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M E D I C A L G R A N D R O U N D S

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VIRAL MYOCARDITIS

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During a six month period in 1988, the Cardiology consultation service was asked to evaluate 27 patients with new onset, or newly recognized congestive heart failure, principally for consideration of endomyocardial biopsy to exclude inflammatory myocarditis. In the past 128 months, 96 endomyocardial biopsies have been performed in the Cardiac Catheterization Laboratory at this institution, and in most cases, the indication for this procedure was to exclude myocarditis as an etiology for undiagnosed ventricular dysfunction. In an informal review of 66 endomyocardial biopsy specimens from Parkland Memorial Hospital for which pathological data was available in 1986, findings consistent with definite, or possible active inflammatory myocarditis were noted in 3. In view of the frequency with which this diagnosis is considered or pursued, the infrequency with which it is definitively diagnosed, and apparent confusion concerning an appropriate clinical approach, this protocol undertakes a review of currently available clinical and experimental data concerning viral myocarditis.

Epidemiology

Illustrative of the confusion surrounding viral myocarditis, no reasonable estimate of the incidence or prevalence of this disorder is available. Three factors are contributory:

i) sample bias inherent to the populations studied
ii) varying, and variably reliable diagnostic criteria, and
iii) difficulty clearly defining the syndrome of interest.

Available information comes from three perspectives.

Evidence of alterations in myocardial physiology during or following viral infection is common, with such nonspecific findings as "tachycardia inappropriate for fever," and ST-segment and T-wave abnormalities on electrocardiography occurring in as many as 1/3 of patients. Such findings correlate poorly with demonstrable myocardial infection or inflammation, or with objective evidence of ventricular dysfunction. Accepting for diagnosis both:

i) culture or serologic evidence of viral infection, and
ii) either pathological evidence of myocardial inflammation, or evidence of ventricular dysfunction,

myocardial involvement has been identified in up to 12% of patients during enteroviral or influenza epidemics, and in 2% to 5% of sporadic Coxsackievirus infections (Helin, et al., 1968; Grist and Bell, 1969, 1974; Karjalanien et al., 1980; Oseasohn et al., 1959; Lerner, 1975; Orinius, 1968; Daly et al., 1984; Siani et al., 1975; Koontz and Ray, 1971). The incidence of myocardial involvement in viral infections, particularly with enteroviral infection, may be substantially higher in infants (Verlinde et al., 1956; Van Crevelde and De Jager, 1956; Gear and Measrock, 1973).

Pathologic studies have identified histologic evidence of mycarditis in < 1% to 10% of routine autopsies (Abelmann, 1966; Saphir, 1941, 1942; Stevens, 1970; Gore and Saphir, 1947), and in 1% to 21% of young persons after sudden unexplained death (Rasmussen et al., 1978; Mason et al., 1978; Koskenvuo, 1976; Corby, 1960; Kuller et al., 1966; Lake and Helpern, 1968).

Of most interest from the perspective of an Internist is the incidence of myocarditis in patients presenting with cardiac disease. History of recent febrile illness has been reported in up to 19% of patients presenting with dilated cardiomyopathy (Fuster et al., 1981; Daly, et al., 1984; Olsen, 1983). Serologic studies have identified evidence of viral infection, reflected in a four-fold rise in neutralizing antibody titer, anti-type-specific IgG titers greater than or equal to 1:512 or IgM titers greater than 1:32, in up to 30% of patients presenting with dilated cardiomyopathy (Kawai, 1971; Cambridge, et al., 1979; Daly, et al., 1984).

Studies examining endomyocardial biopsies from patients presenting with dilated cardiomyopathy have yielded widely disparate results. The dramatic variability in the frequency with which myocarditis is diagnosed on histologic grounds, ranging in such studies from 0-67%, presumably reflects both differences in populations studied and in diagnostic criteria applied. Biopsy evidence of myocarditis has also been reported in 15-22% of patients with unexplained ventricular dysrhythmia (Nippoldt, et al., 1982; Strain, et al., 1983a, 1983b). In general, populations selected for clinical predictors of myocarditis including brief duration of symptoms, historical or serologic evidence of antecedent viral infection or co-existing pericarditis have demonstrated a somewhat higher incidence of histologic myocarditis than unselected series of dilated cardiomyopathy. Even in biopsy series limited to patients with myocarditis suspected on clinical grounds, however, only 17-29% have had confirmatory biopsies (Mason, et al., 1980; Nippoldt, et al., 1982; Olsen, 1980).

The true incidence of viral myocarditis is thus unknown. Subclinical myocardial infection and inflammation may accompany a substantial fraction of acute viral illnesses. Transient, detectable myocardial or electrophysiologic abnormalities probably occur in a few percent. Clinically overt disease likely occurs in less than 1%, and persistent disease in a fraction of those. For reasons discussed below, no reasonable estimate of the incidence of active, or etiologic myocarditis in patients presenting with newly recognized cardiomyopathy is currently possible.

Etiologic Agents

A wide variety of infectious agents have been associated with inflammatory myocarditis, but the majority of recognized cases in the United States for which a causative organism can be identified are viral (Table 1a, Kopecky and Gersh, 1987). Myocarditis has been reported in association with several families of both RNA and DNA viruses (Table 1b, Woodruff, 1980), but for most of these agents, the incidence of clinically apparent myocarditis is extremely low. While an accurate index is difficult, the picornaviruses (including the enteroviruses Coxsackie and Echo) and the orthomyxovirus family (Influenza) have been implicated in the majority of recognized cases (reviewed in Woodruff, 1980).

Causes of Myocarditis*

INFECTIOUS	NONINFECTIOUS
Viruses	Drugs (see next table)
Bacteria	Hypersensitivity states
Mycoplasma	Collagen diseases
Protozoa	Rheumatic fever
Parasites	Radiation
Fungus	Heavy metal
Varicella	Insect stings
Rickettsia	
Spirochetes	
"Idiopathic" (probably viral)	

Table 1: Etiologic agents in human myocarditis.

A: Kopecky and Gersh (1987)
B: Woodruff (1980)

Classification	*Virus*
RNA core	
Picornavirus	Coxsackie A and B
	Echo
	Polio
	Hepatitis A
Orthomyxovirus	Influenza A and B
Paramyxovirus	Rubeola
	Mumps
Togavirus	Chikungunya
	Dengue
	Yellow fever
	Rubella
Rhabdovirus	Rabies
Arena virus	Lymphocytic choriomeningitis
DNA core	
Pox virus	Variola
	Vaccinia
Herpes virus	Varicella zoster
	Cytomegalo
	Ebstein-Barr
Adenovirus	
Hepatitis B virus	

Serologic studies in patients with myocarditis (by diagnostic criteria of varying stringency) have implicated type-B Coxsackievirus in roughly half (Daly, et al., 1984; Sainani, et al., 1968, 1975; Grist and Bell, 1974; Smith, 1976; Koontz and Ray, 1971), and most of the available clinical and experimental observations concerning myocarditis are derived principally from disease caused by this agent.

Clinical Presentation

Vagaries of diagnosis make constructing a picture of the clinical spectrum of viral myocarditis difficult. The prototypic presentation associated with type-B Coxsackievirus, seen most commonly in epidemic infections in infants, is an acute myopericarditis presenting as a febrile illness, frequently with typical pericardial chest pain, pericardial effusion and a variable degree of ventricular dysfunction (Gersh, 1985; Woodruff, 1980; Kopecky and Gersh, 1987). In some cases, the degree of acute ventricular dysfunction is profound, resulting in severe congestive heart failure and circulatory collapse (Whitehead, 1965; Kopecky and Gersh, 1987; Kawai, et al., 1987). Except in infants during enteroviral epidemics, the prototypic presentation is, however, rare. In most cases, myocarditis is diagnosed either as incidental evidence of myocardial involvement during the course of a viral illness, or from serologic or pathologic findings obtained in the course of investigation of newly recognized cardiac disease. In patients presenting with cardiac disease, there are no specific clinical, laboratory, or hemodynamic findings which reliably identify myocarditis (Kunkel,

et al., 1978; Baaudrup and Olsen, 1981; Strain, et al., 1983; Zee-Cheng, et al., 1984; Segal, et al., 1978; Sainani, et al., 1968, 1975; Grist and Bell, 1974; Smith, 1966).

