REVIEW ARTICLE

VIRAL MYOCARDITIS

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Viral Myocarditis

A Review

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INFILTRATION of the myocardium with inflammatory cells occurs during infection with a variety of viruses. Usually the infiltrate is composed of mononuclear cells that are focally or diffusely scattered among the myofibers. Necrosis of myofibers is an important feature of this lesion, and the presence of both myofiber necrosis and inflammation may aid in differentiating myocarditis from other infiltrative lesions in the heart, such as those seen in patients with leukemia and malignant lymphoma.

Cardiac changes which can be attributed to viral infection are not necessarily limited to the myocardium but may also involve the endocardium and epicardium. Therefore, although myocarditis and pericarditis are considered to be separate clinical entities, viral pericarditis is nearly always associated with underlying myocardial lesions [1] leading to the use of the term "myopericarditis."[2] In this review the terms *myocarditis, pancarditis,* and *myopericarditis* are used interchangeably.

The disease we now know as viral myopericarditis was recognized over a century ago, long before modern techniques of virus isolation and serology permitted a specific etiologic diagnosis. As early as 1854 idiopathic pericarditis was an acknowledged disease,[3] and by the late 1800s a similar entity was noted to occur during epidemics of mumps [4] and pleurodynia [5] (now known to be due to the Coxsackievirus and the echovirus [6,7]). The observation that pericarditis and myocarditis occur in association with other conditions such as infectious mononucleosis,[8,9] influenza,[10,11] measles (rubeola),[12,13] smallpox,[14] and poliomyelitis [15] was also made prior to a clear understanding of the viral agents responsible for these diseases.

The present review deals with several aspects of viral myocarditis in man and then characterizes the myocarditis produced in animals infected with the Coxsackie B viruses. We have elected to review experimental models of Coxsackie B viral disease because 1) these agents are considered to be the commonest cause of human myopericarditis and 2) most of our understanding of the mechanisms of pathogenesis of viral myocarditis de-

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Table 1—Viral Infections Associated With Myocarditis in Humans

Classification	Virus	References*
RNA core		
Picornavirus†	Coxsackie A‡	16, 17
	Coxsackie B	2, 18–23
	ECHO	24, 25
	Polio	15, 26–31
Orthomyxovirus	Influenza A + B	11, 32–37
Paramyxovirus	Rubeola	13, 38, 39
	Mumps	4, 40, 41
Togavirus	Chikunguna	42
	Dengue	42
	Yellow fever§	43, 44
	Rubella§	45
Rhabdovirus	Rabies	46, 47
Arenavirus	Lymphocytic choriomeningitis	48
DNA core		
Poxvirus	Variola	49, 50
	Vaccinia	51–54
Herpesvirus	Varicella-zoster	55–58
	Cytomegalo	59, 60
	Epstein-Barr	49, 61 62
Adenovirus	Adeno	63–65
RNA and DNA cores		
Unclassified	Hepatitis	66–68

* Selected cases.
† Virus family.
‡ Coxsackie, ECHO, and polio are enteroviruses.
§ May produce myocardial necrosis without inflammation.

rives from experiments with these viruses. No emphasis will be placed on models of isolated endocarditis induced by viruses. Lastly, this review indicates how some experimental observations have influenced the rationale for therapy of viral myopericarditis and outlines areas needing further investigation.

Virology

Etiology

Myocarditis is found after infection of humans with a wide range of viruses (Table 1). RNA viruses predominate, with picornaviruses being the most commonly identified agents. Coxsackie B viruses are members of this group and in about half the cases where the diagnosis was reasonably es-

tablished myopericarditis was associated with infection by these agents.[2,65,69] Other picornaviruses such as Coxsackie A, echo and polio are also known to cause myocarditis (Table 1).

Only a small number of viruses listed in Table 1 have been isolated from the hearts of infected patients; these agents include Coxsackie B,[70–75] polio,[29] ECHO,[76] and vaccinia.[53] Most often the association of a particular viral infection with heart disease has been based on serologic studies, the isolation or identification of the virus in tissues or fluids other than the heart or pericardial fluid (ie, biopsy specimens, urine, feces, cerebrospinal fluid), or recognition of a characteristic clinical picture.

Diagnosis

Although some infections, such as rubeola and mumps, are clinically quite characteristic, in most instances laboratory tests were needed to identify the agents listed in Table 1. Isolation and/or identification of the virus in the myocardium or pericardial fluid is extremely helpful in determining the etiology of myocarditis. The accuracy of the diagnosis is enhanced when two or more diagnostic techniques are used. Criteria for evaluating the comparative usefulness of laboratory tests in determining the infectious agent in viral myopericarditis have been outlined by Lerner.[77] Brief descriptions of the three major approaches to viral diagnosis are listed below. Details of these procedures are described elsewhere.[78,79–82]

Isolation and Identification

Generally virus will only be isolated if specimens are obtained during the first few days of the illness. Failure to isolate an agent does not, however, exclude it as the cause of the lesion, nor does isolation of virus from noncardiac tissues necessarily mean it is involved in the production of myocarditis. Tissue or fluid specimens are obtained from various body sites depending on the group of agents implicated by the clinical picture. The most common approach is to recover infectious virus from these specimens by inoculating cultured cells, embryonated avian eggs, or susceptible animals. Agents that replicate in these tissues are then identified by the recognition of characteristic cytopathic effects or by the use of immunologic reagents.[78,79,81]

Direct Tissue Examination

As an alternative to viral isolation, tissues or fluids may be examined directly for viruses or pathognomonic changes, with the use of light and electro-microscopic techniques. Historically, certain viral diseases, such as cytomegalovirus infection [83,84] and rabies,[85] were routinely diagnosed by

the recognition of characteristic inclusion bodies in infected cells. Less specific but highly suggestive changes are the intranuclear inclusions seen in herpes simplex and varicella zoster viral infection [86,87] and the intracytoplasmic inclusions seen in vaccinia and smallpox.[85] Multinucleated giant cells are found in tissues during infection with herpes virus, measles virus, myxo-paramyxo viruses, and respiratory syncytial virus.[88] A finding of Warthin–Finkeldey giant cells in lymphoid tissues, especially in the appendix and tonsils [89,90] is particularly suggestive of measles and can lead to a diagnosis during the prodromal stage of the disease.[91] Still other cytologic findings during viral infection have been outlined by Craighead.[88]

Viral antigens can also be detected in a variety of tissues by the use of both direct and indirect immunofluorescence techniques. These methods are available for the rapid diagnosis of viral infection and can be applied to both antemortem and postmortem tissues and fluids. However, the reliability of test results depends on the proper preparation of specimens, the quality of the reagents used, the experience of the investigator, and the use of proper equipment.[80,92] Particular difficulties arise in using these techniques on myocardial tissue because nonspecific fluorescence is a major problem.[93] This difficulty is further complicated by the fact that some viruses—for example, picornaviruses—are not routinely studied in the laboratory by these techniques because of lack of specificity and sensitivity of the method and unavailability of good reagents.[92] Moreover, some investigators have found that specific immunofluorescence cannot be detected even when high titers of picornaviruses are present in the heart.[94,95] Therefore, claims that these viruses can be identified in human hearts with the use of immunofluorescence [96,97] requires independent confirmation.

When electron microscopy is employed in clinical virology, it has a role in both routine and rapid diagnosis.[98–100] Specific agents can be identified by the characteristic morphology or by immunologic techniques.[80,82,101–103]

Serologic Evaluation

Most of the viral infections listed in Table 1 were diagnosed by the demonstration of at least a fourfold rise in specific antibody in paired acute (less than a week) and convalescent (2 weeks or longer) serum specimens. Ordinarily serum antibody is not detected until a few days to a week after the onset of clinical disease.[81] Initially, IgM antibody is present, reaching peak titers by 2–3 weeks and thereafter declining to undetectable levels. In contrast, IgG antibody production peaks later and is the predominant immunoglobulin class after the first month of disease.[104] Characterization of viral antibody production with respect to the Ig classes is therefore useful in determining the stage of infection.

In the past the commonly used tests for detecting and quantitating virus-specific antibodies depended on the ability of antibodies to bind complement, precipitate antigen, inhibit viral interaction with indicator cells (eg, hemagglutination-inhibiting antibody [HI]), or neutralize infectivity.[105] More recently radioimmunoassays (RIA) and enzyme-linked immunoabsorbant (ELISA) assays have been developed to rapidly identify and quantitate specific antibodies. In some instances, IgG is removed from serum by the use of protein A containing *Staphylococcus aureus*, an immunoglobulin-binding reagent, and IgM titers determined by the use of these rapid assay techniques.[82]

The specificity of antibodies detected by different tests varies greatly. This is clearly illustrated in the Coxsackie B virus group, where infection induces neutralizing and HI antibodies that are type specific,[106,107] whereas complement-fixing (CF) antibodies lack this specificity [108] and are of limited value in diagnosing these infections.

Pathology

Prenatal Lesions

Very little is known about the nature of the acute cardiac lesions that appear during viral infection of the fetus. During the first trimester of pregnancy rubella virus induces a focal necrosis of subendocardial fibers. No inflammatory cell infiltrates are described in association with these changes.[110]

In contrast, infection during the last trimester, particularly with viruses of the Coxsackie B group, can lead to a pancarditis with eventual fibroelastic thickening of the endocardium.[110]

Neonatal Lesions

The most commonly recognized cause of viral myocarditis in infants is the Coxsackie B group of viruses.[70–73,111] Lesions produced by these agents are similar to those described after infection with most cardiotropic viruses and therefore serve as prototypes of neonatal disease. However, at least one exception to this may be lesions produced by rubella, where myocardial cell necrosis may be present without an inflammatory response.[45]

Pathologic changes in neonatal Coxsackie B-4 myocarditis vary with the duration of illness; when fatal, this disease seldom lasts longer than 2 weeks. In infants who die early after infection (2–5 days), left ventricular dilatation is present but heart weight is not increased. The endocardium and valves are normal, even though the myocardium is pale. Late in infection (9–11 days of illness) the size of the heart is increased, largely due to dilatation of the left, and occasionally the right, ventricle. The myo-

cardium is soft and pale or mottled dark red and yellow gray, particularly along the left ventricular wall and interventricular septum. Subendocardial hemorrhages may be seen at any stage of the disease, while myocardial hemorrhages usually occur late.[112]

