induced by M-CSF.[58] Moreover, encephalomyocarditis mice treated with immunosuppressive agents and IFN-α have better survival than mice treated with immunosuppressive agents alone.[59]

A recent study examined the effect of IFN-α in humans with biopsy-proven myocarditis or dilated cardiomyopathy.[60] Compared with patients treated with conventional therapy for heart failure, those treated with adjunctive IFN-α were much more likely to have improved left ventricular ejection fraction and exercise tolerance. Side effects of IFN-α treatment were relatively mild and confined to flu-like symptoms, lassitude, and malaise. These symptoms decreased with repeat injections and usually resolved after 10 days of treatment.

Tumor Necrosis Factor

Released by activated macrophages, TNF has been shown to increase myocardial damage in murine models of myocarditis. Smith and Allen[61] showed that TNF is a key mediator in the pathogenesis of inflammation in murine autoimmune myocarditis. Similar results were observed in an animal model of Coxsackievirus myocarditis.[52] Mice that were treated with recombinant TNF-α experienced increased mortality; likewise, mice treated with anti–TNF-α monoclonal antibody revealed increased survival and decreased myocardial damage compared with controls.

Vesnarinone, a recently synthesized positive inotropic agent, has shown promising results in animal models of heart failure. A quinolinone derivative, vesnarinone suppresses TNF-α in a dose-dependent manner.[62] Further, when encephalomyocarditic mice in congestive heart failure are treated with vesnarinone, they experience decreased mortality in comparison to untreated controls. These findings suggest that vesnarinone could play a significant role in cytokine regulation and may be of benefit in the treatment of patients with myocarditis-induced heart failure. However, recent mortality studies have shown a detrimental effect of vesnarinone in pa-

tients with chronic heart failure of a predominant ischemic type.

Antiadrenergics

β-Adrenergic blockade. Although β-blockers have been shown to be of benefit in heart failure and dilated cardiomyopathy, preliminary animal studies suggest that they should be avoided in acute myocarditis. An experimental study done by Rezkalla et al[63] looked at Swiss-Webster mice infected with Coxsackievirus B3. Increased mortality and myocyte necrosis were observed in mice treated with metoprolol compared with those treated with normal saline. Within the first 30 days of infection, only 40% of the metoprolol-treated mice survived compared with 100% of the saline group. The increased number of deaths was associated with greater viral replication on day 10 of infection and more myocardial necrosis on day 30 of infection, as detected by histopathologic studies. Although the mechanism of this effect was not determined, it was felt that β-blockers may interfere with the body's defense against viral replication. Indeed, previous studies have shown that propranolol can inhibit the phagocytic activity of polymorphonuclear cells and monocytes[64] and the activation of lymphocytes.[65]

The negative effects associated with the use of certain β-blockers may result from a lack of $β_2$-adrenergic blockade and/or intrinsic sympathomimetic activity. When Tominaga et al[66] compared carteolol to metoprolol in a murine model of acute, subacute, and chronic myocarditis, they noted that the carteolol group had smaller left ventricular size, wall thickness, myocardial fiber diameter, and histopathologic scores compared with both the metoprolol and control groups. Hence, further studies need to be performed to determine whether the negative effect observed by Rezkalla et al[63] is exclusive to metoprolol. Currently, there are no studies looking at β-blocker use in human models of myocarditis.

α-Adrenergic blockade. Studies performed in the 1970s showed a benefit to using α-blockers in patients with congestive heart failure.[67,68] In 1992, Yamada et al[69] investigated the therapeutic effect of the α1-blocker bunazosin on a murine model of encephalomyocarditis-induced heart failure. Four-week-old Balb/c mice were inoculated with encephalomyocarditis virus and injected daily with either bunazosin or saline. Although bunazosin did not alter the overall survival rate of infected mice, it did show a protective effect if injected from the day of viral inoculation to day 14 when congestive heart failure began. Compared with the control group, the hearts of bunazosin-treated mice revealed lower ra-

tios of heart weight to body weight, left ventricular dimension, and histopathologic grade. Thus, this initial animal study suggests that bunazosin, a balanced arterial and venous vasodilator, has a protective effect against viral myocarditis when started early after infection and continued until the stage of congestive heart failure. Unfortunately, there have been no further studies with α-blockers since the trial of bunazosin.