The most commonly considered clinical presentation is that of an acute dilated cardiomyopathy. Most commonly, myocardial dysfunction is biventricular (Goldman and Bochner, 1980; O'Connell, et al., 1981), but isolated left (Pinamanti, et al., 1988) and right (Dec, et al., 1985; Pinamanti, et al., 1988) ventricular dysfunction have been reported, and left ventricular dysfunction generally dominates the clinical presentation. Similarly, while most reports describe globally depressed systolic function (Yasuda, et al., 1987; Dec, et al., 1985), co-existing or isolated segmental wall motion abnormalities have been identified frequently by currently available imaging technology (Pinamanti, et al., 1988; Miklozek, et al., 1980, 1986). In a few cases, isolated diastolic myocardial dysfunction has been described. Isolated case reports have also described myocarditis presenting with chest pain and segmental myocardial dysfunction mimicking acute myocardial infarction (Gardiner and Short, 1973; O'Neill, et al., 1985; Tsukada, et al., 1986). Whether, in such cases, there is co-existing ischemic injury from coronary thrombosis or vasculitis is unclear. Myocarditis has also been demonstrated by biopsy in several patients with syndromes of anginal chest pain and abnormal exercise electrocardiograms, with normal left ventricular function and epicardial coronary arteries on angiography (Fifer, et al., 1983). Ventricular aneurysms (segmental dyskinesis) have also been described following myocarditis (DeA'Neto, et al., 1980; Tsukada, et al., 1986).

Abnormalities of atrioventricular or interventricular conduction have been identified in a fraction of patients with viral myocarditis (Reyes and Lerner, 1985), including patients presenting with high-grade atrioventricular block requiring pacing (Kawai, et al., 1987), and patients presenting with Stokes-Adams syncopal attacks. Myocarditis has also have been identified in several patients undergoing endomyocardial biopsy for evaluation of unexplained ventricular dysrhythmia (Sugrue, et al., 1984), and has been identified at autopsy in a fraction of patients whose initial manifestation of cardiac disease was sudden death (Mason, et al., 1978; Rasmussen, et al., 1978).

Clinical Course

The clinical outcome in patients with viral myocarditis is, not unexpectedly, strongly correlated with the presenting clinical syndrome. In those patients in whom subclinical heart disease is detected incidentally in the course of evaluation for a viral syndrome, a benign course is almost uniformly observed (Helin, et al., 1968; Karjalainen, et al., 1980; Smith, 1980; Heikkila and Karjalainen, 1982; Bengtsson and Lambuger, 1966). In patients presenting with acute congestive heart failure, early mortality due to progressive congestive heart failure or arrhythmia has been reported in 1-7% (Gardiner and Short, 1973; Smith, 1970). Natural history studies have consistently reported that in the preponderance of patients surviving the acute phase, clinical recovery is the rule (Smith, 1987; Bengtsson and Lamburger, 1966), with a variable fraction demonstrating persistent abnormality of myocardial function, dysrhythmias or electrocardiographic abnormalities (Bergstrom, et al., 1970; Levander-Lindgen, 1965). Prospective studies of patients presenting with acute

enteroviral myopericarditis have reported development of chronic symptomatic dilated cardiomyopathy in 7% (Smith, 1970) and persistent systolic dysfunction or biventricular enlargement in up to 30% (Levi, et al., 1977). Acute myopericarditis has also been reported to follow a relapsing course in some patients (Smith, 1970). A less favorable outcome has been described in adult patients presenting with acute dilated cardiomyopathy (with or without biopsy-proven myocarditis), with less than half demonstrating significant improvement in left-ventricular ejection fraction over 18 months (Dec, et al., 1985). The few longitudinal studies of patients presenting with myocarditis complicating non-enteroviral infections have shown persistent dilated cardiomyopathy less frequently (Hensen and Mufson, 1971; Moore, 1969; Steiner, et al., 1970; Matthews and Griffiths, 1974; Verel, et al., 1976; Roberts and Fox, 1965; Nagaratram, et al., 1971; Obeyesehere and Herman, 1972). Both serologic and biopsy studies of patients with chronic dilated cardiomyopathy have suggested a viral etiology in a variable fraction (see below), often in the absence of an identifiable antecedent acute illness, suggesting that subclinical myocarditis may result in slowly progressive myocardial injury. Currently, no reliable estimate can be made of the frequency with which subclinical myocarditis underlies chronic dilated cardiomyopathy.

Diagnosis

Three general approaches have been employed in pursuing a diagnosis of viral myocarditis in patients presenting with cardiac disease:

i) serologic studies for evidence of antecedent viral infection
ii) imaging techniques to detect infiltration of myocardium by inflammatory cells and/or evidence of myocyte injury, and
iii) myocardial biopsy.

Serologic Studies

Neutralizing antibody titers have been widely employed to detect antecedent viral infection in patients presenting with cardiac disease (reviewed in Reyes and Lerner, 1985). As noted previously, several studies have demonstrated elevated type-specific antiviral antibody titers to group-B Coxsackieviruses in patients with dilated cardiomyopathy (Cambridge, et al., 1979; Kawai, 1971; Kitaura, 1981; Lau, 1983). Criteria accepted as evidence of viral infection in such studies are variable, but include i) absolute neutralizing antibody titers greater than 1:512 (Grist and Bell, 1974) or 1:1024 (Cambridge, et al., 1979), ii) a four-fold rise in neutralizing antibody titer in paired acute and convalescent sera (Reyes and Lerner, 1985; Kawai, et al., 1978; Lerner, et al., 1975) and/or iii) a type-specific IgM titer greater than 1:32 (El-Hagrassy, et al., 1980).

Direct support for the underlying assumption that serologic studies identify patients with myocarditis from the population presenting with undiagnosed heart disease is limited. In only isolated reports, including small numbers of patients, have serologic studies and biopsy findings been compared directly (MacArthur, et al, 1984; Edwards, et al., 1982). In patients presenting with acute myopericarditis, serologic evidence of viral infections is predictive

of histologic myocarditis on biopsy (MacArthur, et al., 1984). At present, however, data are insufficient to determine to what extent high or rising type-specific neutralizing antibody titers predict histologic myocarditis in patients presenting with congestive cardiomyopathy.

Imaging Techniques

While commonly employed techniques for assessing ventricular function including echocardiography (Pinamanti, et al., 1988), radionuclide (Miklozek, et al., 1980) and contrast (Dec, et al., 1985) ventriculography are frequently useful in the initial assessment and longitudinal follow-up of congestive cardiomyopathy, no characteristics identifiable on such studies are useful in the diagnosis of myocarditis. Studies in patients with histologic myocarditis have demonstrated widely varying degrees of ventricular dilation and systolic dysfunction, unilateral or biventricular involvement, and both global and segmental abnormalities of wall motion. Moreover, in some patients with myocarditis demonstrated by biopsy (eg: those presenting with dysrhythmia or conduction defects) ventricular function may be normal (Pinamanti, et al., 1988).

Technetium pyrophosphate scanning is a sensitive technique for detection of myocardial necrosis (Coleman, et al., 1977) and has been reported to identify focal or diffuse myocardial necrosis in some patients with myocarditis (Des A'Neto, et al., 1980). Gallium-67 citrate scanning has been employed to detect accumulation of inflammatory cells in a variety of clinical settings. Robinson et al., (1979) first reported myocardial uptake of ^{67}Ga in 2 patients with unexplained cardiomyopathy. Subsequent studies by several groups have reported widely divergent results, however, with sensitivities ranging from 0% to 83%, and positive and negative predictive values of 0-36% and 0-98%, respectively (Bouhour, et al., 1988; O'Connell, et al., 1981 and 1984; Strain, et al., 1983). Recently, Indium-111 monoclonal antimyosin antibody imaging has been employed to detect myocardial necrosis. Yasuda et al. (1987) have reported the results of ^{111}In-antimyosin scanning in 28 patients with clinically suspected myocarditis. Using right ventricular endomyocardial biopsy as the "gold-standard", these investigators report a sensitivity of 100% and specificity of 58% for the detection of myocarditis.

Magnetic resonance imaging has been reported to accurately identify both necrotic and reversibly injured myocardium. Experience with MRI for detection of myocarditis is insufficient to determine the diagnostic utility of this technique presently. Similarly, SPECT and PET scanning using metabolic tracers have recently been described, but currently available data are anecdotal.