Microscopically, myofiber necrosis and inflammation are the dominant findings. Necrosis of myofibers is either patchy or diffuse and may occur at any site, although left ventricular involvement is common. Necrotic myofibers have been observed as early as 2 days after the onset of illness. By Day 5, myofiber destruction is pronounced, and by Day 9 many myofibers have completely disintegrated, leaving scattered masses of chromatin. By Day 11 necrotic areas are clearly demarcated from the surrounding tissues. Calcification of partially necrotic myocardial cells is observed by Day 9.[112]

Initially the inflammatory infiltrate is composed of polymorphonuclear leukocytes, but by Day 5 or 6 mononuclear inflammatory cells are found. Histiocytes, lymphocytes, and plasma cells markedly increase in number after Day 6 and are the major cellular infiltrates by Day 9. Fibroblasts and granulation tissue appear thereafter.[112]

Adolescent and Adult Lesions

The gross findings after infection of adolescents and adults with picornaviruses, myxoviruses and paramyxoviruses, rhabdoviruses, poxviruses, herpesviruses, and hepatitis viruses are similar and closely parallel those observed in the neonatal coxsackieviral disease. As in neonates, dilatation, softening, and mottling of the ventricular walls are observed, although in some cases there are no gross abnormalities or there are just epicardial or endocardial petechiae. With pericarditis, the epicardium is thickened by fibrin, and a serous effusion is present.[14,29,36,47,55,62,67,76,113,114]

Although there are exceptions, the histologic findings after infection with many viruses are similar. Early in the illness scattered hypereosinophilic myofibers, widespread edema, and only a few inflammatory cells are present. Later, myofibers exhibit loss of striations, clumping of the cytoplasm, fragmentation, and eventually dissolution or dropout. The degenerating or partially necrotic myofibers are usually surrounded by mononuclear cells, such as lymphocytes, plasma cells, and macrophages [29,55,62,67,76,89,113,115] (Figure 1). These mononuclear cells are commonly seen "invading muscle fibers, some of which were broken down completely" [113] and only rarely observed surrounding arterioles in a manner simulating necrotizing arteritis.[115] An exception to this picture is the frequent finding in yellow fever of myocardial cell degeneration in the absence of primary inflammation.[43] Inflammation and necrosis of the bundle

of His has been observed in hepatitis [67] and is a potential finding after infection with many viruses. Acute inflammatory cells, although noted in neonates, are infrequently observed in adults, except after infection with viruses such as influenza,[34,36] where concomitant bacterial infections are common. Chronic and healed lesions due to all viruses contain interstitial fibrosis and evidence of loss of myofibers.

Epidemiology

Incidence

The true incidence of acute viral heart disease in the general population is unknown, in part because of difficulties involved in establishing the diagnosis of both myocarditis and the specific viral infection.

Current estimates of the prevalence of viral myopericarditis come from studies on 1) the incidence of idiopathic myocarditis in a series of autopsies, 2) the frequency of cardiovascular symptoms or clinically diagnosed disease during epidemics, and 3) the appearance of clinical disease occurring in one location over a period of years. Evidence indicates that perhaps 5% or more of a viral-infected population experiences cardiac involvement. Moreover, the frequency of the disease is influenced by several factors such as epidemics or sporadic outbreaks, seasons, host age, pregnancy, and the sex of the patient.

There are two different patterns of occurrence of myocarditis in autopsy series. In studies on unselected cases involving 1) 40,000 consecutive autopsies,[49] 2) 417 young adult and middle aged male victims of sudden accidental death,[116] and 3) 214 children who had died a sudden violent death,[117] the overall prevalence of myocarditis of suspected viral origin ranged from 2.3% to 5.0%.

In the second pattern of disease, the incidence of acute idiopathic myocarditis in cases of sudden unexpected, nonaccidental death was much higher. A 3-year study of 90 Minnesota children from birth to 17 years of age who had died suddenly in the 1970s showed that 17% had this cardiac lesion.[117] A Japanese survey of 47 sudden deaths in schoolchildren during the same decade showed that 21% had myocarditis.[118]

Abnormalities in the electrocardiogram (ECG) are frequently observed during viral infection and have been used to estimate the frequency of clinical disease. ECG findings seen in myopericarditis include sinus tachycardia, ST-T segment abnormalities, ventricular conduction disturbances, and extra systoles and have been reported in up to 40% of patients with infectious mononucleosis,[113] in 12–31% of individuals with poliomyelitis,[30,119,120] and in 20–30% of patients with rubeola.[39,121] Houck emphasized cautious interpretation of such findings, because the ECG

changes may be only indirectly related to the infection and not definitive evidence of cardiac disease.[122]

Factors Affecting Incidence

Several conditions can affect the frequency of heart disease due to viruses. Picornaviral infections have been selected to illustrate the influence of these conditions or factors on the incidence and severity of viral myocarditis.

Epidemics and Institutional Outbreaks

There is evidence that during widespread Coxsackie B viral infection the occurrence of myocarditis and pericarditis is increased above interepidemic levels. During a Coxsackie B-5 epidemic in England in 1965 virus was isolated from at least 1160 patients.[123] Symptoms of cardiac disease were present in at least 5% of the patients (from 900 reports). In the same year epidemics of Coxsackie B-5 virus were observed in Scotland, Finland, and Australia and were also associated with an increased incidence of viral myopericarditis [16,17,124,125]; as much as 12% of the infected population that sought medical care manifested acute cardiac disease.[17] Myocarditis is also a prominent finding during epidemics of poliomyelitis and influenza.[26,33,36,126]

Picornaviral infections also appear as explosive outbreaks in institutions, especially in nursing homes. These episodes are usually associated with widespread infection in the surrounding community. During outbreaks of Coxsackie B viral disease lethal myocarditis occurs in up to 50% of infected infants.[18,111]

Seasons

Many viral infections have a characteristic seasonal distribution. For example, orthomyxoviruses (influenza) are prevalent during the winter months, while picornaviruses, including polio, Coxsackie A and B, and ECHO viruses are isolated during the summer and fall. This phenomenon has been known for years and still remains a valid observation. In 1975, for instance, 100% of the influenza A virus isolations from patients seen at the Nassau County Medical Center in New York were obtained from January to March. Picornaviral isolations made between June and October of the same year represented 98% of the Coxsackie A and B and 85% of the ECHO virus identifications. In contrast, other viruses, particularly members of the herpes group, such as herpes simplex and varicella zoster, were observed with nearly equal frequency throughout the year (1975 Yearly

Report, Virology and Rickettsiology Service, Nassau County Medical Center, East Meadow, New York).

The seasonal distribution of picornaviruses influences the frequency of myopericarditis due to these agents. Thus Bornholm's disease, or epidemic pleurodynia, which ordinarily occurs during the summer and early fall is often accompanied by an increased incidence of myopericarditis.[127–130] Similarly, in England during 1965 the incidence of Coxsackie B viral heart disease peaked during July and August.[123] However, climate may influence the seasonal variation of viral infections; there is evidence, for example, that in California the peak incidence of coxsackieviral disease and associated myocarditis is shifted to the fall and early winter.[131]

Host Age

The prevalence of Coxsackie B viral heart disease varies with the age of the host. These agents are known to produce a high incidence of myocarditis and disseminated disease in infected individuals during the first year of life, particularly in the neonatal period.[19,70–73,111] In contrast, myocarditis due to this agent appears to be uncommon in early childhood, even though these individuals are susceptible to infection. This decline in the frequency of coxsackieviral myocarditis after the neonatal period correlates with the general observation that both "nonspecific" and viral myocarditis of childhood decreases markedly after the first 6 months of life.[132] The incidence of coxsackieviral heart disease increases again during late childhood and adolescence.[131] This is supported by the previously cited data obtained during the 1965 epidemic of coxsackievirus B-5 in England. Cardiac cases could be grouped into the following patterns, based on 900 infected patients: 12% were under 10 years of age, and half of these were less than a year old; in contrast, the vast bulk of cardiac conditions was in adolescents and adults, with as much as 35% of the patients ages 10–30 years. As the predominant clinical feature of infection, cardiovascular symptoms were found in 5.0% of all 1-year-olds, 1.0% of those ages 1–9, 6.0% of those aged 10–29, and 14.0% of older adults.[123]

Pregnancy

There is only circumstantial evidence that pregnancy and the postpartum state predisposes of viral involvement of the heart. For example, pregnancy increases the susceptibility to picornaviral infection and the risk of developing severe disease, since the incidence of poliomyelitis requiring hospitalization during the gravid state is enhanced over that of the adult population in general.[136] Moreover, parturition occurring during the acute phase of poliomyelitis increases the risk of developing paralysis,[133] a

stage of disease that is associated with a high incidence of myocarditis.[28,134]

Pregnancy may also increase the frequency of myopericarditis during coxsackieviral infection. Data from a coxsackievirus B-5 epidemic in New York in 1960 suggests that the incidence of heart disease during pregnancy may be greater than the prevalence of pleurodynia,[135] which is ordinarily a common manifestation of Coxsackie B viral infection in adults.[2,6,7,17]

Sex

Coxsackie B viral heart disease in adolescent and adult patients predominates in male patients. In a survey of 164 adolescent and adult cases of Coxsackie B viral myopericarditis reported or cited in reviews between 1957 and 1973, we found that 109, or two-thirds of the patients, were male (Table 2). In one study deletion of postpartum women from the data increased the incidence of disease in men from 60% to 72%.[21] A similar male predominance of adolescent and adult heart disease has been seen after infection with Coxsackie A viruses.[17] In addition, one survey of carditis in 35 fatal cases of poliomyelitis showed a 2.5:1 male-to-female ratio.[26] It is not known whether such sex-related differences are found after infection with other viruses.

Clinical Manifestations

Most of our information concerning the clinical manifestations of viral myocarditis comes from observations made of patients infected with Coxsackie B viruses. Moreover, clinical heart disease due to these agents is characteristic of myopericarditis caused by many other viruses.