Calcium-Channel Blockers

In murine models of encephalomyocarditis, the calcium-channel blockers verapamil and amlodipine have been shown to have a protective effect against myocardial injury. Verapamil, a heart rate-lowering calcium-channel antagonist, has been shown to reduce the clinical and pathologic effects of cardiomyopathy in Syrian hamster[70] and murine Chagas models.[71] In these diseases and in acute murine myocarditis, microvascular spasm is believed to contribute to the development of cardiomyopathy. In a study of DBA/2 mice infected with encephalomyocarditis, verapamil given either intraperitoneally beginning 7 days before infection or orally beginning 4 days after infection was shown to significantly reduce microvascular changes and myocardial necrosis, fibrosis, and calcification leading to cardiomyopathy.[72] Although mortality rates were the same in both verapamil-treated and control mice, the time of death was significantly delayed in the verapamil-treated group. The authors believe that verapamil's seeming lack of an effect on mortality is due to the early deaths (within 14 days of infection) of the animals when verapamil is not believed to have an effect. This was confirmed by pathologic findings: myocardial inflammation and necrosis during the first week was not dramatically different in treated and untreated animals, but was significantly different after day 14 (peak infection). Hence, there may be need for further studies that examine long-term mortality in verapamil-treated and untreated mice.

Although studies have found the calcium-channel blockers diltiazem and verapamil to be detrimental in patients with congestive heart failure,[73,74] a recent double-blind, placebo-controlled clinical trial of amlodipine, a novel dihydropyridine calcium-channel blocker, revealed a 45% reduction in the risk of death in patients with heart failure resulting from nonischemic dilated cardiomyopathy.[75]

Given these promising results, a study was created to compare the effects of amlodipine with diltiazem and placebo in DBA/2 mice infected with encephalomyocarditis.[76] The mice were inoculated with encephalomyocarditis and administered amlodipine, diltiazem, or vehicle orally for 2 weeks. By day 14, 70% of the mice in the amlodipine group had sur-

vived compared with 50% in the diltiazem group and 33% in the control group. Although viral titers were not influenced by amlodipine, the ratios of heart weight to body weight and the histopathologic grades of myocardial lesions were significantly lower in the amlodipine-treated mice.

In follow-up studies, the authors noted that amlodipine inhibited encephalomyocarditis-induced nitric oxide production in a concentration-dependent manner in both a macrophage-cell line and in the hearts of mice with congestive heart failure. Diltiazem did not have this effect. It is hypothesized that the beneficial effects of amlodipine result from inhibition of myocardial nitric oxide production. This is consistent with previous studies of the dihydropyridine calcium-channel blockers,[77] and with studies confirming the role of nitric oxide in myocarditis.[78]

Aminoguanidine and Nitric Oxide Inhibition

As previously mentioned, recent studies have demonstrated that patients with myocarditis have elevated circulating levels of bacterial endotoxins (such as lipopolysaccharides) and proinflammatory cytokines such as IL-1β and TNF-α.[79] The negative inotropic effect of these substances on cardiomyocytes has been shown to be mediated by the *de novo* synthesis of iNOS and the subsequent production of nitric oxide.[79] In two separate studies of experimental autoimmune myocarditis in rats, researchers demonstrated that excess amounts of nitric oxide produced by iNOS contributed to the progression of myocardial damage. Further, aminoguanidine, an inhibitor of iNOS, decreased the pathophysiologic sequelae of myocarditis.

Ishiyama et al[80] induced autoimmune myocarditis in 20 Lewis rats by injection of porcine cardiac myosin. Ten of the 20 rats were given aminoguanidine (400 mg/kg intraperitoneally). The animals were killed 21 days after infection and the severity of myocarditis evaluated by measuring the size of inflammatory lesions and the levels of CK-MB. Histopathologic study revealed extensive myocardial destruction and inflammatory cell infiltration in the untreated rats, but only focal mononuclear infiltrates in the aminoguanidine-treated rats.

In a similar study by Hirono et al,[78] aminoguanidine was systemically administered to myosin-immunized Lewis rats from either days 0 to 10 or days 11 to 21 to elucidate the contribution of iNOS in two different stages of the immune response. Although the administration of aminoguanidine from days 11 to 21 ameliorated both histopathologic and functional changes in the rat hearts, administration between days 0 and 10 had no effect. These observations suggest that nitric oxide has little effect on the initial immune responses induced by antigen challenge; nonetheless, it may play an important role in the effector stage of experimental autoimmune myocarditis. Thus, selective inhibition of iNOS may be an effective treatment strategy for autoimmune myocarditis. However, more research needs to be conducted in this area.