Endomyocardial Biopsy

Since a diagnosis of myocarditis implies active myocardial inflammation producing myocyte injury and death, the development of simple and safe techniques to obtain (usually right) ventricular endomyocardial biopsy specimens suitable for pathological analysis has provided a "gold-standard" for the diagnosis of myocarditis (Mason, et al., 1978; Anderson, et al., 1984). Application of a

biopsy "standard" has highlighted the limitations of clinical and noninvasive diagnostic modalities for the accurate identification of myocarditis in patients presenting with acute dilated cardiomyopathy. While an increased incidence of "positive" biopsies in patients with brief duration of symptoms has been reported (Dec, et al., 1985), in most series, clinical and histologic diagnoses have correlated poorly, with biopsies "positive" in only 17-29% of clinically suspected cases (Mason, et al., 1980; Nippoldt, et al., 1982; Olsen, 1980). While early experience suggested that study of "biopsy-proven" myocarditis would resolve much of the confusion surrounding this diagnosis, widely discrepant observations persist. Kopecky and Gersh (1987) compiled statistics concerning the incidence of myocarditis on biopsy in 14 published series including 1,380 patients (Figure 1). In most cases, the indication for biopsy was undiagnosed cardiomyopathy. The incidence of "positive" biopsies ranged from 0-67%.

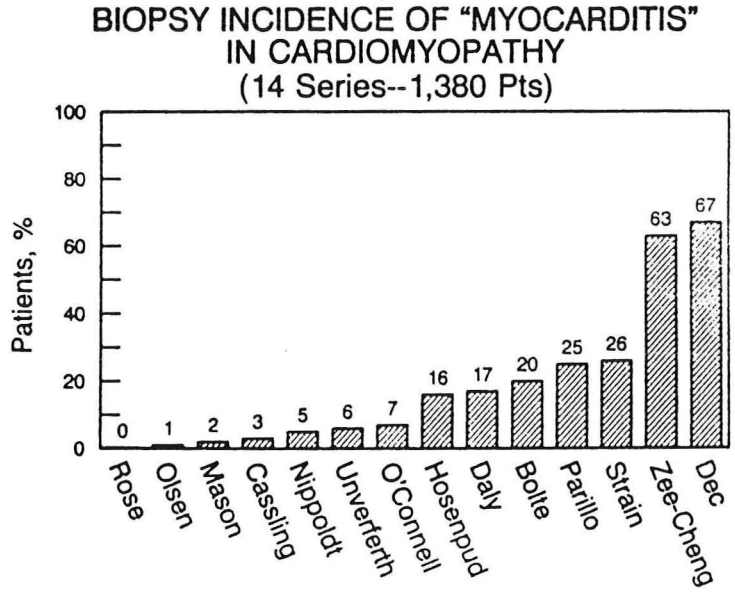


Figure 1: Incidence of Myocarditis on Endomyocardial Biopsy in 14 published series (Kopecky and Gersh, 1987).

Potential reasons for this wide divergence include differences in patient populations, time from onset of symptoms and probably most importantly, differences in histologic criteria.

In an attempt to standardize pathologic criteria, a panel of cardiac pathologists have proposed consensus criteria (the Dallas Classification System, Aretz, et al., 1987)(Table 2), requiring "myocyte necrosis, or degeneration, or both associated with an inflammatory infiltrate adjacent to the degenerating or necrotic myocytes". A variety of clinical conditions can be associated with myocardial accummulation of inflammatory cells, and thus could be confused with myocarditis on histologic examination of biopsy specimens. Characteristics of several such disorders are shown in Table 3.

Dallas Classification System

Initial Biopsy:

Active myocarditis:	Inflammatory cell infiltration of myocardium and myocyte necrosis or degeneration, with or without fibrosis.
Borderline myocarditis:	Less intense inflammatory cell infiltration, or absence of myocyte necrosis
No evidence of myocarditis	

Subsequent Biopsies:

Persistent myocarditis:	Unchanged or increased inflammation and necrosis compared to initial biopsy.
Resolving myocarditis:	Decreased inflammation, no ongoing necrosis, reparative changes.
Resolved myocarditis:	Inflammation/necrosis absent, residual fibrosis, hypertrophy.

TABLE 2: Classification of Myocarditis on Endomyocardial Biopsy (Aretz et al., 1986).

INFLAMMATORY INFILTRATES AND DIFFERENTIAL DIAGNOSES IN MYOCARDITIS

Lymphocytic	Neutrophils Present
Idiopathic	Idiopathic
Viral	Viral (Early)
Toxic	Pressor effect
Collagen vascular disease	Ischemia
Sarcoidosis	Bacterial Infections
Kawasaki's disease	
(Lymphoma)	

Eosinophils Present	Giant Cells Present
Hypersensitivity	Idiopathic
Parasitic infestation	Sarcoidosis
Hypereosinophilic syndromes	Hypersensitivity
?Idiopathic	Rheumatic fever
	Rheumatoid diseases
	Granulomatous infections

TABLE 3: Differential Diagnosis of Conditions Associated with Inflammatory Cell Infiltration of Myocardium (Aretz et al., 1987).

The difficulty associated with interpretation of biopsy specimens is illustrated by a blinded study in which 16 biopsy specimens from patients with suspected myocarditis were reviewed independently by 7 cardiac pathologists (Shanes, et al., 1987). Extensive interobserver variability was observed (Figure 2).

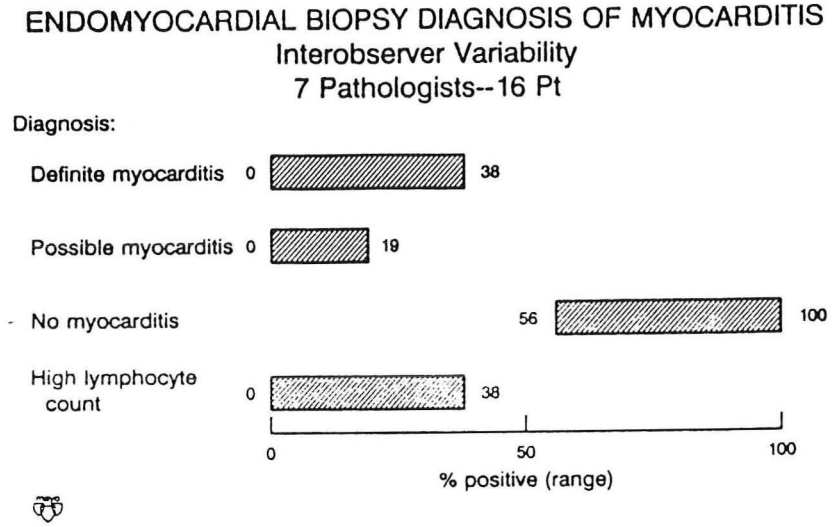


Figure 2: Variation in lymphocyte count and diagnosis of myocarditis in 16 patients reviewed by seven highly skilled cardiac pathologists (from Shanes, et al., 1987).

Attempts to achieve greater uniformity by applying quantitative criteria for lymphocytic infiltration have proven controversial (Finoglio, et al., 1983; Edwards, et al., 1982; Parillo, et al., 1984; Aretz, et al., 1986), as "abnormal" numbers of lymphocytes have been reported in up to 87% of hearts with dilated cardiomyopathy explanted during transplantation (Tazelaar and Billingham, 1985). Moreover, in one study (Zee-Cheng, et al., 1984), minimal lymphocytic infiltration did not preclude a favorable response to immunosuppressive therapy. Because of these observations, the Dallas Classification System requires both lymphocytic infiltration of the myocardium and active myocyte necrosis within the inflammatory foci for a diagnosis of active myocarditis.

Recently, the addition of tissue immunofluorescence studies to routine light microscopic analysis has been proposed. Myocardial staining for IgG and C_3 have been reported to correlate strongly with mononuclear cell infiltration (sensitivity 12/14, specificity 12/15, Hammond, et al., 1987). These same authors also reported that identification of myocyte necrosis (reflected by discontinuity of nuclear or plasma membranes) by electron microscopy, while not correlating with histologic myocarditis by light microscopy, was a strong predictor of subsequent clinical course in patients with dilated cardiomyopathy. Table 4 shows the correlation of myofilament loss with mortality over an 18 month mean followup period in this study.