Acute Disease

Neonatal

Infants, most often from 5 to 10 days of age, manifest an acute illness after an incubation period of approximately 2–8 days. Early in infection pyrexia, tachycardia, and inactivity are frequent findings. These may be followed in severely ill subjects by tachypnea, cyanosis, and rapidly progressive circulatory collapse. In less susceptible patients, the initial illness may be followed by a subacute phase lasting up to a week, before cardiac failure occurs. An occasional infant will experience subclinical infections.[111,147]

Early in infection roentgenography and auscultation may reveal a nor-

Table 2—Sex-Related Differences in the Pattern of Virus-Associated Immune-Mediated Diseases

Sex	Disease or infection	Indices of enhanced disease		Primary immunologic mechanism implicated in pathogenesis	References
		Clinical	Experimental		
Male	Coxsackie B viruses	66% of heart disease in adolescents and adults			2, 16, 20, 21, 114, 124, 136–146*
			Accelerated deaths in CD-1 and BALB/c mice		228,† 253
			From 66–90% of diabetes in SJL/J and NIH Swiss mice		280
				T-cell–mediated	215, 216, 218, 223, 223, 224, 227
Female	Lupus erythematosis	More than 80% cases of disease in young adults			300, 301
			Accelerated deaths in NZB/NZWF_1 mice		
				B-cell mediated	303–305

* Total of 164 cases surveyed.
† Also see Text-figure 3B.

mal heart size. Later, cardiomegaly may be seen, along with a precordial systolic murmur and gallop rhythms as well as the occurrence of multiple arrhythmias.[111]

Abnormalities in the ECG including diffuse ST-T abnormalities typical of myopericarditis are often present but may be transient. However, ECGs are not of prognostic value in the acute phase of myocarditis, although frequent monitoring is generally recommended in severely ill patients.[147]

Clinical manifestations of systemic disease are common after infection with many viruses. With Coxsackie B viruses signs and symptoms are related to multiorgan involvement, including the brain and meninges, liver, pancreas, and adrenals. The average mortality rate is as high as 50% in some series of cases.[111,147]

Adolescent and Adult

In contrast to the abrupt, severe, and often fatal disease seen in the neonatal period, viral myopericarditis in adolescents and adults usually has a delayed onset and is rarely fatal. After Coxsackie B viral infection, for example, initial symptoms are often of an upper respiratory (typically influenzalike) or gastrointestinal illness. Acute heart disease is usually not noted until about a week to 10 days later and has a presentation that can mimic the characteristic picture of pericarditis, coronary artery occlusion, or progressive heart failure. A fourth group of patients does not present with heart disease but manifests miscellaneous signs and symptoms such as fever, myalgia, and headache; and cardiac involvement is often only suspected because of typical ECG changes.[65,148]

The most common symptom in acute myopericarditis is chest pain,[2] although myocarditis without pericardial involvement can be painless.[149] Other clinical findings include tachycardia, arrhythmias, murmurs, rubs, cardiomegaly (due to ventricular dilitation or pericardial effusion), an elevated erythrocyte sedimentation rate, and ECG changes that include conduction disturbances and ST-T, Q-T, and Q wave abnormalities.[2,21,124] Although myocarditis can be silent and only diagnosed by subtle changes in the ECG,[1,148,150,151] in some cases the ECG may be normal.[63,139,140]

Death can occur due to arrhythmias or congestive heart failure,[2,21,114,143] but this sequela is uncommon.

As in the neonatal infection, manifestations of systemic disease are also noted. In Coxsackie B viral infections these include findings compatible with pleurodynia, meningitis, hepatitis, orchitis, lymphadenopathy, and splenomegaly.[2]

Subacute, Chronic, and Recurrent Diseases

An intriguing, clinically important, though controversial, concept in cardiology is the idea that during viral myocarditis inflammation and necrosis can become chronic. Chronic myocarditis is known to occur in parasitic disease in man [152,153] and has also been seen experimentally after inoculation of adolescent mice with coxsackievirus B-3.[154,155]

Interestingly, idiopathic interstitial myocarditis affecting a wide age range of patients and characterized by a "protracted downhill course" over a period of months to years, ending with death in heart failure has been recognized for decades.[156–158] This "pernicious" form of disease is observed infrequently; Saphir and Kline reported an incidence of 6 out of 255 cases of myocarditis found at autopsy.[158]

Evidence that viral myocarditis may be subacute or chronic derives from studies of patients infected with Coxsackie B viruses and is illustrated in the following cases.

Case 1—Subacute Myocarditis: A 42-year-old white man developed palpitations following a short febrile episode that was associated with myalgia. Over the next month orthopnea developed in this man and progressed in severity. Near the end of the month he began a regimen of daily swimming. After 1 week of exercising he was hospitalized in critical condition and diagnosed as having congestive heart failure with cardiomegaly, pericardial effusion, arrhythmias, and myocarditis. His condition deteriorated rapidly, and he died on the eighth hospital day or approximately 40 days after initial symptoms. At autopsy the heart weighed 500 g and showed hypertrophy of the left ventricle. Microscopic examination revealed scattered infiltrates of lymphocytes and other monocytes in the myocardium. Interstitial fibrosis was also present, along with a mixed pattern of myofiber atrophy and hypertrophy. Coxsackievirus B-4 was isolated from a myocardial biopsy obtained during the last week of life, and a specific neutralizing antibody titer of 1/64 was detected in the postmortem serum.[74]

Case 2—Chronic Myocarditis: A 45-year-old black man was hospitalized 1 year antemortem with diagnoses of congestive heart failure, cardiac murmurs, and pancarditis of recent onset. Six and 2 weeks before admission chest pains developed that were characteristic of pericarditis, and the patient then experienced an upper respiratory infection. Studies on acute and convalescent serum demonstrated a rise in neutralizing antibody to coxsackievirus B-4 from 1:8 to 1:256 over a 2-week period. During an 8-week hospitalization he was maintained on a regimen of bed rest, salt restriction, and diuretics. His cardiac condition improved with treatment,

but chest pain and evidence of congestive heart failure persisted for several months after discharge. The patient failed to follow instructions to maintain bed rest after discharge, and recurring chest pain and progressive cardiac decompensation marked his last year of life. He died in acute congestive heart failure. At autopsy the heart was pale and flabby, and there was an extensive inflammatory cell infiltrate, myofiber degeneration, and fibrosis. Numerous dense particles measuring 270 Å in diameter were present in the myocytes. No severe coronary artery disease was noted.[159]

Case 1 portrays a coxsackievirus myocarditis that persisted for about 6 weeks. Case 2 is compatible with a viral myocarditis that had a more chronic course. However, the latter history also suggests that coxsackieviral infection was superimposed on an established pericarditis. In both cases the failure to restrict physical activity for an extended period may well have contributed to the progression of the disease. This same mechanism may have played a role in other reported cases of chronic coxsackieviral heart disease.[2,139] Physical activity or exercise is known to exacerbate viral myocarditis and experimentally can convert an acute benign lesion into progressive and lethal disease.[160]

Corticosteroids also augment the severity of viral myopericarditis. If these drugs are administered early during murine coxsackieviral infection, acute benign disease is converted into disease with inordinately elevated and persistent virus titers in the heart, extensive cardiac necrosis, and a high incidence of mortality.[161–163] It is possible, then, that the use of these agents in man can be associated with severe disease if administered before infectious virus is cleared by the host.

Case 3—Chronic Myocarditis Treated With Steroids: A 28-year-old white man who manifested symptoms of an upper respiratory tract infection 4 weeks before hospitalization was treated with large doses of corticosteroids beginning on the eleventh hospital day. At that time clinical findings compatible with a myopericarditis were present, and soon thereafter paired serum samples showed a significant rise in neutralizing antibodies to coxsackievirus B-2. During subsequent months, while he was maintained on daily betadexamethasone therapy, he experienced episodes of pericardial effusion and low grade fever; eventually there developed a heart murmur, with evidence of congestive heart failure. He died approximately 3½ months after initial symptoms while still on steroid therapy. At autopsy the heart weighed 400 g, and the pericardium was thickened by fibrosis and was adherent to the epicardium. Microscopically, a "mild chronic myocarditis" with collections of both lymphocytes and eosinophils was found.[22]

In this case it is not known whether infectious virus was still present in the host when corticosteroids were administered. It is possible that the course of the disease would have been the same with or without this therapy. Nevertheless, severe and potentially lethal coxsackievirus myocarditis associated with steroid therapy is common enough to warrant our attention [140,144,164,165] (Figure 2).

Instead of subacute or chronic disease, recurrent myopericarditis develops in some individuals after an initial viral infection.[2] These recurrences may be mild, leading to full recovery, or may be serious, leading to heart failure and death. Reinfection with the same virus is unlikely; so recurrent myocarditis is probably due to either other viruses or nonviral agents. In addition to bacteria and parasites, agents such as mycoplasma, chlamydia, and rickettsia can produce cardiac disease that closely mimics infection with viruses.[148,163–169] Inadequate or inappropriate therapy may be factors favoring recurrence of lesions in the heart.

Case 4—Recurrent Myocarditis: A 59-year-old male truck driver developed acute severe Coxsackie B-4 myocarditis and was treated with digitalis, diuretics, salt restriction, and bed rest for 1 month. After discharge he returned to work, driving a truck 10–14 hours a day. Within 1 year the patient was rehospitalized with symptoms resembling the initial illness. Severe myocarditis and cardiomegaly were diagnosed, and the patient died within 10 days. At autopsy the heart weighed 500 g, and the myocardium was pale and soft. Histologic studies revealed acute and chronic changes in the heart; scattered throughout the myocardium were foci of lymphocytes and myofiber necrosis. In other areas patches of fibrosis were present. An anamnestic rise in antibody to coxsackievirus B-4 was not detected, and the identity of the organism that may have been responsible for the terminal myocarditis was not ascertained.[146]

Complications and Sequelae

Complications of viral myopericarditis can occur early or late (after 6 weeks) in the disease. Some early complications such as pleural effusion, arrhythmia, cardiomegaly and congestive heart failure were noted previously and have been observed after infection with a wide range of viruses. Other acute complications of viral heart disease are listed in Table 3.