Angiotensin-Converting Enzyme Inhibitors

As previously discussed, ACE inhibitors constitute part of the conventional treatment regimen for patients with myocarditis-related heart failure. However, recent experimental studies indicate that captopril in particular may be effective therapy for acute viral myocarditis.

In mice with acute Coxsackievirus B3 myocarditis, captopril administered at 0.05 mg/g intraperitoneally reduced heart weight and myocardial necrosis when administered within the first 6 days of infection.[81] When administered later in the course of infection (day 10), captopril was still shown to reduce heart weight. The beneficial effects of captopril were confirmed by Suzuki and Matsumori[82] in a murine model of encephalomyocarditis. When the drug was administered to mice at doses of 10, 30, or 100 mg/kg, survival increased and myocardial injury decreased in a dose-dependent manner.

Whether the other ACE inhibitors have a similarly beneficial effect is unclear. When captopril was compared with enalapril in a murine model of myocarditis, only the former improved survival and limited cardiac injury.[83] Moreover, captopril is the only ACE inhibitor known to reduce myocardial inflammation and necrosis. This effect may result from the oxygen-radical scavenging properties of captopril.[84] The other ACE inhibitors have been shown to decrease cardiac mass, an effect that may be secondary to a decrease in afterload or to the inhibition of protein synthesis.

TCV-116, a new angiotensin II type 1 receptor antagonist, was recently studied in a murine model of encephalomyocarditis.[85] When treatment with 10 mg/kg was started one day before inoculation, the survival of mice was improved slightly, although not enough to be statistically significant. Heart weight and myocardial necrosis, however, were significantly reduced. The authors concluded that angiotensin II plays an important role in viral myocarditis and that angiotensin II receptor antagonists have a cardioprotective effect.

Antiviral Agents

Antiviral therapy has been shown to benefit murine models of viral myocarditis provided that treatment is started early in the course of infection. In 1985 Matsumori et al[86] first showed that mice treated with

ribavirin from the first inoculation with encephalomyocarditis had prolonged survival and less myocardial damage than controls. Kishimoto et al[87] had similar results using a murine model of Coxsackievirus myocarditis. When ribavirin administration was begun on day 1 of infection, viral replication and myocardial damage were reduced and survival increased. When treatment was started on day 4 of illness, no difference was seen between the experimental and control mice.

Recently a new class of antiviral compounds (isoxazoles) has been synthesized. These drugs have a broad antipicornavirus activity and function by inhibiting viral uncoating. WIN 54954, a newly synthesized disoxaril analogue, was recently shown to inhibit Coxsackievirus B3 replication in vitro when given at a minimal inhibitory concentration.[88] Further, in A/J mice with Coxsackievirus B3 myocarditis, WIN 5454 (100 mg/kg orally twice daily) given from days 0 to 5 of infection conferred significant protection from enteroviral mortality. At 3 weeks after infection, more than 80% of the mice given WIN 54954 were alive compared with only 10% of the control group. The drug did not have any effect on Coxsackievirus B3 antibody titers, macrophage infiltration, or the expression of surface lymphocyte subset markers. Unfortunately, no studies have looked at the effect of WIN 54954 treatment after viral inoculation. Given the initial promising effects of WIN 54954 on survival and the fact that it does not appear to interfere with cellular and humoral immunity, the drug may be a promising candidate for further trials.

Transplantation

Cardiac transplantation remains the last resort for patients with chronic myocarditis who develop refractory heart failure. In the acute phase transplantation should be avoided, as many patients will recover completely with conventional treatment. Moreover, with acute autoimmune forms of myocarditis (such as giant cell myocarditis), there is a high incidence of recurrence in the transplanted heart.[19] In the past it was believed that patients with myocarditis undergoing transplantation fared worse than patients undergoing transplant for other reasons. This idea was based on a study that reviewed a small series (n = 12) of patients.[89] Recent evidence contradicts this previous report. A 1995 study that used multivariate analysis to compare transplant recipients with myocarditis with those with other diagnoses revealed no significant difference in outcome.[90] Although this study had a much larger sample size that the original one, attempts were not made to independently confirm the diagnosis of myocarditis in the explant.