Mononuclear cell infiltration	Myofilament loss (grade)	Patient alive*	Patient dead*	% mortality
Present	0-1+	6	1	14
	2-3+	4	3	43
Absent	0-1+	38	4	10
	2-3+	15	8	35

TABLE 4: Prognostic significance of myofilament loss by EM in idiopathic dilated cardiomyopathy and myocarditis (Hammond, et al., 1987).

Diagnosis of myocarditis by endomyocardial biopsy is further complicated by the potential for sampling error. While some patients demonstrate diffuse lymphocytic infiltration of the myocardium, frequently associated with a more fulminant clinical course (Billingham, 1987), numerous studies have reported focal involvement of the myocardium in the inflammatory process with the potential for "falsely negative" biopsies. Multiple samples (3-5) only partially address this issue (Aretz, et al., 1986). The significance of focal lymphocytic infiltration of the myocardium is debated, with some authors maintaining that only diffuse involvement correlates with clinically significant myocardial dysfunction (Billingham, 1987), and that focal lymphocytic accumulations occur in a significant fraction (4-10%) of normal hearts (Tazelaar and Billingham, 1987; Stevens and Grand, 1970; Linder, et al., 1985), while others have reported progressive dilated cardiomyopathy in patients with focal involvement (eg., Dec, et al., 1985) and have suggested that focal myocarditis may represent an early, less fulminant or chronic stage of the disease. Extent and severity of lymphocytic infiltration have correlated with subsequent clinical course in some (Finoglio, et al., 1983) but not all (Dec, et al., 1985; Hammond, et al., 1987) series.

Attempts to identify a causitive organism in biopsy specimens by culture or immunologic techniques (Reyes and Lerner, 1985) have been almost uniformly unrewarding. More recently, application of hybridization techniques to detect viral nucleic acid in biopsy specimens has been reported (Bowles, et al., 1986).

At the present time, a convincing diagnosis of myocarditis is predicated upon histologic demonstration of both inflammatory cell infiltration of myocardium and myocardial cellular necrosis. It is likely that focal involvement of myocardium introduces some sampling error, and thus a histologic "gold-standard" may miss an unknown fraction of the disease. Immunocytochemistry may improve the yield marginally, and electron microscopy may provide additional prognostic information. Biopsy alone, however, does not establish a viral etiology. Serologic studies, particularly type-specific IgM titers, can provide supportive evidence of recent viral infection, and implicate a causative organism. Current data do not convincingly support a role for noninvasive imaging modalities in the diagnosis of viral myocarditis.

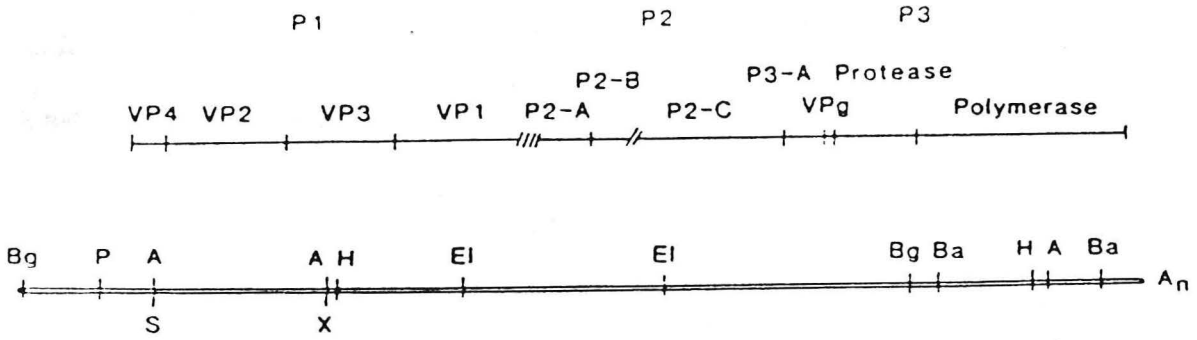
Pathophysiology of Myocarditis

Virtually all of the available experimental data concerning the pathophysiology of myocarditis is derived from studies of murine myocarditis produced by group-B Coxsackie or related viruses. Since Coxsackievirus is the most commonly implicated agent in human myocarditis, this model may have considerable relevance to the clinical disease.

Biology of Coxsackie Virus

Group-B Coxsackievirus (6 serotypes numbered 1-6) is a small, non-encapsulated, icosahedral, single-stranded RNA virus of the genus enterovirus (with Coxsackie A, Echo and Polioviruses) in the picornavirus family. The viral genome is a 7396 nucleotide polyadenylated RNA. A full-length cDNA copy of the Coxsackie B_3 (Nancy) genome has been cloned (Lindberg, et al., 1987), and the complete nucleotide sequence determined. The genome contains a single, long, open-reading frame (positions +741 to +6558) encoding a 2185 amino acid polyprotein, from which mature virally encoded proteins are derived by post-translational cleavage. A structural map of the Coxsackie virus genome, and the identified functions of virally encoded proteins are shown in Figure 3.

Coxsackie B_3 (Nancy)



P1: VP1: capsid protein
VP2: capsid protein, ? principal neutralizing epitopes
VP3: capsid protein
VP4: capsid protein, ? cell-surface receptor binding

P2: P2A: tyrosine-glycine protease, P220 CBP cleavage
P2B: unknown
P3C: ? membrane bound replication complex

P3: P3A: VPg precursor, unknown function
VPg: membrane binding of genome: myristate - VPg- RNA
P3C: glutamine-glycine protease
P3D: RNA-dependent RNA polymerase

FIGURE 3: Structure of the Coxsackievirus B_3 genome showing coding regions for viral proteins and partial restriction map; known functions of virally encoded proteins.

Lytic Coxsackie Virus Infection

Group B Coxsackieviruses infect a wide range of mammalian cells in culture. Infection is mediated by attachment of virus particles to specific cell surface receptors (Zajac and Crowell, 1969; Crowell, 1966; Krah and Crowell, 1985; Mapoles, et al., 1985). The cellular receptor site for Group B Coxsackie virus has been identified as an Mr 275,000 multicomponent membrane glycoprotein complex (Crowell and Siak, 1978), which also functions as the receptor for adenovirus 2, but is distinct from the receptor for other enteroviruses (Crowell, 1976; Lonberg-Holm et al., 1976). One component of the Coxsackie virus receptor has been purified from HeLa cell membrane fractions (Mapoles, et al., 1985), and is a 49 kd intrinsic membrane glycoprotein. Recently, the 45 kd cell surface receptor for Polio virus has been cloned, and identified as an integral membrane glycoprotein of the immunoglobulin superfamily (Mendelsohn, et al., 1989). Whether the 49 kd Coxsackievirus receptor has a similar structure is currently unknown.

Receptor-bound enterovirus particles are thought to enter cells via receptor-mediated endocytosis. Uncoating requires an acid environment (Madshus, et al., 1984) and is thought to occur in lysosomes. The infectious viral genome remains covalently bound to a virally encoded peptide Vpg, which functions to mediate attachment of the viral genome to the cell membrane via myristate covalently linked to its aminoterminus (reviewed in Kuhn and Wimmer, 1987). After uncoating, the viral genome functions as mRNA resulting in production of the viral polyprotein. Processing of the polyprotein occurs initially by autocatalytic events, and subsequently via proteolytic cleavage catalyzed by two virally encoded proteases P2-A (a tyrosine glycine-specific protease) and P3-C (a glutamine-glycine specific protease, Toyoda, et al., 1986; Bernstein, et al., 1985).

Lytic infection by Coxsackievirus occurs as a result of inhibition of host cell function. The virally encoded P2-A protease catalyzes the cleavage of cellular P220 CAP binding protein required for efficient translation of ^{7}mGppp capped (cellular) mRNA, but not the (uncapped) viral RNA, producing a selective inhibition of host cell translation which is functionally important in lytic infection (Bernstein, et al., 1985). Replication of the viral genome occurs in the cytoplasm in a membrane associated complex, catalyzed by the virally encoded RNA-dependent RNA polymerase (reviewed in Rueckert, 1985).