In addition, chronic complications and sequelae of viral myopericarditis due to the same agents are outlined in Table 3; many were mentioned in a review by Abelman.[170] As shown in this table, long-term or permanent damage to heart after viral infection can be reflected in residual cardiomegaly, persistent abnormalities in the ECG, and reduced

Table 3—Complications and Sequelae of Viral Myopericarditis*

Virus	Complications and Sequellae†	References‡
Coxsackie B	Hemopericardium	2, 171, 172
Coxsackie B, Epstein Barr (EB)	Constrictive pericarditis	2, 130, 141, 174
Coxsackie B, EB, polio, ECHO, vaccinia	Persistently abnormal ECG	1, 2, 21, 124, 175–177
Coxsackie B, polio	Reduced working capacity	2, 176
Rubella	Ventricular aneurysm	178
Coxsackie B, herpes	Congestive cardiomyopathy (?)	179, 180
Coxsackie A + B, EB, polio, influenza, adeno, rabies, varicella, hepatitis, mumps, vaccinia, smallpox	Death§	28, 33, 40, 50, 57, 62, 64, 67, 74, 111, 114, 173, 181–184

* After postnatal infection.
† Other than pleural effusion, acute arrhythmias, cardiomegaly, and congestive heart failure.
‡ Selected cases.
§ Occasionally due to acute circulatory collapse.

working capacity. Severe complications are most often related to the degree of myocardial inflammation and necrosis. For example, in Smith's study of 42 cases of Coxsackie B viral myopericarditis, heart size returned to normal in all 20 patients where "pericarditis" predominated, while 3 of 22 patients with clinical "myocarditis" experienced long-term cardiomegaly. Moreover, 6 patients, all in the "myocarditis" group, had abnormal electrocardiograms for from 6 months to 6 years. Only patients with "myocarditis" did not recover completely from the disease; 2 of these died from cardiac complications.[2]

It is also possible that viruses are at least one cause of congestive cardiomyopathy. High titers of neutralizing antibody to Coxsackie B viruses are more commonly found in patients with a recent onset of cardiomyopathy than in age and sex-matched controls with other cardiac diseases.[179] In a similar study significantly higher titers of antibody to both herpes and Coxsackie B viruses were detected in patients with congestive cardiomyopathy, compared with control subjects.[180]

Experimental Observations

For over three decades animal models have been used to study clinical and pathologic changes in viral myocarditis as well as mechanisms of both host defense and pathogenesis. At first, viruses or filterable agents obtained from animals were used [185,186]; subsequently, viruses were isolated

from human subjects and adapted to animals. Viruses obtained from patients with myocarditis were ordinarily cardiotropic [71,187]; otherwise, cardiotropicity was established by serial passages in animal hearts.[162] Coxsackie B viruses have been the agents most often used in such models, which is fortunate, since these viruses are not only the most commonly identified cause of viral myopericarditis in man,[2,65,69] but readily replicate *in vivo* and *in vitro*.[188,189] Moreover, infection of animals with Coxsackie B viruses leads to the production of lesions in multiple organs, which closely resemble human disease.[190–195]

Originally, various animals, including mice, hamsters, monkeys, and chimpanzees, were used to study coxsackieviral disease.[161,196–200] Since then, however, mice have become the primary host in experimental systems analyzing group B virus-induced lesions, and the murine model has provided most of the data upon which we base our current understanding of the immunology and pathophysiology of viral myocarditis. Therefore, observations made of mice infected with Coxsackie B virus will be discussed in detail. A limited number of studies of group B viral infection of hamsters and primates will also be summarized, because they contribute to our understanding of mechanisms of viral induced cardiac dysfunction and provide information concerning clinicopathologic correlations.

Biology of Coxsackie B Viruses

As noted earlier, Coxsackie B viruses are members of the picornaviruses. This family contains several genera, including the enteroviruses, rhinoviruses and caliciviruses. All are small RNA viruses (20–40 nm in diameter) that are nonenveloped and have an icosahedral capsid.[189,201]

Enteroviruses, which include Coxsackie A and B, polio, and ECHO viruses, cause systemic infection in man after ingestion and replication in the gastrointestinal tract. These agents are resistant to low pH and are not destroyed by gastric secretions. Coxsackieviruses are therefore transmitted by the fecal-oral route, although infection through the respiratory tract is also common.[191,192]

Enteroviruses multiply to high titer in many animal cell lines within 5–6 hours of infection. Coxsackie B viruses attach to target cells by receptors that are not shared with other members of the enteroviruses group. Thus all 6 Coxsackie B viruses compete for the same receptor, while polioviruses attach to a different receptor on target cells. These receptors are essential before viral replication can occur and therefore may determine tissue tropism.[202] Enteroviral infection is an inefficient process. A large number of particles (60–100) are required for infection of a cell; however, once absorption, uncoating, and penetration occurs, replication of new in-

fectious particles is rapid. Replication is confined to the cytoplasm and is directed by the single-stranded RNA genome that also serves as the messenger RNA for protein synthesis by the ribosomes.[189,203]

Macromolecular synthesis is rapidly suppressed in enterovirus-infected cells. Cell protein, RNA, and DNA synthesis are shut down after synthesis of viral-coded proteins. Cytopathic effects become visible in tissue culture cells within a few hours of infection. However, the cause for early cytopathic changes and eventual cell death are not completely understood. Infected tissue culture cells are usually lysed at the time mature virus particles are released. Thus enteroviruses are considered to be lytic agents and produce lesions directly by virus induced cytolysis.[189,203,204]

Characteristics of Murine Coxsackie B Viral Disease

Viral Replication, Inflammation, and Host Defense

Coxsackie B viruses replicate and/or produce inflammatory lesions in the heart, pancreas, liver, spleen, and brain in several strains of mice.[77,194,195,205–207] When originally isolated from man, coxsackieviruses were considered to be selectively pathogenic for suckling (less than 2-week-old) mice.[196,197,205,207–209] Subsequent studies showed that viral replication and disease also occurred in weanling (2–3-week-old) and adult (4-week-old or older) mice after infection by several routes.[77,154,192,193,206]

Studies in weanling and adult animals using the Coxsackie B group have shown that parenteral infection results in viremia and then replication in target organs. Viremia is detected within 24 hours and usually persists till Day 3.[210] The virus grows in the various target organs with maximum levels achieved by Day 3 or 4.[194,210] After maximum virus growth is established, host defense mechanisms become operative, since virus titers begin to decline in target organs and are usually undetectable by 7–10 days.[194]

Current evidence favors the view that neutralizing antibody and mononuclear inflammatory cells play a major role in terminating viral growth during primary coxsackieviral infection. The role of interferon is at present poorly understood, since although severe Coxsackie B viral disease can be aborted in mice by manipulations that result in the production of interferon early in infection,[211] these viruses are poor inducers of interferon *in vivo* and *in vitro*.[212]

A variety of experiments have shown that neutralizing antibody is critical in primary host defense during coxsackieviral disease. Thus, virus is readily neutralized by antibody *in vitro*,[213] and infection is aborted by transfer of antibody to mice within 24 hours of viral inoculation.[214] Moreover, the kinetics of antibody production correlate with the decline in

virus titers in the blood and target organs. Serum neutralizing antibody is first detected in mice 5 days after infection,[163,215] immediately following the termination of viremia,[210] and reaches high levels by Day 7, when virus titers are significantly reduced.[194] Production of this early antibody, presumably IgM, is not dependent on T cells, since mice depleted of T lymphocytes nevertheless exhibit normal levels of neutralizing antibody during the first week of infection.[216,217]

Even so, there is evidence that the inhibition of coxsackieviral growth in target organs cannot be attributed solely to neutralizing antibody. Thus, in CD-1 mice virus replicates in the heart, pancreas, and liver, but recovery occurs. However, the infection is lethal if cortisone acetate is given at the time of virus inoculation. In such animals abnormally high and persistent titers of virus are found in the heart and other tissues, despite the fact that serum neutralizing antibody levels are normal and are detected early after infection. In contrast, there is a marked reduction in the accumulation of mononuclear inflammatory cells in the hearts of these animals.[163]

The accumulation of mononuclear inflammatory cells in infected tissues is a hallmark of Coxsackie B viral disease.[77,192,194,210] Mononuclear cells that migrate into infected organs may play a critical role in suppressing viral growth. The precise cell population responsible for this function is unknown, but it is unlikely to be T lymphocytes, since adult mice depleted of T cells by treatment with antithymocyte serum (ATS) or by adult thymectomy, lethal irradiation, and bone marrow reconstitution (TXBM) do not exhibit any defect in the ability to inhibit coxsackieviral growth.[216] Similarly, viral growth is readily suppressed in nude mice.[218] It is not unexpected, therefore, that thymectomized mice do not experience severe coxsackieviral disease.[216,218,219]

Indirect evidence suggests that macrophages that infiltrate infected tissues are involved in suppressing viral replication. First, macrophages predominate in the mononuclear cell infiltrate observed in the heart 5–10 days following coxsackieviral infection of mice.[194,216] Second, suckling BALB/c mice, which usually experience lethal coxsackieviral B-3 disease, are protected from severe illness when infused with macrophages from the peritoneal cavity of uninfected adult animals.[220] Undiluted immune serum administered before infection also protects against lethal disease,[214] but diluted antibody is effective only when administered in conjunction with syngeneic adult peritoneal exudate cells.[220] These findings imply that the interaction between macrophages and neutralizing antibody limits the spread of virus in target organs and is responsible for the termination of

infection. The role of other mononuclear cells has not been investigated; for example, the contribution of natural killer (NK) cells [221] in primary host defense is unknown.

Models of Myocarditis

Myocarditis occurs in mice of most age groups after infection with several Coxsackie B viruses inoculated by various routes.[77,154,155,191,192,194,208,210]

The production of myocarditis after Coxsackie B viral infection depends on several factors. These include 1) the tropicity of the virus, 2) the age of host, and 3) the strain of animal used. The importance of the first two factors are illustrated in the studies of Grodums and Dempster. They passaged mouse-adapted Coxsackie B virus Types 1–5 through mouse brains *in vivo*; all viruses were found to produce an encephalitis in suckling inbred albino mice. In contrast, these types differed with respect to the production of cardiac disease; Types 2, 3, and 4 induced minimal heart lesions, whereas extensive myocarditis was produced by Types 1 and 5. Although the Type 3 strain produced minimal heart disease in suckling mice, it nevertheless induced extensive lesions in weanling and adult animals. Moreover, in adult mice all 5 virus types produced myocarditis, but none elicited encephalitis.[222,193]

In addition, variants of the same virus may differ in the capacity to induce myocarditis in mice of the same age. Thus the strain of coxsackievirus B-3 used in this laboratory (CVB-3_M) produces moderate to severe heart lesions in adult CD-1 mice,[194,216] while a variant of the same virus type maintained in the laboratory of Dr. Richard Crowell (Hahnemann Medical College (CVB-3_O) induces few or no cardiac lesions in the same animals.[223] Both viruses multiply in CD-1 mice, although CVB-3_M replicates to a significantly higher titer in the heart.[223]

The influence of the animal strain on susceptibility to viral myocarditis induced by coxsackieviral infection has received little attention; yet there is evidence that this factor is also important. For example, Coxsackie B-3 virus produces extensive cardiac necrosis and inflammation in adult CD-1 mice,[194] moderate myocarditis in BALB/c animals,[216] and few if any changes in Albany mice (unpublished observations). Similarly, Grodums and Dempster found that Albany mice (originally obtained from the Division of Laboratories and Research, New York Department of Health, Albany) are relatively insusceptible to heart disease after Coxsackie B-3 viral challenge, compared with infected inbred albino mice.[192]

Most studies on the histologic characteristics of the heart lesions induced by Coxsackie B viruses have been carried out in weanling and adult mice. In adult animals myocarditis is usually transient. Early degenerative

changes such as swollen and hypereosinophilic myofibers are generally not observed until the third day of disease, the time of peak viral replication in the heart. Focal and diffuse collections of lymphocytes and macrophages appear in the myocardium by Day 5 and are prominent by Day 7.[215] Characteristically, these infiltrates are found surrounding partially necrotic myofibers (Figure 3).[77,192,194] Usually, cardiac inflammation and necrosis persist for several days after heart virus titers are markedly reduced and are rarely observed after 2 weeks.[192,215] Cardial residua include myofiber dropout, interstitial fibrosis, and focal calcification (unpublished observations).