Another recent study of heart transplantation in patients with end-stage Chagas heart disease also calls into question previous reports that cited an increased likelihood of disease recurrence in patients who undergo transplantation for treatment of Chagas myocarditis. DeCarvalho et al[91] noted a lower frequency of recurrence in patients with Chagas myocarditis compared with age- and sex-matched controls. Moreover, they noted no signs of disease recurrence in the allografts. Hence, recent findings suggest that cardiac transplantation may be a more viable option for patients with dilated cardiomyopathy than was previously thought. Nonetheless, the risks and complications of transplant, along with the promising new armamentarium of medical treatment, still leave this a last, albeit lifesaving, option.

CONCLUSION

It would certainly behoove us to find effective therapies for myocarditis. Idiopathic dilated cardiomyopathy accounts for 25% of all cases of heart failure in the United States.[92] Heart failure itself accounted for $38 billion or 5.4% of federal healthcare dollars in 1991.[9]

Although the past century has considerably expanded our understanding of myocarditis, our treatment strategy remains essentially unchanged. In the future, if we are to effectively target the illness, the following needs to be accomplished:

First, more precise diagnostic methods are needed, so that the illness is identified before the patient has progressed to cardiac failure. Unfortunately, the varied, often nonspecific, clinical presentation of myocarditis will evade even the most astute diagnostician. Even if cardiac troponin 1 and creatine phosphokinase (CPK) levels prove to be sensitive and specific indicators of disease, the individual physician must have cause to suspect myocarditis before ordering such tests.

Second, more accurate methods of distinguishing autoimmune from viral forms of myocarditis are necessary, so that appropriate treatment is initiated. As the MTT demonstrated,[30] current histologic techniques alone are not adequate. Polymerase chain reaction and other molecular biology techniques may hold more promise in terms of detection of viral genome.

Third, in keeping with the above, guidelines will be needed to define treatment regimens for different types of myocarditis (viral, autoimmune, etc.). Clearly, immunosuppressive agents should not be used in patients with viral myocarditis. However, they may be highly effective in autoimmune forms of the illness. Antiviral agents may be beneficial in treating viral myocarditis if started early in the course of infection. As the literature points out,

IFN-α and immunoglobulins have shown tremendous potential in myocarditis treatment. Further clinical trials will be needed to verify the efficacy of these treatments before recommendations can be made.

As previously mentioned, the ongoing ESETCID trial[29] attempts to address these issues by treating patients with immunosuppression, hyperimmunoglobulin, and IFN-α for autoimmune, cytomegaloviral, and enteroviral myocarditis, respectively. The trial will hopefully shed much light on this complex topic.

REFERENCES

1. Rezkalla SH, Kloner RA: Management strategies in viral myocarditis. *Am Heart J* 1989;117:706–708.

2. Olinde KD, O'Connell JB: Inflammatory heart disease: pathogenesis, clinical manifestations, and treatment of myocarditis. *Annu Rev Med* 1994;45:481–490.

3. Maisch B, Herzum M, Hufnagel G, Schonian U: Immunosuppressive and immunomodulatory treatment for myocarditis. *Curr Opin Cardiol* 1996;11:310–324.

4. Wenger NK, Abelmann WH, Roberts WC: Myocarditis. In, Hurst JW, Schlant RC (eds.): *The Heart. Seventh edition.* New York: McGraw Hill, 1990;1256–1277.

5. Maze SS, Adolph RJ: Myocarditis: unresolved issues in diagnosis and treatment. *Clin Cardiol* 1990;13:69–79.

6. Pisani B, Taylor DO, Mason JW: Inflammatory myocardial diseases and cardiomyopathies. *Am J Med* 1997;102:459–469.

7. Caforio ALP, McKenna WJ: Recognition and optimum management of myocarditis. *Drugs* 1996;52:515–525.

8. Takada H, Kishimoto C, Hiraoka Y: Therapy with immunoglobulin suppresses myocarditis in a murine Coxsackievirus B3 model. *Circulation* 1995;92:1604–1611.

9. Brown CA, O'Connell JB: Myocarditis and idiopathic dilated cardiomyopathy. *Am J Med* 1995;99:309–314.

10. Patel R, Frishman WH: Cardiac involvement in HIV infection. *Med Clin North Am* 1996;80:1493–1507.

11. Schulze K, Schultheiss HP: The role of ADP-ATP carrier in the pathogenesis of viral heart disease. *Eur Heart J* 1995;16(Suppl O):64–67.

12. Pummerer C, Grassl G, Neu N: Cellular immune mechanisms in myosin-induced myocarditis. *Eur Heart J* 1995;16(Suppl O):71–74.