Analysis of the structure of viral capsid proteins VP1-VP4 of several picornaviruses have identified 4 regions of marked sequence divergence. Epitope mapping experiments have demonstrated that these hypervariable regions are important antigenic sites (Rossmann, et al., 1985), and correspond to epitopes recognized by neutralizing antisera. Of these, a divergent region of the VP-2 capsid protein has been identified as the most important epitope recognized by neutralizing antisera against Coxsackievirus B_3 (Beatrice et al., 1980). Capsid protein VP-4 is apparently not exposed on the virion surface, and is not recognized by neutralizing antisera raised against intact virion particles (Hogle, et al., 1985; Rossmann et al., 1985). On the basis of the recently determined three-dimensional structure of Poliovirus (Hogle, et al., 1985), and mutational analysis in rhinovirus (Colonno, et al., 1988), it has been suggested that capsid protein VP-4 may be involved in specific cell surface receptor binding (Crowell, et al., 1986).

Murine Myocarditis Model

Both human and murine myocardial cells express cell surface receptor for, support replication of, and are subject to lytic infection by Coxsackieviruses (Reyes and Lerner, 1985). Mice infected with several strains of Coxsackievirus develop a diffuse necrotizing myocarditis (Gauntt, et al., 1979). This murine model of viral myocarditis has been extensively studied by several groups, and is the basis for most current concepts concerning the pathogenesis of human viral myocarditis. Mice infected with Coxsackievirus B-1 or B-4 develop an acute necrotizing myocarditis within 96 hours. Peak myocyte necrosis parallels the cellular accumulation of virus, resolves in parallel with disappearance of the infectious viral particles from myocardial tissue, and is thus felt to reflect the acute lytic viral infection (El Khatib, et al., 1979). Induced deficiency of cellular immunity (thymectomy, irradiation, antithymocyte serum) increases the extent of acute myocardial injury (Khatib, et al., 1983).

Myocarditis also occurs in mice infected with CVB3. In contrast to CVB1/4 infection, however, thymectomy, bone marrow irradiation, or antithymocyte serum treated mice develop minimal myocarditis despite intact viral replication, suggesting that myocardial injury results from a T-cell dependent immunologic mechanism (Woodruff and Woodruff, 1974). In vitro studies have demonstrated that CVB3 infection induces a population of virus-specific cytotoxic T-lymphocytes capable of lysing myocardial cells in culture (Wong, et al., 1977a, 1977b, 1977c; Huber, et al., 1980, 1981). Studies with T-lymphocytes isolated from CVB3 infected animals and fractionated by cell surface antigenic determinants have demonstrated that the T-lymphocyte subset Lyt1-2+ (cytolytic/suppressor) cells, but not Lyt1+ (helper) cells are lytic for myocytes in culture, and that adoptive transfer of cytolytic/suppressor cells into CVB3 infected, T-cell deficient mice restores myocarditis (Guthrie, et al., 1984). In situ immunohistologic experiments have shown accumulation of both Lyt2+ and L3T4+ (helper) T-cell subsets in inflammatory foci in CVB3 infected animals (Godeny and Gauntt, 1987). Subsequently, in vitro experiments have identified two populations of T-cells in CVB3 infected mice capable of producing myocytolysis in culture. L3T4+ cells isolated from CVB3 infected animals are lytic for CVB3 infected but not uninfected myocytes in culture (termed virus- specific cytotoxic T-lymphocytes or VSCTLs). VSCTLs presumably recognize neoantigens expressed in response to viral infection. In contrast, Lyt2+ cells from infected animals specifically lyse only uninfected myocytes (termed autoreactive cytotoxic T-lymphocytes or ACTLs), and presumably recognize a native myocardial cell antigen which is modified or suppressed in virally infected cells (Estrin and Huber, 1987). Co-culture of murine spleen cells on monolayers of CVB3-infected myocardial cells induce a population of L3T4+ VSCTLs. Similarly, co-culture of spleen cells with uninfected myocytes induces a population of T-cells with ACTL activity. Why exposure of spleen cells to autologous myocardial cells in vitro results in generation of ACTLs is unclear. Presumably, this observation reflects either expression of a neoantigen in the cultured myocytes, the effects of high antigen load, or loss of suppressor activity for self-antigens normally found in vivo.

Additional data concerning the mechanism of CTL-induced myocardial injury is derived from specific inhibitor studies. In vitro generation of VSCTLs but not ACTLs is inhibited by antibody directed against the class II MHC antigen Ia^d, by antibody directed against IL-2 receptor (anti-Tac) and low-dose cyclosporine

(presumably impairing IL-2 release, Andrus and Lafferty, 1982; Bunjes, et al., 1981). In contrast, ACTL generation is inhibited by antibody directed against class I MHC antigen (K^dD^d). Thus, VSCTLs appear class II MHC restricted and IL-2 dependent, while ACTLs are MHC class I restricted (Estrin and Huber, 1987).

Suppressor Activity in Myocarditis

Coxsackievirus-induced murine myocarditis demonstrates important sex and viral strain specificity. Male and pregnant female Balb/c mice infected with CVB3 virus develop myocarditis. Virgin female mice similarly infected do not develop myocarditis despite similar myocardial viral titers. Treatment of virgin female mice with antithymocyte serum or low-dose cyclophosphamide abolishes this resistance to myocarditis. Additionally, lymphocytes from virgin female mice protect male mice against development of myocarditis after adoptive transfer in vivo, and inhibit generation of ACTLs in in vitro co-culture experiments (Job, et al., 1986). These data suggest that the resistance of virgin female mice to CVB3-induced myocarditis results, in part, from suppressor activity. Additional mechanisms are apparently operative. The resistance of female mice to myocarditis can be overcome by higher innoculum or prior administration of progestational or androgenic steroids. Additionally, at limiting innoculum, males and testosterone-treated females demonstrate 20-fold higher myocardial virus titers, suggesting additional effect(s) of sex steroids on receptor expression or viral replication (Lyden, et al., 1987).

Viral strain differences provide additional evidence for suppressor activity protective against CVB3-induced myocarditis. Both myocarditic ($CVB3_m$) and nonmyocarditic ($CVB3_0$) strains of group B type 3 Coxsackievirus have been identified (Gauntt, et al., 1979). These variants demonstrate no identifiable differences in infectivity, capsid antigens or viral replication in vitro or in vivo. Myocytes infected in vitro with $CVB3_m$ or $CVB3_0$ express non-crossreactive neoantigens on the cell surface, but myocytes infected with either variant are recognized by autologously sensitized VSCTLs (Huber and Job, 1983). $CVB3_0$ infected mice treated with low-dose cyclophosphamide develop myocarditis. Additionally, spleen cells from $CVB3_0$ infected mice inhibit induction of myocarditis in $CVB3_m$ infected mice after adoptive transfer suggesting that $CVB3_0$ induces virus-specific suppressor activity. Subtyping experiments have demonstrated that these suppressor cells are Lyt 2+ subclass T-lymphocytes (Estrin, et al., 1986).

Host Strain Diversity and the Genetics of Susceptibility

CVB3-induced murine myocarditis demonstrates important host strain specificity. Lodge et al. (1987) examined CVB3-induced myocarditis in three mouse strains: Balb/c, DBA/2 and A. Balb/c and DBA/2 mice developed acute myocarditis which resolves after 2 weeks. A-strain mice, in contrast, develop more severe myocarditis which persists beyond 56 days. Depletion of Lyt 2+ cells inhibits myocarditis in Balb/c mice, but potentiates myocarditis in A-strain mice. Depletion of L3T4+ cells does not affect Balb/c myocarditis, but significantly reduces the severity of myocarditis in DBA/2 mice. Depletion of both lymphocyte subsets inhibits myocarditis in A-strain mice (Figure 4). These

results suggest the operation of distinct strain-specific pathogenic mechanisms. As noted previously, myocarditis in Balb/c mice results primarily from ACTLs via an MHC class I restricted mechanism. DBA/2 myocarditis in contrast, appears T-helper subclass (L3T4+) dependent, while myocarditis in A-strain mice presumably involves additional pathophysiologic factors.