In contrast, in weanlings cardiac inflammation and necrosis may become chronic. Thus, in Swiss mice infected at 14–17 days of age myocarditis persists for weeks to months, even though infectious virus cannot be recovered from the heart after the first week of disease. As in the acute infection in adults, cardiac lesions heal with interstititial fibrosis and calcification of myofibers. Hypertrophy of myocardial cells is also observed, and ongoing inflammation and evidence of healing may appear simultaneously.[154,155]

Mechanisms of Pathogenesis of Myocarditis

Evidence has been obtained in studies on Coxsackie B viral heart disease in mice that at least two mechanisms may be operative in the production of cardiac necrosis during myocarditis.

Virus-Mediated Destruction of Myofibers

Coxsackie B viruses readily replicate in tissue culture and are cytocidal in these systems.[204,224] These viruses also replicate in murine myofibers *in vitro*,[225–227] but there is evidence that these cells are less susceptible to lysis than conventional cultures, since infection of neonatal BALB/c myofibers does not result in lysis over a period of 21 hours, as determined morphologically and by ^{51}Cr release studies.[227]

Nevertheless, several lines of evidence support the idea that Coxsackie B viruses can have a direct effect on myofibers *in vivo*. First, as noted earlier, after viral infection scattered, partially necrotic myofibers appear in the murine heart by Day 3, even though no inflammatory infiltrates occur until Day 5.[210,215,228] Second, studies of multiple sections of hearts taken at the time of maximum myocarditis (7–8 days) reveal that some necrotic myofibers are unaccompanied by inflammation (unpublished observations). Third, extensive cardiac necrosis is observed in immunosuppressed mice, even though the expected mononuclear inflammatory cell infiltrate in the heart is abolished or severely reduced. In this situation, the exten-

Table 4—Effect of T Lymphocyte Depletion on Coxsackievirus B-3 Induced Myocarditis in Mice

Experiment	Mouse strain	Number of animals	Treatment before injection	Cardiac lesion score	
				Inflammation	Necrosis
1	CD-1	10	NRS, × 3	2.4	2.1
	CD-1	10	ATS, × 3*	0.7‡	0.7‡
2	BALB/c	5	T × BM + T	2.2	2.1
	BALB/c	5	T × BM	0.8‡	0.7‡

Adult animals were inoculated with $10^{4.0}$–$10^{5.0}$ tissue culture dose 50 (TCD_{50}) virus intraperitoneally and killed 6 days later. The severity of cardiac lesions was scored on a scale of 0–4 as described previously,[194] with 0 indicating no histologic evidence of inflammation or necrosis and 4, widespread mononuclear cell infiltration and necrosis. Values were obtained after examination of multiple sections from each heart; average scores are shown.

* Animals given injections of antithymocyte serum (ATS, 0.5 ml intraperitoneally) daily for three days and infected with virus 2 hours after the last dose. NRS = normal rabbit serum.[216]

† Adult thymectomized, lethally irradiated mice reconstituted with bone marrow cells (T×BM) or bone marrow and thymus cells (T×BM + T).[216]

‡ Significantly less ($P < 0.05$) than average scores obtained with control mice.

sive necrosis of myofibers correlates with the elevated and persistent titers of virus found in the hearts of these animals.[163,194]

Cell-Mediated Destruction of Myofibers

Coxsackieviruses lack envelopes, do not bud from infected cells, and do not induce membrane changes detectable by antiviral antibody.[189,201,204,227,229] As a consequence, virus-induced cytolysis [230] and not immunologic mechanisms [1] were traditionally considered to play the major role in pathogenesis of heart disease due to these agents. The finding that coxsackievirus B infection of weanling mice produced a chronic myocarditis without concomitant persistence of replicating virus provided the first experimental evidence that mechanisms unrelated to viral cytolysis were involved in the production of heart disease.[154]

Direct evidence for participation of immunologic mechanisms, especially those mediated by T cells, in the production of virus-induced heart disease have been obtained from studies using adult mice. First, animals pretreated with ATS and then infected with Coxsackie B-3 fail to develop a normal inflammatory response; the number of mononuclear cells infiltrating the heart on Day 7 is reduced when compared with infected controls (Figures 3 and 4). Moreover, the extent of cardiac necrosis in the ATS-treated animals is significantly decreased, compared with that observed in infected normal rabbit serum treated (NRS) animals (Table 4). Differences in the extent of myofiber damage are unrelated to impairment of viral replication; virus growth is comparable in both groups.[216]

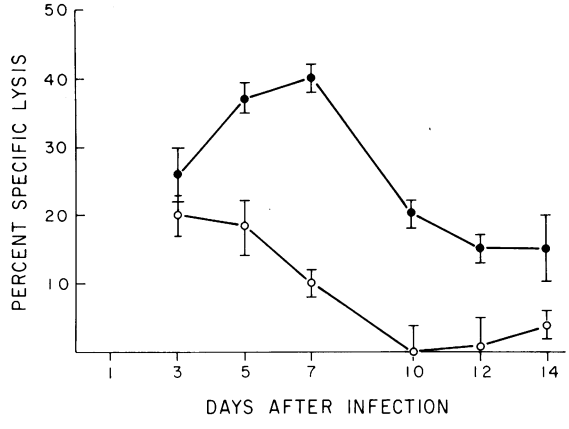
Additional support for the idea that T-cell–mediated reactions play a role in cardiac inflammation and necrosis during viral infection have been obtained with the use of TXBM animals. In such animals coxsackievirus produces minimal inflammation and necrosis, even though myocarditis occurs in the irradiated and bone-marrow–and thymus-cell–reconstituted (TXBM + T) control animals (Table 4).[216] Similarly, myocarditis is absent or diminished in nude BALB/c mice 6–7 days after infection with virus.[215,218]

The data indicate that T cells are critical to the development of the mononuclear cell infiltrates and myofiber necrosis in hearts after coxsackieviral infection. T cells could exert these effects via several mechanisms. First, they could be responsible for the accumulation of activated macrophages, which could damage myofibers or impair cardiac function.[231] Second, T lymphocytes may be required for the production of antibody, which is essential for antibody-dependent cell-mediated cytotoxicity (ADCC)[232] or lysis of myofibers by antibody and complement.[233] Third, myofibers could be damaged by the direct action of cytotoxic T cells.[234]

Cytotoxic T Lymphocytes: Experiments in adult male BALB/c mice have shown that coxsackievirus B-3 infection elicits production of spleen cells capable of lysing virus-infected myofibers and fibroblasts *in vitro.* The activity of syngeneic immune cells against infected targets is detected as early as Day 3 and peaks between Days 5 and 7 of infection (Text-figure 1).[229] Maximum reactivity against infected myofibers is noted by Day 5 (Table 5).[237] The cytotoxic cells exhibit viral specificity since lysis of infected myofibers and fibroblasts is greater than that observed using uninfected targets.[227,229] In addition, reciporocal assays performed using both Coxsackie and vaccinia viruses provide evidence of viral specificity. Thus immune spleen cells obtained from mice infected with vaccinia virus are not active against cells infected with coxsackievirus B-3. Likewise, coxsackievirus-immune cells are not cytotoxic for vaccinia-infected targets.[229]

It can be said that the cytotoxic reaction in male mice is mediated by T cells, because lysis of targets is abolished by treatment of effector cells with anti-thy 1.2 serum and complement (Table 6). Also, virus-specific reactivity is H-2–restricted; immune effectors exhibit little or no activity against infected allogeneic targets. In contrast, no impairment of reactivity occurs when immune spleens are treated with anti-Ig serum and complement or when adherent cells are removed. Thus, there is no evidence that B cells or macrophages exert a major role in cytotoxicity.[225,227]

Cytotoxic T cells are generated during infection of mice with several viruses.[235–242] Studies on lymphocytic choriomeningitis (LCM) virus infec-



TEXT-FIGURE 1—Kinetics of *in vivo* generation of male BALB/c spleen cell cytotoxic activity against coxsackievirus-infected (●—●) and uninfected (○—○) syngeneic neonatal fibroblasts. Fibroblasts were seeded into 6-mm wells and 5–6 days later used in cytotoxic assays that are described in detail elsewhere.[229] Briefly, coxsackievirus-infected and uninfected targets were labeled with ^{51}Cr and then incubated with 1) nonimmune spleen cells, 2) viral immune spleen cells, or 3) media alone. The cultures were incubated at 37 C at an effector-to-target cell ratio (E/T) of 150:1. After 18 hours the amount of ^{51}Cr in the supernatant and cells was measured and the percentage of lysis calculated by the use of standard formulas. The percentage of specific lysis represented the percentage of lysis by immune spleen cells minus the percentage of lysis by nonimmune cells. The difference between specific lysis of infected and that of uninfected target cells was significant on Days 5, 7, and 10. Lysis of uninfected fibroblasts by immune cells on Days 3 and 5 was significantly greater than lysis by nonimmune spleen cells. Data reprinted with permission of the publishers.[229]

tion of mice provide evidence that these cells are capable of inducing lesions in infected tissues.[243] It is therefore possible that cardiotropic viruses other than the Coxsackie B group can generate T cells or even non-T effector cells during infection that react against the myocardium.

The T lymphocytes generated during coxsackieviral infection apparently recognize both viral and H-2 specificities on target cells. This is similar to the restriction of T-cell–mediated lysis demonstrated during infection with other viruses. Thus maximal reactivity during LCM and ectromelia virus infection requires that the effector and the target cells share the K or the D end of the H-2 gene complex.[244] Findings with these viruses have led to the idea that the generation of cytotoxic T cells is not stimulated by the virion itself, but by infected host cells, with the T cells recognizing membrane antigens composed of both viral and H-2 determinants on the stimulator cell.[244] Thus cytotoxic T cells have been considered to have receptors for modified H-2 antigens or "altered self" or to process receptors for recognition of both self- and virus-induced antigens.