13. Strauer BE, Kandolf R, Mall G, Maisch B, Mertens T, Schwartzkopff B, Schultheiss HP: Myocarditis-cardiomyopathy. *Acta Cardiol* 1996;4:347–371.

14. Khaw BA, Narula J, Sharaff AR, Nicol PD, Southern JF, Carles M: SR-Ca²⁺ ATPase an autoimmunogen in experimental myocarditis. *Eur Heart J* 1995;16(Suppl O):92–96.

15. Cooper LT, Berry GJ, Rizeq M, Schroeder JS: Giant cell myocarditis. *J Heart Lung Transplant* 1995;14:394–401.

16. Kodama M, Matsumoto Y, Fujiwara M, Masani F, Izumi T, Shibata A: A novel experimental model of giant cell myocarditis induced in rats by immunization with cardiac myosin fraction. *Clin Immunol Immunopathol* 1990;57:250–262.

17. 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.

18. Caforio ALP: Role of autoimmunity in dilated cardiomyopathy. *Br Heart J* 1994;72(Suppl 6):S30–S34.

19. Kong G, Madden B, Spyrou N, Pomerance A, Mitchell A, Yacoub M: Response of recurrent giant cell myocarditis in a transplanted heart to intensive immunosuppression. *Eur Heart J* 1991;12:554–557.

20. Puigbo JJ: Chagas' heart disease: clinical aspects. *Cardiologia* 1968;52:91–96.

21. Kiel RJ, Smith FE, Chason J, Khatib R, Reyrs MP: Coxsackievirus B3 myocarditis in C3H/Hej mice: description of an inbred model and the effect of exercise on virulence. *Eur J Epidemiol* 1989;5:348–350.

22. Stevenson LW: Diseases of the myocardium. In, Bennett JC, Plum F (eds.): *Cecil's Textbook of Medicine. Twentieth edition. Philadelphia: WB Saunders,* 1996;327–336.

23. Cossart YE, Burgess YA: Fatal Coxsackievirus B myocarditis in an adult. *Med J Aust* 1965;1:337–339.

24. Glajchen D: Myocarditis due to Coxsackievirus infection in an adult. *Br Med J* 1961;52:870–871.

25. Matsumori A, Kawai C: Immunomodulating therapy in experimental myocarditis. *Springer Semin Immunopathol* 1989;11:77–88.

26. Matsumori A, Yamada T, Kawai C: Immunomodulating therapy in viral myocarditis: effects of tumour necrosis factor, interleukin-2 and anti-interleukin-2 receptor antibody in an animal model. *Eur Heart J* 1991;12(Suppl D):203–205.

27. Tomioka N, Kishimoto C, Matsumori A, Kawai C: Effects of prednisolone on acute viral myocarditis in mice. *J Am Coll Cardiol* 1986;7:868–872.

28. Shanes JG, Gahli J, Billingham ME, Ferrans YJ, Fenoglio JJ, Edwards WD, et al: Inter-observer variability in the pathological interpretation of endomyocardial biopsy results. *Circulation* 1987;75:401–405.

29. Maisch B, Hufnagel G, Schonian U, Hengstenberg C: The European study of epidemiology and treatment of cardiac inflammatory disease (ESETCID). *Eur Heart J* 1995;16(Suppl O):173–175.

30. Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billingham ME, Moon TE: A clinical trial of immunosuppressive therapy for myocarditis. *N Engl J Med* 1995;333:269–275.

31. Mason JW, Billingham ME, Ricci DR: Treatment of acute inflammatory myocarditis assisted by endomyocardial biopsy. *Am J Cardiol* 1980;45:1037–1044.

32. Maisch B, Schonian U, Hengstenberg C, Herzum M, Hufnagel G, Bethge C, et al: Immunosuppressive treatment in autoreactive myocarditis: results from a controlled trial. *Postgrad Med J* 1994;70(Suppl 1):29–34.

33. Fenoglio JJ, Ursell PC, Kellog CF, Drusin RE, Weiss MB: Diagnosis and classification of myocarditis by endomyocardial biopsy. *N Engl J Med* 1983;308:12–18.

34. Dec WG, Palacios IG, Fallon JT, Southern JF: Active myocarditis in the spectrum of acute dilated cardiomyopathies. *N Engl J Med* 1985;312:885–890.