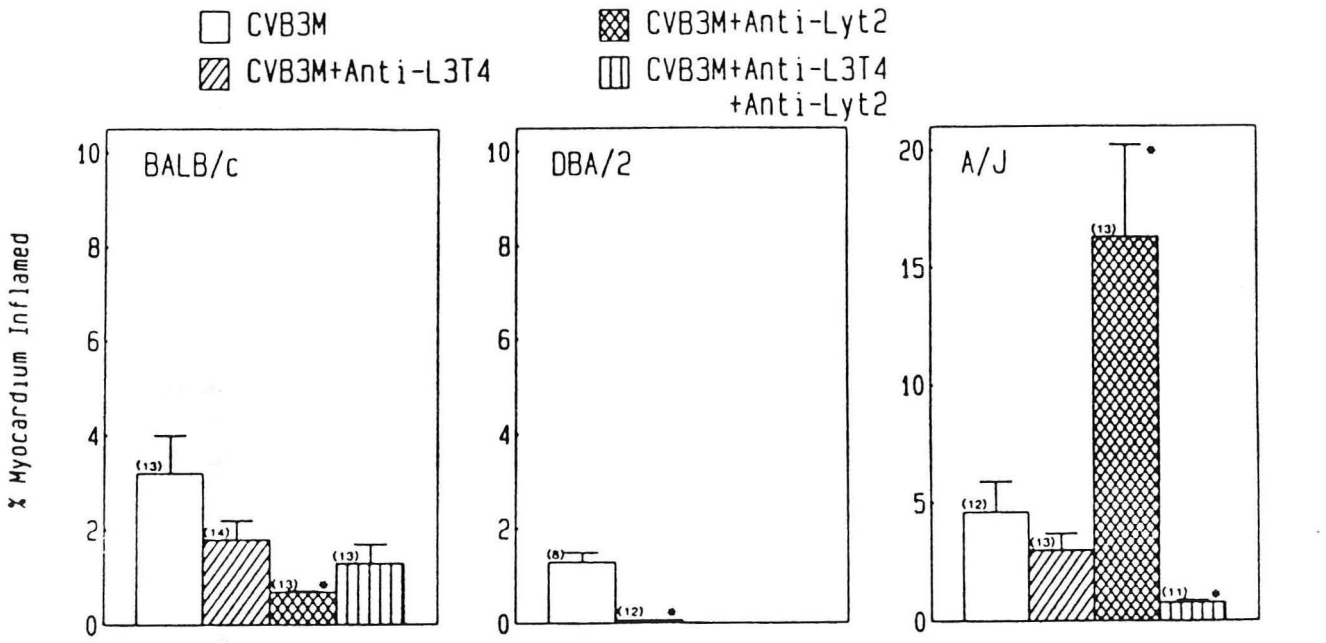


FIGURE 4: Effect of depletion of specific T-lymphocyte subsets on the severity of myocarditis in Balb/c, DBA/2 and A/J mice (Lodge, et al., 1987).

Rose et al.(1987) examined susceptibility to CVB3 induced myocarditis in multiple genetically defined mouse strains selected to comprise 2 experimental groups.

i) Mouse strains differing in general genetic background but sharing a single MHC haplotype, and
ii) Strains with similar non-H_2 (MHC) genetic background, but different H_2 haplotypes.

Mice were sacrificed at varying intervals after CVB3 infection, the character and severity of myocarditis classified pathologically, and myocardial virus and neutralizing serum titers determined. These authors identified two phases of myocarditis in CVB3 infected animals. An early phase of less than 2 weeks duration, which while of variable severity, occurred in all strains and temporally paralleled myocardial virus production. A later phase of myocarditis was observed in only some strains. Severity of early phase myocarditis, and occurrence of late phase myocarditis correlated strongly with host-strain non-MHC genetic background. Higher circulating virus loads, reflecting reduced or delayed clearance of infectious virus were observed in susceptable mouse strains. MHC haplotype appeared to exert only a minor influence on the severity of early myocarditis.

Humoral Immunity

Clearance of infectious CVB3 virus from circulation and myocardial tissue parallels the appearance of type-specific neutralizing antibodies (Rose, et al., 1987; Lodge, et al., 1987). Mouse strains with diminished or delayed antibody response demonstrate more severe acute myocarditis, and a higher incidence of chronic myocarditis (Rose, et al., 1987).

In theory, antibodies directed against virally encoded antigens could play an important role in the pathogenesis of myocarditis. Antibodies reacting with myocardial cell associated viral antigen could mediate compliment-dependent, or antibody-dependent T-cell mediated myocytolysis. Alternatively, circulating immune complexes could be deposited in myocardium and induce an inflammatory reaction. No direct experimental evidence exists to support either of these mechanisms currently.

CVB3 infection has been demonstrated to induce appearance of antibodies reactive against native myocardial antigens (heart reactive antibodies or HRA, Maisch, et al., 1980; Lodge et al., 1987; Rose, et al., 1987). Antibodies directed against both sarcolemma (Rose, et al., 1987) and cardiac myosin (Neu, et al., 1987) have been identified. Circulating HRA have been correlated with persistent myocarditis in A-strain mice (Rose, et al., 1987). Similar HRA, however, have been observed in association with a variety of diseases producing myocardial injury, and, in most cases, it remains unclear whether HRA are causally related to myocarditis, or the innocent result of myocyte necrosis. Recent studies suggest that in some mouse strains, antibody dependent complement mediated myocytolysis is pathogenically important, since complement depletion in CVB3 infected DBA/2 or A-strain mice is protective (Lodge, et al., 1987). IgM antibodies directed against both viral and myocardial antigens are observed uniformly after experimental CVB3 infection, but IgG HRA are observed only in animals with persistent myocarditis (Rose et al., 1987; Lodge et al., 1987). Moreover, immunohistochemistry demonstrates IgG deposition only in the hearts of animals with persistent myocarditis (Lodge et al., 1987).

Persistence of Viral Infection

After CVB3 infection in most mouse strains, myocardial viral titers peak between 5 and 7 days, and infectious virus cannot generally be isolated after 15 days. In A-strain mice, myocardial titers decline more slowly, but generally disappear between 4 and 8 weeks (Lodge et al., 1987; Kishimoto, et al., 1985, 1986). Similarly, viral antigens are generally undetectable in myocardium after 2 weeks. Recently, however, viral RNA has been demonstrated in myocardium of the CVB3 infected mice up to 56 days after infection by *in situ* hybridization using probes generated from a cloned CVB3 cDNA (Kandolf et al., 1987). Hybridization was localized to regions of the myocardium demonstrating foci of inflammatory cells and active myocyte injury. These findings suggest that, at least in part, persistent viral infection may underlie chronic experimental CVB3 myocarditis. The molecular mechanisms underlying viral persistence, particularly the existence of detectable viral genome in the absence of infectious viral particles or identifiable viral antigens, are currently unknown. Moreover, the

extent to which persistent or latent viral infection plays an important pathogenic role in experimental myocardial injury remains undetermined.

Effects of Immunosuppressive and Antiviral Therapy on Experimental Myocarditis

Because of the preponderance of data implicating immune mediated myocyte injury in the pathogenesis of myocarditis, several groups have examined the effects of immunosuppressive therapy on the course of myocardial inflammation in this model. Treatment of mice infected with CVB3 with low-dose cyclophosphomide (Rager-Zisman and Allison, 1973) or glucocorticoids (Woodruff, 1979) paradoxically increases the extent of lymphocytic infiltration of the myocardium and myocardial cell necrosis. Subsequent studies have shown that virgin female mice, normally resistant to CVB3 induced myocarditis, develop severe disease after such immunosuppressive therapy (Job et al., 1986). Similarly, mice infected with $CVB3_0$, a normally nonmyocarditic strain of Coxsackievirus, develop disease after immunosuppressive therapy (Estrin, et al., 1986). In both cases, resistance to myocarditis is linked to virus-induced suppressor T-cell activity. Immunosuppressive therapy inhibits generation of suppressor activity in these settings, presumably accounting for the paradoxical effect of these agents on the severity of myocardial inflammation.

Similarly, cyclosporine A in doses which significantly inhibit alograft rejection, is not protective against myocarditis in Balb/c mice infected with CVB3 (Estrin et al., 1986). Myocarditis in this strain is not dependent upon either L3T4+ (helper) T-lymphocytes or interleukin-2, presumably accounting for this observation. Cyclosporine A does inhibit myocarditis in mouse strains (eg: A, DBA/2) in which humoral immunity appears to play a significant pathogenic role (Estrin et al., 1988).