Table 5—Cytotoxicity of Day Five Viral-Immune and Nonimmune Spleen Cells Against Infected and Uninfected Murine Myofibers

Experiment	Spleen cell donor	% Lysis of myofibers*	
		Uninfected	Infected
1	Nonimmune	6.0 ± 3.0	5.5 ± 12.5
	Immune	43.3 ± 4.7†	75.0 ± 5.1‡
2	Nonimmune	−13.0 ± 1.0	−3.5 ± 0.5
	Immune	21.7 ± 6.4†	66.0 ± 8.6‡

The cytotoxic assay employed was similar to that described in Text-figure 1 with the exceptions that myofibers were maintained in 6-mm wells for only 48 hours before use, and the effector-to-target cell ratio was 100:1.

* Mean value ± SE.

† Significantly greater ($P < 0.05$–0.01) than the percentage of lysis by nonimmune spleen cells in same experiment.

‡ Significantly greater ($P < 0.05$) than all other values in same experiment. (From Huber et al.[227])

The nature of the viral antigen on Coxsackie-infected myofibers that is recognized by effector T cells is not known. Nevertheless, some characteristics of this antigen can be surmised. First, it is probably part of the target cell plasma membrane, since successful elicitation of T cell cytotoxicity in other viral systems depends on the incorporation of viral and H-2 antigens into the same lipid bilayer.[245] Second, it is not readily detected on infected cells by neutralizing antiserum known to contain antibodies directed against structural components of the virus capsid. In these studies viral antigens have not been detected on infected myofibers with the use of standard immunofluorescence techniques.[225] nor has it been possible to block T-cell-mediated killing of coxsackievirus-infected myofibers or fibroblasts by pretreating targets with antiviral serum.[227,229] In addition, the hearts of coxsackievirus-infected mice contain a KCl-extractable antigen with a molecular weight of around 50,000 that stimulates the production of mi-

Table 6—Cytotoxic Activity of Immune Spleen Cells Against Murine Myofibers after Treatment of Effectors with Anti-Ig or Anti-Thy 1.2 Serum and Complement

Animal number	% Specific lysis* of infected myofibers after treatment of effector cells with:			
	Medium	C	Anti-Ig + C	Antithy 1.2 + C
1	14.0	21.3	13.3	1.0†
2	24.8	13.3	17.0	−7.7†
3	18.3	16.3	20.6	−1.5†
4	17.3	24.0	23.3	−8.7†

Cytotoxic assays were performed as described in Table 5. Procedures for treatment of effector cells with antiserums were described in detail previously.[227] Spleen cell preparations were depleted of macrophages by an adherence technique.

* Percentage of lysis by immune spleen cells minus percentage of lysis by nonimmune cells.

† Significantly less ($P < 0.01$) than all other values for same animal. (From Huber et al.[227])

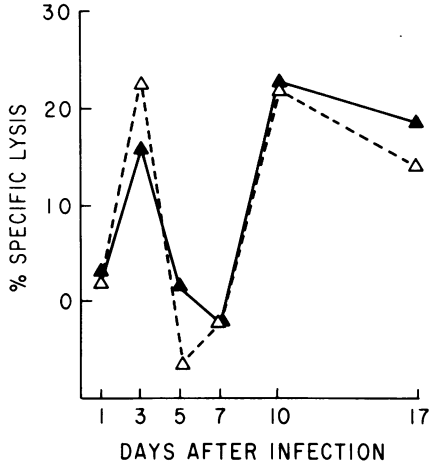
gration inhibitory factor by immune peritoneal exudate cells. In this system also, antigen does not bind specific neutralizing antibody.[223,246] Together, these findings make it unlikely that T cell reactivity is directed against intact viral particles absorbed to the membranes of target cells. These results do not exclude the possibility that structural virion antigens are components of the target cell membrane determinants recognized by T cells. Although experiments with coxsackieviruses were the first to demonstrate that infection with a nonbudding, nonenveloped virus could elicit cytotoxic T cells, this has also recently been achieved with reoviruses. Observations with reoviruses show that the gene that codes for hemagglutinin protein is the predominant gene determining specificity of the effector cells.[247] Coxsackieviruses also hemagglutinate, and it is possible that the structural component responsible for this function or some other capside subunit not reactive with neutralizing antibody is recognized by cytotoxic T cells.

Influence of Sex on Production or Expression of Cytotoxic Cells: Interestingly, male and female animals have been found to differ markedly in the capacity to generate coxsackievirus-specific effector cells. In male mice the immune cell response is brisk with cytotoxic activity against infected myofibers and fibroblasts detected on Day 3 and reaching a peak by Days 5–7 (Test-figure 1). This peak is followed by low levels of reactivity in the second week. During the initial week of infection cytotoxic cells are also generated that lyse uninfected target cells. This response occurs early (by Day 3) is always less than the virus-specific activity and decays after Day 7.[229,248,249]

In contrast, female mice exhibit a different pattern of reactivity. First, little if any virus-specific cytotoxicity is found using female cells obtained during the first week of infection. Usually when activity is detected it is directed against both infected and uninfected targets (Text-figure 2). Second, the female response is cyclic; spleen cells manifesting activity are detected early in infection (ie, Day 3) but are absent by Day 7 and then reappear during the second week of disease. Third, although male virus-specific activity is mediated by T lymphocytes exclusively, female immune spleen cells active against infected targets appear to be heterogeneous and composed of both T and non-T cells. Forth, the magnitude of the female response against infected targets, as measured by the percentage of chromium released, is always less than that exerted by male animals.[248,249]

Sex-related differences in reactivity appear to be a property of the virus inasmuch as the responses of male and female mice infected with vaccinia virus are essentially the same.[248]

Although the cause for differences in male and female reactivity after



TEXT-FIGURE 2—Kinetics of *in vivo* generation of female BALB/c spleen cell cytotoxicity against viral infected (▲—▲) and uninfected (△—△) syngeneic neonatal fibroblasts. The cytotoxic assay used was identical to that in Text-figure 1. The spleen cell-to-target ratio was 150:1. Significant specific lysis of both infected and uninfected targets was found with the use of spleen cells obtained 3, 10, and 17 days after infection. Immune cells exhibited a comparable degree of reactivity against both infected and uninfected fibroblasts. Data reprinted with permission of the publishers.[248]

coxsackieviral infection is not known, several possibilities can be considered. First, a difference in cytotoxic activity in males and females could be due to a lower concentration of effector cells in female mice. Dose response studies using various effector-to-target cell ratios have failed to support this idea.[248]

Second, the amount of virus administered, although adequate for the induction of virus-specific cytotoxic T cells in males, may have been inadequate for the generation of similar cells in the female animals. However, variation in the dose of virus used for infection failed to elicit virus-specific effector cells.[248]

Third, it is possible that suppressor cells or serum factors control the generation or expression of cytotoxic cells recruited during coxsackieviral infection. The regulatory mechanism for expression of reactivity is likely to be complex, because there is evidence that helper cells are also involved in the generation of cytotoxicity during viral infection.[250] Since in some systems cultured but not fresh lymphoid cells demonstrate cytotoxicity,[251] we carried out experiments in which Day 7 coxsackieviral immune female spleen cells were incubated at 37 C for 3 days and then assayed for activity. Uncultured 7-day cells demonstrated no activity, whereas the cultured cells were cytotoxic, but both infected and uninfected targets were lysed. It appears that cytotoxic cells preferentially survive incubation or that factors or cells capable of depressing lymphocyte reactivity are lost *in vitro*.[248] Even so, the cytotoxic cells appeared to lack virus-specific activity, suggesting that the population mediating such a response is lacking in the female spleen or cannot be uncovered by *in vitro* culture.

Fourth, it is assumed that infected host cells and not virions are respon-

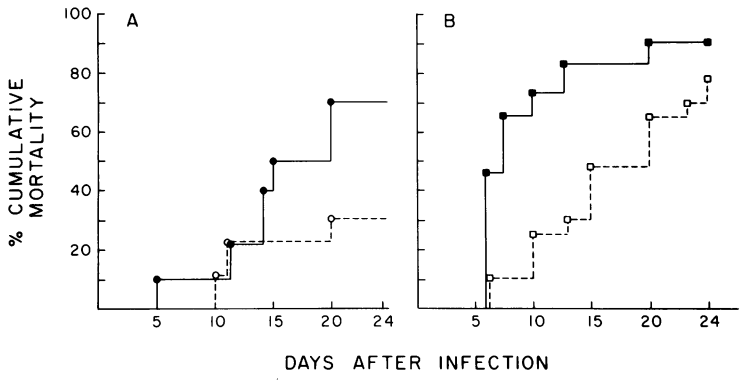
sible for the stimulation of virus-immune spleen cells. A more rapid elimination of virus from female tissues could diminish the number of infected parenchymal cells available to stimulate virus-specific effectors. Support for this idea derives from the finding that virus growth in the spleen declines more rapidly in female mice. Concomitantly, serum-neutralizing antibody titers peak earlier in these animals, a factor that would limit the spread of virus.[248]

"Autoreactive" Cytotoxic Cells: An unexpected finding to emerge from studies on cell-mediated immunity in coxsackieviral disease was the observation that spleen cells are generated that are capable of lysing uninfected myocardial cells and fibroblasts *in vitro* (Text-figures 1 and 2, Table 5).[217,219,248]

Several characteristics of the autoreactive cells have been identified. First, these cells arise soon after viral infection (as early as Day 3), and the reactivity detected continues for several days thereafter. Second, the female response is more vigorous than that of males. Thus marked autoreactivity is present in females on Days 3 and 4 and increases to Day 6. On the other hand, male activity is lower on Days 3 and 4 but gradually increases to levels slightly less than in female mice by Day 6. Third, all autoreactive cells detected 3 days after infection fail to express surface antigens characteristic of T cells or B cells and lack of the properties of macrophages. These early "autoreactive" cells have the characteristics of natural killers.[248,249]

The mechanism by which coxsackieviral infection elicits the production of effector cells with the capacity to lyse uninfected targets is not known. Such effector cells cannot as yet be identified with certainty as autoimmune, because activity has only been investivated with the use of neonatal myofibers or fibroblasts. It is known, however, that NK cells lyse a wide range of targets, and there is no evidence as yet that there is any greater target cell specificity to the NK cell response induced by coxsackieviruses than that already exhibited by these cells when induced by other viral and nonviral agents.[252]. Nevertheless, the appearance of "autoreactive" as well as viral-specific cytotoxic cells during coxsackieviral infection suggests that a heterologous population of effector cells with various specificities are generated and that some, if not all, possess the capacity to produce cardiac necrosis.