35. Anderson JL, Fowles RE, Unverferth DV, Mason JW: Immunosuppressive therapy of myocardial inflammatory disease: initial experience and further trials to define indications for therapy. *Eur Heart J* 1987;8(Suppl J):263–266.

36. Kilbourne ED, Wilson CB, Perrier D: The induction of gross myocardial lesions by a Coxsackie (pleurodynia) virus and cortisone. *J Clin Invest* 1956;35:362–367.

37. Sekiguchi M, Yu Z-X, Hasumi M, Hiroe M, Morimoto S, Nishikawa T: Histopathologic and ultrastructural observations of acute and convalescent myocarditis: a serial endomyocardial biopsy study. *Heart Vessel* 1985;1(Suppl 1):143–153.

38. Schultheiss HP, Kuhl U, Janda I, Schanwell M, Strauer BE:

Immunosuppressive therapy in myocarditis. *Herz* 1992;17:112–121.

39. Edwards WD, Holmes DR, Reeder GS: Diagnosis of active lymphocytic myocarditis by endomyocardial biopsy: quantitative criteria for light microscopy. *Mayo Clin Proc* 1982;57:419–425.

40. Maisch B, Herzum M, Hufnagel G, Bethge C, Schonian U: Immunosuppressive treatment for myocarditis and dilated cardiomyopathy. *Eur Heart J* 1995;16(Suppl O):153–161.

41. Zhang S, Kodama M, Hanawa H, Izumi T, Shibata A, Masani F: Effects of cyclosporine, prednisolone and aspirin on rat autoimmune giant cell myocarditis. *J Am Coll Cardiol* 1993;21:1254–1260.

42. Herzum M, Ruppert V, Kuytz B, Jomaa H, Nakamura I, Maisch B: Coxsackievirus B3 infection leads to cell death of cardiac myocytes. *J Mol Cell Cardiol* 1994;26:907–913.

43. Cooper LT, Berry GJ, Shabetai R: Idiopathic giant cell myocarditis: natural history and treatment. *N Engl J Med* 1997;336:1860–1866.

44. Costanzo-Nordin MR, Reap EA, O'Connel JB, Robinson JA, Scanlon PJ: A nonsteroid anti-inflammatory drug exacerbates Coxsackie B3 murine myocarditis. *J Am Coll Cardiol* 1985;6:1078–1082.

45. Rezkalla SH, Khatib G, Khatib R: Coxsackievirus B3 murine myocarditis: deleterious effects of nonsteroidal anti-inflammatory agents. *J Lab Clin Med* 1986;107:393–395.

46. Rezkalla SH, Khatib R, Khatib G, Smith F, Walsh J, Sowers J, Kloner R: Effect of indomethacin in the late phase of Coxsackievirus myocarditis in a murine model. *J Lab Clin Med* 1988;112:118–121.

47. Groothius JR, Simoes EAF, Levin MJ, Hall CB, Long CE, Rodriguez WJ, et al, for the Respiratory Syncytial Virus Immune Globulin Study Group: Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. *N Engl J Med* 1993;329:1524–1530.

48. Clarkson SB, Bussel JB, Kimberly RP, Valinsky JE, Nachman RL, Unkeless JC: Treatment of refractory immune thrombocytopenic purpura with an anti-Fc receptor antibody . *N Engl J Med* 1986;314:1236–1239.

49. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al: A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med* 1991;324:1633–1639.

50. Drucker NA, Colan SD, Lewis AB, Beiser AS, Wessel DL, Takahashi M, et al: Gamma-globulin treatment of acute myocarditis in the pediatric population. *Circulation* 1994;89:252–257.

51. McNamara DM, Rosenblum WD, Janosko KM, Trost MK, Villaneuva FS, Demetris AJ, et al: Intravenous immune globulin in the therapy of myocarditis and acute cardiomyopathy. *Circulation* 1997;95:2476–2478.

52. Sasayama S, Matsumori A, Matoba Y, Matsui S, Yamada T, Shioi T, et al: Immunomodulation: a new horizon for medical treatment of heart failure. *J Cardiac Fail* 1996;2(Suppl 4):S287–S294.

53. Shioi T, Matsumori A, Sasayama S: Persistent expression of cytokine in the chronic stage of viral myocarditis in mice. *Circulation* 1996;94:2930–2937.

54. Matsumori A, Sasayama S: Immunomodulating agents for the management of heart failure with myocarditis and cardiomyopathy: lessons from animal experiments. *Eur Heart J* 1995;16(Suppl O):140–143.