The effects of three forms of antiviral therapy on experimental myocarditis have been examined. Ribovirin, a nucleoside analog with broad antiviral activity against both RNA and DNA viruses, significantly decreases murine myocardial virus replication, and decreases the severity of both lymphocytic infiltration and myocyte necrosis (Matsumori, et al., 1985). While passive immunization of mice prior to CVB3 infection is protective against virally induced myocarditis, administration of neutralizing antisera in the setting of established myocarditis does not favorably influence the course of the disease (Rager-Zisman and Allison, 1973). This observation is consistent with the observation of co-existent myocarditis and an autogolously generated neutralizing antibody response in Balb/c mice. Whether passive immunization would favorably alter the course of myocarditis in mice (A-strain) with delayed antibody response and viral clearance is unknown.

More recently, vaccination of mice against CVB3 prior to viral infection (Matsumori, et al., 1987) and treatment of mice with recombinant α-interferon (Matsumori, et al., 1984) have been demonstrated to convey resistence to both infection and subsequent myocarditis.

Agents which alter arachidonic acid metabolism have variable effects on the course of experimental myocarditis. Indomethacin administration early in the course of myocardial inflammation exacerbates CVB3 induced myocarditis

(Rezkalla, et al., 1988). In vitro experiments have implicated the lipoxygenase metabolite LTC_4 in the pathogenesis of decreased myocardial contractility accompanying lymphocytic infiltration of myocardium (Lieros, et al., 1988), but the effects of lipoxygenase inhibitors or LTC_4 receptor blockade have not been examined in intact animals.

Evidence for an Immune Pathogenesis in Clinical Myocarditis

Abnormalities of Cellular Immunity: Immunohistochemical studies of biopsy specimens from patients with myocarditis have demonstrated that macrophages and T-lymphocytes comprise the majority of infiltrating inflammatory cells (Hammond and Anderson, 1983). Both helper and suppressor/cytotoxic T-lymphocyte subsets have been identified.

Several groups have reported defects in cell mediated immunity in patients with myocarditis, including deficiencies in both suppressor (Fowles, et al., 1979; Eckstein, et al., 1982) and natural killer cell (Anderson, et al., 1982, 1985) function. These defects, however, have not been uniformly observed (Anderson, et al., 1981; Fletcher and Wegner, 1968; Lowry, et al., 1985a, 1985b), and significant overlap exists with normal controls. Moreover, similar alterations in cellular immunity have been reported in patients with congestive heart failure of several etiologies (Kopecky and Gersh, 1987), suggesting they may reflect an epiphenomenon.

Histocompatibility typing of patients with dilated cardiomyopathy has identified over-representation of the B27 and DR_4 haplotypes previously associated with other disorders of presumed autoimmune etiology (Anderson, et al., 1984). Prior studies have associated HLA haplotypes and susceptibility to Coxsackievirus associated autoimmunity (Fohlman, et al., 1987), and correlated nucleotide sequence polymorphisms in class II MHC loci with a variety of presumably autoimmune diseases (Todd, et al., 1988). Detailed genetic susceptibility studies in patients with "confirmed" myocarditis are lacking.

It has been suggested that vascular endothelium may play an important role in the pathogenesis of myocarditis as an antigen-presenting cell (in the context of Ia, Burger and Vetto, 1982), but supportive data are not currently available.

Humoral Immunity: Several groups have reported circulating heart reactive antibodies in a fraction (10-30%) of patients with dilated cardiomyopathy (Camp, et al., 1969; Kirsner, et al., 1973; Das, et al., 1972). Maisch, et al. (1982a) have reported antisarcolemmal antibodies in patients with a history of myopericarditis which mediate complement-dependent myocytolysis in vitro. These antibodies could be absorbed with viral antigens, suggesting that the antisarcolemmal specificity reflects cross-reaction with viral epitopes (Maisch, et al., 1982b). Deposition of immunoglobulin in the myocardium of patients with dilated cardiomyopathy has been reported (Hatte and Melbeye, 1976; Bolte and Schultheiss, 1978), and correlated with the degree of left ventricular dysfunction (Bolte, et al., 1980), but it remains unclear whether this reflects a pathogenic role or is a nonspecific marker of myocardial injury.

Persistent Viral Infection

Attempts to isolate infectious viral particles from the myocardium of patients with myocarditis, or to detect expression of virus-specific antigens in pathological or biopsy specimens have been virtually uniformly unrewarding (reviewed in Reyes and Lerner, 1987). Molecular cloning of cDNA copies of the genomes of several strains of Coxsackievirus (Iizuka, et al., 1987; Lindberg, et al., 1987; Jenkins, et al., 1987), and the development of sensitive hybridization techniques, now make it possible to detect specific viral nucleic acid sequences in clinical specimens. Bowles, et al. (1986) examined RNA extracted from biopsy specimens from 21 patients with dilated cardiomyopathy, with or without histologic evidence of myocarditis. In 9 of 17 samples from patients with inflammatory cells infiltrating myocardium, these authors observed specific hybridization of probes corresponding to Coxsackie B viral sequences to myocardial RNA. In several cases, biopsy specimens were obtained "late" in the course of the disease. While subject to some technical reservations, these observations suggest that, in some cases, myocarditis and/or dilated cardiomyopathy are associated with persistent viral infection, during which infectious viral particles or viral antigens may not be detectable by standard techniques.

Implications of Experimental Myocarditis for Human Disease

Taken in aggregate, the observations concerning Coxsackievirus induced murine myocarditis present a confusing picture, with a diversity of "clinical" course, response to "therapeutic" intervention, and apparent pathophysiologic mechanism reminiscent of the literature concerning human myocarditis. This picture is clarified by the realization that, while arising from a single pathogenic organism, murine myocarditis is not a single disease. Rather, at least three "clinical", and four pathophysiologic syndromes can be identified (Figure 5).

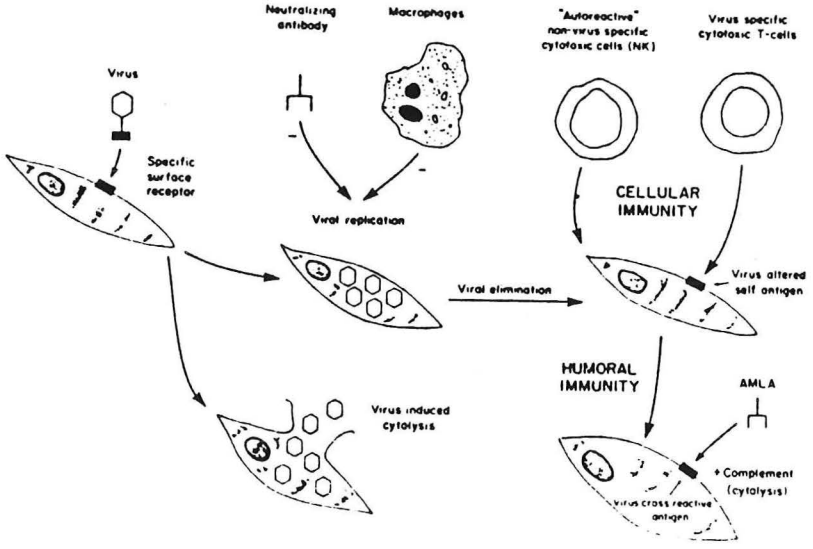


Figure 5: Schematic representation of possible mechanisms for virus-associated myocardial injury. NK = null killer; AMLA = antimyolemma antibody (Kereiakes and Parmley, 1984).

The first, termed here for discussion *acute viral myocarditis*, results from the direct cytopathic effects of viral infection. Modeled by CVB1 or 4 infections, this acute syndrome, a few days in duration, resolves spontaneously with clearance of the virus. While the acute injury may produce varyingly severe residual ventricular dysfunction reflecting the extent of myocardial necrosis, there is no ongoing myocyte injury. Immunosuppressive therapy in this setting is detremental, as viral clearance is reduced and a more fulminant infection results. Some forms of antiviral therapy appear at least partially protective.

The second "clinical" syndrome, termed here *subacute immune myocarditis*, is modeled by CVB3 infection of Balb/c or DBA/2 mice. The onset of myocardial injury lags temporally with respect to viral replication, and parallels the appearance of cytotoxic T-lymphocytes which mediate the myocyte injury. Two pathophysiologic variants exist. In Balb/c mice, myocardial injury results primarily from CTLs recognizing native myocyte antigen(s). The process is MHC class I restricted, and appears to reflect a deficiency in suppressor activity. Nonspecific immunosuppressive therapy appears to exacerbate the deficiency in suppressor activity, and adversely affects the course of the disease. Cyclosporine A is ineffective. In contrast, T-lymphocyte depletion may be protective.