Correlation of Cytotoxicity With Systemic Disease: There is substantial evidence that the cytotoxic T cells generated during coxsackievirus infection are not only critical to the development of heart lesions but also play a role in generalized disease. For example, in adult BALB/c animals Coxsackie B-3 virus infection is a lethal disease; and death, which is not necessarily due to myocarditis, is dependent on normal T cell function (Text-



TEXT-FIGURE 3—Cumulative mortality of male and female BALB/c mice following infection with coxsackievirus B-3 intraperitoneally ($10^{4.5}$–$10^{5.0}$ TCD_{50}). The results presented in A were obtained with 10 female 14–16-week-old mice per group: TXBM+T (●—●) and TXBM (○—○). In the experiment illustrated in B, 26 male (■—■) and 27 female (□—□) age-matched 11–12-week-old mice were employed. Significant differences in the incidence of mortality were found in A after Day 14 and in B on Days 6–15.

figure 3A).[215,216] Moreover, the prevalence of death in infected adult BALB/c mice correlates with the fact that there is a sex-related difference in the pattern of virus-induced immune spleen cell activity against infected targets.[248,249] Thus male mice that generate a vigorous virus-specific cytotoxic response during the first week of infection show a high incidence of mortality at this time. As shown in Text-figure 3B, animals begin to die by Day 6 (40–50%); and by Day 13, 85% of the male mice have expired. In contrast, female BALB/c animals generate cytotoxic cells at low levels early after infection, exhibit no activity on Day 7, and then demonstrate renewed reactivity after Day 10.[248] As shown in Text-figure 3B, the incidence of death is only 10% by Day 7 but jumps to almost 50% by Day 15, and closely approximates that occurring in males by Day 24.

The data showing a sex-related difference in the incidence of mortality after Coxsackie B-3 viral infection correlates with observations made by Berkovich using type B-1 virus. In that study twice as many male as female CD-1 mice died by the tenth day of infection.[253] This 2 : 1 male:female ratio not only closely approximates our own observations but also the incidence of clinical coxsackieviral heart disease in male and female patients (Table 2).

Factors Augmenting Disease

Drugs

Corticosteroids administered to mice at the time of or soon after Coxsackie B virus challenge, markedly alter the course of infection, trans-

forming a benign disease into one that is characterized by abnormally high titers of virus in target organs, including the heart; extensive necrosis of myofibers, pancreatic acini, and hepatic parenchyma; gross cardiac lesions; and a high incidence of death.[161-163] Nevertheless, there is no evidence that the early viral antibody response is impaired in steroid-treated mice, since concentrations of serum neutralizing antibody during the first week of disease are normal. However, the migration of mononuclear inflammatory cells into the hearts of these animals is markedly reduced.[163] As noted earlier, the accumulation of mononuclear inflammatory cells into the myocardium of Coxsackievirus-infected mice is a prominent feature of the disease and is thought to play a role in suppressing viral growth. Presumably cortisone acetate can prevent the immigration of mononuclear cells to infected hearts by several mechanisms; it is known that corticosteroids interfere with the release of monocytes from the bone marrow and the mobilization of these cells into inflamed tissues.[254-256]

Steroids also affect other monocyte and macrophage functions. These drugs decrease the production of chemotoxic factors by lymphocytes,[257] abort the interaction of migration inhibitory factor with macrophages,[258] and impair phagocytosis and clearance or inactivation of antigen by macrophages and the reticuloendothelial system.[259,260] Macrophages possess surface receptors for corticosteroids, and recent studies have provided evidence that these hormones can impair cell enzyme activity involved in activation and participation in inflammatory responses.[261-263]

Since early anticoxsackieviral antibody synthesis is not impaired by steroids [163] and host defense is T cell-independent,[216] it is unlikely that the well-known lytic effect of this hormone on murine lymphocytes [264,265] is a factor in enhanced susceptibility to severe disease. Steroid-induced impairment of interferon production could play a role in the enhancement of lesions, since these drugs suppress interferon formation after infection with some viruses.[266] This does not occur with coxsackieviruses; in fact, after cortisone acetate treatment of infected mice, elevated serum interferon titers are present and are proportional to the enhanced growth of virus in the spleen.[267] Nevertheless, interferon has been implicated in the activiation of both macrophages and natural killer cells,[268-270] and it is possible that the enhanced viral growth found in cortisone-treated coxsackievirus-infected mice is partly the result of suppression of the interaction of interferon with inflammatory cells. Alternatively, corticosteroids could induce the suppression of mononuclear cell activity.[271]

Another consideration is that corticosteroids enhance the severity of disease by a direct effect on target cells. For example, the heart has receptors for these agents,[272] and studies have shown that steroids affect the me-

tabolism of most tissues to which they bind. After long-term corticosteroid therapy, abnormal electrocardiographic changes and diffuse mitochondrial alterations are seen in the rabbit myocardium.[273] Electrocardiographic changes have also been observed in patients on chronic steroid therapy.[273] In addition, it is possible that corticosteroids alter myofibers sufficiently to increase their susceptibility to infection.

The severity of coxsackieviral disease is also increased by cyclophosphamide (CY). The infection is characterized by inordinately high titers of virus in several organs, including the heart and pancreas, marked necrosis of myofibers, and a persistent viremia. The CY-treated animals are immunosuppressed inasmuch as IgG serum-neutralizing antibody cannot be detected; IgM antibody, however, appears early in disease. In contrast, levels of interferon in the peripheral blood are higher than in animals not treated with CY.[214,274]

Cyclophosphamide also appears to reduce the mononuclear inflammatory cell response normally observed in coxsackievirus-infected hearts,[274] an effect that may contribute to the severe disease seen after treatment with this drug.

It is unlikely that the administration of corticosteroids or CY to mice after clearance of virus from infected tissues can exacerbate coxsackieviral infection. This statement is supported by the finding that daily treatment of 14–18-day-old Swiss mice, compared with uninfected animals, with cortisone or CY beginning 18 days after coxsackievirus B-3 infection fails to increase mortality. Nevertheless, the administration of these drugs is associated with more deaths than observed in untreated control animals.[275,276]

Physiologic Factors

Coxsackieviral infection during the third trimester of pregnancy is associated with severe visceral lesions and enhanced mortality.[277,278] Similar findings have been made with another cardiotropic picornavirus, encephalomyocarditis virus; higher virus titers in the heart and more extensive cardiac necrosis and inflammation are found in pregnant mice than in nonpregnant control animals.[279]

In young adult and older mice the sex of the animal may also determine the severity of coxsackieviral disease. This point was illustrated earlier by two examples. First, two thirds of the deaths in CD-1 mice infected with coxsackievirus B-1 occurs in male animals. After castration the resistance of the highly susceptible males is increased, while that of the females is decreased; mortality rates in both groups are then similar.[253] Second, BALB/c mice experience more severe disease after infection with coxsa-

ckievirus B-3; twice as many males as females die during the first week of disease. By 24 days, however, female deaths closely approximate the number in males (Text-figure 3B).

Exercise also augments the virulence of Coxsackie B viruses. When weanling mice are forced to swim during acute viral infection, mortality is increased from 5.5% to 50%. Virus titers in the hearts of swimming animals are markedly elevated, and extensive cardiac necrosis occurs, associated with cardiac hypertrophy. Even when swimming is begun as late as the ninth day of infection, some increase in mortality is noted.[160] Exercise also increases the severity of murine Coxsackie A viral infection. In this case, also, cardiac size is increased and virus titers are elevated, but the disease is less severe than that seen after infection with Coxsackie B viruses.[280]

The nutritional status of the host is another factor that affects the severity of disease. Thus, although healthy young adult CD-1 mice ordinarily tolerate Coxsackieviral infection, in marasmus the disease is lethal; the extent of virus growth in the heart is increased, and there is a defect in the capacity of the animals to clear infectious virus from the tissues. The precise mechanism of this effect is unknown but it has been found that in marasmic animals the lymphoid tissues are atrophic, the inflammatory response in the heart is negligible, and the production of neutralizing antibody is reduced. In addition, there is an extreme degree of myocardial necrosis, and many necrotic foci are calcified. These latter findings are of interest because they demonstrate that unrestricted replication of virus in the heart can by itself result in the destruction of myofibers. In any event, resistance to infection rapidly increases after the animals are placed on a normal diet, an effect associated with a reversal of the abnormalities in lymphoid tissues. Although a defect in early antibody production is found in severe marasmus, serum-neutralizing antibody does not necessarily correlate with the increased resistance to viral infection seen in animals changed to a normal diet at the time of viral challenge, thus demonstrating a lack of correlation between neutralizing antibody synthesis and suppression of coxsackieviral replication in tissues.[194,213]

Adult mice fed a hypercholesterolemic diet and infected with coxsackievirus B-3 also experience a high mortality rate. This severe disease, however, is not associated with elevated titers of virus in target organs, and the mechanism of pathogenesis is not clear.[281]

Environmental Influences

Persistent exposure of adult mice to a temperature of 4 C results in severe Coxsackie B viral disease with elevated organ virus titers, extensive

lesions, and death.[282] Whole-body ionizing irradiation of suckling animals 29–120 hours before coxsackieviral challenge leads to a similar augmentation of infection.[283] Severe disease is not elicited if the animals are exposed to cold or irradiation 48 hours after viral challenge. In all likelihood, early host defense mechanisms are depressed by these environmental factors.

Characteristics of Hamster Coxsackie B Viral Disease

Coxsackieviruses readily replicate in hamsters; these animals were originally used in the isolation of these agents from human material.[196] When inoculated with B-3 virus, suckling (12-day-old) and weanling (22-day-old) hamsters develop a myocarditis. Virus replicates rapidly and reaches peak titer in the blood by Day 2 and in the heart by Day 3. As in the mouse, host defense mechanisms became operative after the third day because viral growth progressively declines in the heart thereafter. Myocardial necrosis and inflammation are observed after peak virus titers are found. Although cardiac lesions have not been extensively described, they are observed 6 and 7 days after infection and have "substantially subsided" by the eighteenth day. By the ninetieth day of infection all hearts examined are normal. As many as 45% of the suckling and 10% of the weanling animals die from the disease.[284]

In hamsters infected when sucklings or weanlings abnormalities in cardiac muscle mechanics occur that persist after recovery from the acute infection. Thus, in animals infected as sucklings, the maximum peak tension of isometrically contracting trabecular carnae is depresesd 17–34% when examined 18 and 90 days after infection. By 180 days muscle tension returns to normal. In contrast, although animals infected during the weanling period exhibit some reduction in muscle tension (15%) 18 days later, muscle contraction is normal after 90 days.[284] The data suggest that depending on host age, infection with a cardiotropic virus reduces cardiac muscle contractility and compliance and that such a physiologic defect may persist for several weeks.