55. Huber SA, Polgar J, Schultheiss P, Schwimmbeck P: Augmentation of pathogenesis of Coxsackievirus B3 infections in mice by exogenous administration of interleukin-1 and interleukin-2. *J Virol* 1994;68:195–206.

56. Kishimoto C, Kuroki Y, Hiraoka Y, Ochiai H, Kurokawa M, Sasayama S: Cytokine and murine Coxsackievirus B3 myocarditis: interleukin-2 suppressed myocarditis in the acute stage but enhanced the condition in the subsequent stage. *Circulation* 1994;89:2836–2842.

57. Kishimoto C, Crumpacker CS, Abelmann WH: Prevention of murine Coxsackie B3 viral myocarditis and associated lymphoid organ atrophy with recombinant human leukocyte interferon alpha A/D. *Cardiovasc Res* 1988;22:732–738.

58. Hiraoka Y, Kishimoto C, Takada H, Suzaki N, Shiraki K: Colony-stimulating factors and Coxsackievirus B3 myocarditis in mice: macrophage colony-stimulating factor suppresses acute myocarditis with increasing interferon-alpha. *Am Heart J* 1995;130:1259–1264.

59. Kanda T, Nagaoka H, Kaneko K, Wilson JE, McManus BM, Imai S, et al: Synergistic effects of tacrolimus and human interferon-alpha A/D in murine viral myocarditis. *J Pharm Exp Ther* 1995;274:487–493.

60. Miric M, Vasiljevic J, Bojic M, Popvic Z, Keserovic N, Pesic M: Long-term follow up of patients with dilated heart muscle disease treated with human leucocytic interferon alpha or thymic hormones. *Heart* 1996;75:596–601.

61. Smith SC, Allen PM: Neutralization of endogenous tumor necrosis factor ameliorates the severity of myosin-induced myocarditis. *Circ Res* 1992;70:856–863.

62. Sasayama S, Matsumori A: Vesnarinone: a potential cytokine inhibitor. *J Cardiac Fail* 1996;2:251–258.

63. Rezkalla SH, Kloner RA, Khatib G, Smith FE, Khatib R: Effect of metoprolol in acute Cox sackievirus B3 murine myocarditis. *J Am Coll Cardiol* 1988;12:412–414.

64. Djaldetti M, Van Der Lijn E, Mittelman M, Hart J: Propranolol's effect on phagocytizing ability of polymorphonuclears and monocytes (letter). *Drug Intell Clin Pharm* 1985;19:583.

65. Hallengren B, Forsgren A, Melander A: Influence of β-adrenoceptor blocking agents on lymphocyte function in vitro. *Br J Clin Pharmacol* 1982;13:543–546.

66. Tominaga M, Matsumori A, Okada I, Yamada T, Kawai C: Beta blocker treatment of dilated cardiomyopathy: beneficial effect of carteolol in mice. *Circulation* 1991;83:2021–2028.

67. Awan NA, Miller RR, Mason DT: Comparison of effects of nitroprusside and prazosin on left ventricular function and the peripheral circulation in chronic refractory congestive heart failure. *Circulation* 1978;57:152–159.

68. Cohn JN, Franciosa JA: Vasodilator therapy of cardiac failure. *N Engl J Med* 1977;297:27–31, 254–258.

69. Yamada T, Matsumori A, Okada I, Tominaga M, Kawai C: The effect of alpha-1 blocker bunazosin on a murine model of congestive heart failure induced by viral myocarditis. *Jpn Circulation J* 1992;56:1138–1145.

70. Factor SM, Minase T, Cho S, Dominitz R, Sonnenblick EH: Microvascular spasm in the cardiomyopathic Syrian hamster: a preventable cause of focal myocardial necrosis. *Circulation* 1982;66:342–354.

71. Morris SA, Weiss LM, Factor SM, Bilizekian JP, Tanowitz H, Witiner M: Verapamil ameliorates clinical, pathologic and biochemical manifestations of experimental chagasic cardiomyopathy in mice. *J Am Coll Cardiol* 1989;14:782–789.

72. Dong R, Liu P, Wee L, Butany J, Sole MJ: Verapamil ameliorates the clinical and pathological course of murine myocarditis. *J Clin Invest* 1992;90:2022–2030.

73. Goldstein RE, Boccuzzi SJ, Cruess D, Nattel S: Diltiazem increases late-onset congestive heart failure in postinfarction patients with early reduction in ejection fraction. *Circulation* 1991;83:52–60.