In DBA/2 mice, myocyte injury is dependent upon L3T4+ (T-helper) lymphocytes. Both humoral and cellular immune mechanisms may be important. VSCTLs of the L3T4+ subclass can mediate myocytolysis via an MHC class II restricted, IL-2 dependent mechanism. Additionally, these animals generate heart-reactive IgG antibodies, and show myocardial deposition of immunoglobulin. Compliment depletion is at least partially protective, suggesting a significant humorally mediated component. The effects of immunosuppressive therapy on DBA/2 myocarditis have not been reported, but <u>in vitro</u> experiments suggest that cyclosporine A may be beneficial.

Finally, a syndrome of *chronic viral myocarditis* occurs in A-strain mice infected with CVB3. These animals show deficient viral clearance, and persistent infection is demonstrable, at least by hybridization techniques. The mechanism of myocyte injury is uncertain but is dependent upon both Lyt-2+ and L3T4+ lymphocytes, and correlates with chronic circulating heart-reactive IgG antibodies. Depletion of suppressor subclass T-lymphocytes exacerbates myocarditis. Cyclosporine A is protective.

The diversity of myocarditis in man suggests that a similar spectrum of pathophysiologic processes exist. The apparently very common findings of transient, often subclinical and generally benign abnormalities of ventricular function or electrophysiology accompanying a viral syndrome, seem in this context, likely to result from direct viral cytopathic effects. The acute myopericarditis syndrome, and the syndrome of truly acute dilated cardiomyopathy with the inflammatory cell infiltration on endomyocardial biopsy, follow a course similar to *subacute immune myocarditis* in mice. Finally, if viral myocarditis is an important cause of chronic dilated cardiomyopathy, chronic low-grade myocardial injury associated with persistent viral infection may be responsible. Identification of viral nucleic acid sequences in myocardium from patients with dilated cardiomyopathy long after the initial manifestations of cardiac disease support this possibility. The potential that clinical myocarditis, even that

produced by a single causative agent, could be pathophysiologically diverse carries important implications for therapy.

Therapy

Principles of the management of ventricular dysfunction, congestive heart failure, and ventricular dysrhythmia have been reviewed in recent Medical Grand Rounds by Drs. Firth, Malloy and Kremers.

Based on the evidence suggesting that immune mechanisms may play an important role in the pathogenesis of myocardial injury, and on an early report of dramatic improvement in myocardial function in a patient with "biopsy-proven" myocarditis treated with corticosteroid therapy (Mason, et al. 1980), a number of authors have advocated immunosuppressive therapy for patients with myocarditis. The available data concerning the efficacy of such therapy is limited to uncontrolled reports involving small numbers of patients, variable diagnostic criteria, differing therapeutic regimens, and frequently soft end-points. A summary of nine such reports assembled by Kereiakes and Parmley (1984) is shown in Figure 6.

Reference	*No. of patients*	*Sex*	*Treatment Agent, Dose (no. of patients)*	*Clinical course*			*Comments*
				Imp	*NSC*	*Det*	
Mason et al.[11]	10	5M	P 40-60 mg/m²(2)	1	1		One death from opportunistic infection
		5F	A 75 mg/m²				
			P 40-60 mg/m²(8)	4	3	1	
Zee-Cheng et al.[17]	11	5M	A + P(9)*,†	5	1	2	One death from sepsis; positive treatment response correlated with low-grade/ "chronic" inflammation
		6F	A + P + ATG(1)	1			
			P + CTX(1)*				
Edwards et al.[21]	10	7F	P 40-60 mg/day(5)	3	2		Clinical course similar whether treated or not
		3F	No treatment(5)	3	2		
Daly et al.[39]	9	2M	P†(1)		1		Late deterioration without inflammatory changes on EMB in 2 patients
		7F	A + P(8)	7	1		
Melvin et al.[42]	3	3F	P 50 mg/m²(3)	3			Peripartum cardiomyopathy
			A 75 mg/m²				
Fenoglio et al.[110]	34	22M	P or A + P†(18)	7	11		Positive treatment response correlated with low-grade/"chronic" inflammation
		12F	No treatment(4)	2	2		
Weiss et al.[111]	13	NI	A + P†(13)	8	5		Positive treatment response correlated with low-grade/"chronic" inflammation
Strain et al.[112]	7	NI	A + P†(7)	5	2		Discordance of clinical and histologic response
Williams et al.[113]	6	NI	A + P†(6)	5		1	Late deterioration without inflammatory changes on EMB in 2 patients
Totals‡			82 (treated)	49	27	4	
Percentage				(60%)	(33%)	(5%)	

Abbreviations: P = prednisone; A = azathioprine; ATG = antithymocyte globulin; CTX = cytoxan; NI = not indicated; EMB = endomyocardial biopsy; NSC = no significant change; Imp = improved; Det = deteriorated.

*Follow-up not given on one patient.

†Dosage not indicated.

‡Follow-up not given on two patients.

FIGURE 6: Results of Immunosuppressive Therapy for Myocarditis in 9 Published Series (Kereiakes and Parmley, 1984).

Sixty percent of the 82 treated patients in these combined series demonstrated some index of improvement during treatment with variable combinations of azathioprine, prednisone and cytoxan. Significantly, 5 of 9 untreated patients in these series also demonstrated improvement. More recently, Hosenspud et al. (1985) reported no beneficial effect (by invasive hemodynamic criteria) of prednisone and azathioprine in six patients with "biopsy-proven" myocarditis. Similarly, Dec et al. (1985) retrospectively examined 27 patients with acute dilated cardiomyopathy. In 18 patients, biopsy specimens were "positive" for myocarditis. Nine patients were treated with immunosuppressive therapy, of which four demonstrated objective improvement in left ventricular function. Six of 18 untreated patients also improved (p = N.S.). If only those patients whose biopsies met "Dallas Criteria" for myocarditis are considered, 3 of 7 patients treated with immunosuppressive therapy and one of four untreated patients demonstrated improvement in left ventricular function over a mean followup of 18 months. Notably, 6 of 16 patients with negative biopsies by Dallas Criteria also improved. Kereiakes and Parmerly (1984) have noted that in the available reports, improvement in response to immunosuppressive therapy has been observed more commonly in patients with chronic or low-grade myocarditis than in those with high-grade or acute inflammatory changes.

The effects of more selective immunosuppressive therapy (eg. cyclosporine A) on the course of myocarditis have not been determined, although a prospective controlled clinical trial (the Multicenter Myocarditis Trial) is in progress.

There is a single, anecdotal report of the treatment of a patient with "biopsy-proven" myocarditis with monoclonal antibody reactive against the CD3 T-lymphocyte surface antigen (OKT-3) with dramatic improvement in congestive heart failure (Gilbert et al., 1988).

Approach to the Patient with Suspected Myocarditis

Ultimately, a decision to pursue a diagnosis of myocarditis in a patient presenting with new, or newly recognized symptoms of cardiac disease is predicated upon the assumptions that:

i) the incidence of myocarditis is sufficiently high to justify the attendant risk and/or expense;

ii) histologic and/or serologic studies can reliably identify or exclude myocarditis;

iii) a diagnosis of myocarditis will provide therapeutically or prognostically useful information, and

iv) immunosuppressive therapy initiated for myocarditis is effective and safe.

It seems likely that myocarditis is responsible for at least a few percent of dilated cardiomyopathies. A significant question exists, however, concerning the reliability of histologic diagnoses from endomyocardial biopsy specimens. The course of patients with myocarditis is variable, and is not clearly different

than that of patients with undiagnosed dilated cardiomyopathy. Evidence that immunosuppressive therapy beneficially affects the course of myocarditis is unconvincing.

Consequently, I believe that currently available clinical data support neither the routine use of serologic studies or endomyocardial biopsy in the evaluation of patients with dilated cardiomyopathy, nor the noninvestigational therapy of myocarditis with immunosuppressive agents. The ongoing Multicenter Myocarditis Trial may, in the near future, provide better guidance. Future application of DNA amplification technology to identify viral nucleic acid in biopsy specimens may, at least for enteroviral myocarditis, provide the hard diagnostic endpoint needed to clarify the currently confusing clinical literature.

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