74. Ferlinz J, Gallo CT: Responses of patients in heart failure to

long-term oral verapamail administration (abstract). *Circulation* 1984;70(Suppl II):II-305.

75. Packer M, O'Connor CM, Ghali JK, Pressler ML, Carson PE, Belkin RN, et al, for the PRAISE Study Group: Effect of amlodipine on morbidity and mortality in severe chronic heart failure. *N Engl J Med* 1996;335:1107–1114.

76. Wang WZ, Matsumori A, Yamada T, Shioi T, Okada I, Matsui S, et al: Beneficial effects of amlodipine in a murine model of congestive heart failure induced by viral myocarditis. *Circulation* 1997;95:245–251.

77. Szabo C, Thiemermann C, Vane JR: Dihydropyridine antagonists and agonists of calcium channels inhibit the induction of nitric oxide synthase by endotoxin in cultured macrophages. *Biochem Biophys Res Commun* 1993;196:825–830.

78. Hirono S, Islan O, Nakazawa M, Yoshida Y, Kodama M, Shibata A, Izumi T, Imai S: Expression of rat inducible nitric oxide synthase in rat experimental autoimmune myocarditis with special reference to changes in cardiac hemodynamics. *Circ Res* 1997;80:11–20.

79. Stein B, Frank P, Schmitz W, Scholz H, Thoenes M: Endotoxin and cytokines induce direct cardiodepressive effects in mammalian cardiomyocytes via induction of nitric oxide synthase. *Mol Cell Cardiol* 1996;28:1631–1639.

80. Ishiyama S, Hiroe M, Nishikawa T, Abe S, Shimojo T, Ito H, et al: Nitric oxide contributes to the progression of myocardial damage in experimental autoimmune myocarditis in rats. *Circulation* 1997;95:489–496.

81. Rezkalla SH, Kloner RA, Khatib G, Khatib R: Beneficial effects of captopril in acute Coxsackievirus B3 murine myocarditis. *Circulation* 1990;81:1039–1046.

82. Suzuki H, Matsumori A: Myocardial injury due to viral myocarditis improved by captopril in mice (abstract). *Circulation* 1990;82(Suppl III):III-674.

83. Suzuki H, Matsumori A, Kawai CH: Effects of angiotensin converting enzyme inhibitors on myocardial injury and congestive heart failure due to myocarditis in mice: comparison with captopril and enalapril (abstract). *Circulation* 1991;84(Suppl III): III-634.

84. Rezkalla SH, Raikar S, Kloner R: Treatment of viral myocarditis with focus on captopril. *Am J Cardiol* 1996;77:634–637.

85. Tanaka A, Matsumori A, Wang W, Sasayama S: An angiotensin II receptor antagonist reduces myocardial damage in an animal model of myocarditis. *Circulation* 1994;90:2051–2055.

86. Matsumori A, Wang H, Ablemann WH, Crumpacker CS: Treatment of viral myocarditis with ribavirin in an animal preparation. *Circulation* 1985;71:834–839.

87. Kishimoto C, Crumpacker CS, Abelmann WH: Ribavirin treatment of murine Coxsackievirus B3 myocarditis with analyses of lymphocyte subsets. *J Am Coll Cardiol* 1988;12:1334–1341.

88. Fohlman J, Pauksen K, Hyypia T, Eggertsen G, Ehrnst A, Ilback NG, Friman G: Antiviral treatment with WIN 54954 reduces mortality in murine Coxsackievirus B3 myocarditis. *Circulation* 1996;94:2254–2259.

89. O'Connell JB, Dec WG, Goldenberg IF, Starling RC, Mudge GH, Augustine SM, et al: Results of heart transplantation for active lymphocytic myocarditis. *J Heart Transplant* 1990;9:351–356.

90. O'Connell JB, Breen TJ, Hosenpud JD: Heart transplantation in dilated heart muscle disease and myocarditis. *Eur Heart J* 1995;16(Suppl O):137–139.

91. DeCarvalho VB, Sousa EFL, Vila JHA, daSilva JP, Caiado MR, Araujo SR, et al: Heart transplantation in Chagas' disease. *Circulation* 1996;94:1815–1817.

92. Sole MJ, Liu P: Viral myocarditis: a paradigm for understanding the pathogenesis and treatment of dilated cardiomyopathy. *J Am Coll Cardiol* 1993;22(Suppl A):99A–105A.