Characteristics of Primate Coxsackie B Viral Disease

Chimpanzees and cynomolgous monkeys are susceptible to infection with Coxsackie B viruses administered by parenteral and oral routes.[71,187,198,199] All six members of the Coxsackie B virus group induce systemic disease in cynomolgous monkeys, although myocarditis has not been reported with B-6.[200]

Extensive studies on the clinical and pathological characteristics of coxsackievirus B-4 myocarditis have been carried out in young adult cynomolgous monkeys. After intravenous infection with a cardiotropic

strain, viremia lasts for 2 days. Fever (40 C) is occasionally present from the third to seventh day, and infectious virus can be isolated from the myocardium from the sixth to the fifteenth day. The left ventricular wall and interventricular system are the sites most likely to contain virus, although it can also be isolated from extracardiac tissue such as the brain, spinal cord, liver, and spleen for 4–10 days after challenge.[187]

The pathologic changes closely mimic human disease. On gross examination, the heart is dark red–brown, but after fixation the myocardium shows a yellow–gray mottling. The endocardium and pericardium are normal. Microscopic changes depend on the duration of infection. By Day 6 there are foci of myofibers that are homogeneously and intensely eosinophilic, have lost striations and nuclei, or are fragmented. The cellular reaction is minimal, and edema is prominent. By Day 8 myocardial lesions are patchy, diffuse, or confluent and involve the entire thickness of the ventricular walls. The myocardium contains a mononuclear cell infiltrate composed of lymphocytes, plasma cells, and histiocytes; and the adjacent myofibers are in various stages of necrosis. Cellular infiltration of the epicardium also occurs. Between Days 8 and 10 healing in the form of granulation tissue becomes evident in the myocardium, and by Day 10 calcification of myofibers can occur.[187]

Despite the occurrence of severe myocarditis in these animals, electrocardiograms obtained biweekly were not abnormal.[187]

Therapy

Therapy is symptomatic and supportive and is aimed at relieving pain and treating complications. Bed rest or at least reduction in work and exercise is recommended [150]; the rationale for this recommendation is based on experiments demonstrating that the severity of picornaviral disease is increased by exercise [160,280,285] and on clinical observations showing that physical activity may exacerbate infection with viruses.[74,286]

Immunosuppressive drugs have been used in the treatment of myocarditis and pericarditis. For example, corticosteroids have been employed when cardiac symptoms persist. It was proposed years ago that these drugs be used in the management of viral heart disease if hypersensitivity played a role in pathogenesis [1] and if the drugs were administered in the "postinfectious" phase of illness. This period has been estimated to be about 10 days after the onset of symptoms with infection by picornaviruses ie, Coxsackie and ECHO,[287] which are the primary causes of myopericarditis in man.[2,21,25,65,69] This timing is based on the assumption that such virus infections are transient, while in fact these agents have been isolated from patients for as long as 24–63 days after initial symp-

toms.[74,142,289] Poliovirus, also a picornavirus, can be excreted by the host for more than a month.[289] In addition, in many cases of viral myopericarditis, the etiologic agent is never identified and the duration of infection is unknown. Severe experimental disease can occur if infectious virus is present when corticosteroids are given.[161-163,29] Similarly, enhanced, often fatal coxsackieviral disease [22,140,144,164,165] with persistence of virus in the heart or pericardial fluid [140,144,165] has been noted in patients treated with these agents. Adverse effects of steroids have been reported even when these drugs were administered a month after the onset of symptoms of coxsackieviral myocarditis, at a time when high titers of serum-neutralizing antibody were present.[164] It has been suggested, therefore, that steroids be restricted to life-threatening situations such as cardiovascular collapse.[150,293]

Clinical and Theoretic Considerations

Clinical

The diagnosis of myopericarditis requires an awareness by the physician of the various clinical manifestations and a high degree of suspicion; the diagnosis is often missed and only made at autopsy. Some cases may be subclinical, and there is evidence that standard tests such as the ECG do not invariably detect active disease and may be unreliable in determining prognosis during the acute stages of inflammation. New procedures are needed to supplement techniques used in diagnosis and evaluation of heart lesions. For example, gallium imaging [294] may become useful in monitoring the extent and/or duration of cardiac inflammation.

In most cases of viral myocarditis the responsible agent is never identified. The failure to recognize the virus and to know the period of replication creates problems in the management of the disease and prevents one from determining its true incidence and population distribution. Moreover, insight into the relationship of viral myocarditis to cardiomyopathy requires more detailed virologic data.

At present, therapy is symptomatic and supportive. Specific forms of treatment may derive from a clearer understanding of the pathophysiology of the disease and the development of tests that can more accurately gauge the severity and duration of lesions. It is possible that broad spectrum antiviral agents might be useful in the treatment of the acute disease, while agents with specificity for distinct lymphoid cell populations might be of value during periods of active inflammation, as is suggested by the experimental demonstration that ATS markedly reduces the severity of coxsackieviral heart disease.[216]

The role of cytotoxic cells in the production of human heart disease

needs to be investigated. The use of *in vitro* assays for detection of cytotoxic cells [227,229] or MIF production [223] provides the opportunity for the evaluation of cellular immune responses in patients during acute disease and convalescence and for the determination of the existence of relationships between the nature of cytotoxic cells, the sex of the patient, and the severity and duration of the lesions.

Information is also needed that can help us determine whether there is a population group that is particularly susceptible to viral myocarditis. The relationship of HLA types to clinical disease might be of value in identifying such individuals in view of evidence that cell-mediated immunity has been implicated in the pathogenesis.

Theoretic

As outlined in this review, there is strong evidence that immunologic mechanisms are involved in the production of myocardial necrosis and inflammation elicited during coxsackievirus infection. Since these agents ordinarily induce only acute disease, the experiments by Wilson et al [154] were of particular interest, because they showed that infection of adolescent mice with coxsackievirus could result in chronic myocarditis. Inflammation persisted long after infectious virus was cleared from the tissues, suggesting that an "autoimmune" reaction was responsible for the lesions. This view has now been supported by the demonstration that coxsackieviral infection generates a population of effector cells that are cytotoxic for uninfected myofibers.[227] At least one of these "autoreactive" cell populations belongs to the natural killer cell group.[249] It is not known, however, what antigen or antigens these or other chronic inflammatory cells recognize on myofibers. In any event, chronic myocarditis is of interest because this lesion could lead to cardiomyopathy. Inflammatory cells might appear as the result of an autoimmune reaction or in response to persistence of viral antigen. Animal viruses known to produce latent or chronic infections would be the most likely candidates for induction of this disease. Nevertheless, the viruses most often implicated in cardiomyopathies belong to the picornavirus group,[179,180] agents which usually cause only acute infections. However, the persistence of infectious picornaviruses in tissues might occur under conditions where host defense mechanisms are suppressed; this could be caused by treatment with cortisone [161-163] or CY [214,274] or result from viral-induced immune depression,[295,296] an underlying immunodeficiency disorder,[297] or exercise.[74,160] Another consideration is that recurrent acute infections with different viruses or other infectious agents could be a cause of congestive cardiomyopathies.

As already noted, Coxsackie B viral myopericarditis in adolescence and adulthood predominates in males (Table 2). A similar association of severe systemic disease with the male sex has been found in animals. For example, an accelerated death rate occurs in male mice infected with Coxsackie B-1 or B-3 [228,253] (Text-figure 3B), an effect which can be prevented by castration.[253] In addition, a diabetic-like disease induced by Coxsackie B-4 infection of mice also predominates in males.[298] There is evidence that T-cell–mediated reactions are involved in the pathogenesis of the disease, due to these agents and closely related viruses.[217,299] An *in vitro* correlate of these findings is the demonstration that male mice have a stonger T cell effector response than female mice.[248,249] It therefore appears that sex-related factors influence the pathophysiology of picornaviral disease and that these factors may be immunologically determined. Moreover, a striking difference in the prevalence, severity, and mechanism of immune-mediated lesions can be observed clinically and experimentally in males and females after exposure to different antigens. In coxsackievirus-infected individuals, where the evidence indicates that lesions are T-cell–mediated, the parenchymal disease is more severe in males. In contrast, systemic lupus erythematosus is found primarily in females, and experimental findings indicate that B-cell–mediated reactions are involved in pathogenesis, particularly in the production of renal lesions (Table 2).

Summary

Numerous viruses have been implicated as a cause of myocarditis in man, although only a few of these agents have actually been isolated from the hearts of infected individuals. There is evidence that susceptibility to infection and severity of disease are influenced by multiple factors, including the host's age, sex, seasons, the occurrence of epidemics, pregnancy, exercise, and the mode of therapy. Genetic factors probably play a role, but their contribution has not yet been evaluated.

The true incidence of viral myocarditis or myopericarditis is not known, although in epidemics or in highly susceptible groups cardiac disease may occur in 5% or more of the infected population. An accurate assessment of the extent of disease has not been possible because of difficulties involved in the diagnosis of myopericarditis and in the isolation of the etiologic agent. Work on Coxsackie B viral infection, the most commonly identified cause of human myopericarditis, shows that the clinical manifestations are variable; and while the disease is usually acute and severe in the neonate, it is most often acute and benign in adolescents and adults but can pursue a subacute or chronic course in such individuals and result in complications and permanent sequelae. There is also evidence

that some individuals may experience bouts of myocarditis caused by different viruses, and the possibility exists that recurrent infections may play a role in the pathogenesis of cardiomyopathies.

It is remarkable that the murine model of Coxsackie B viral myocarditis closely resembles human disease, particularly with respect to the characteristics of the lesions, and the conditions or factors which enhance the severity of the disease. In addition, the accelerated appearance of severe infection in male mice correlates with the predominance of severe disease in males in adolescence and adulthood. These similarities have provided the impetus for detailed studies of mice infected with coxsackieviruses. The evidence indicates that both the production of neutralizing antibody and the arrival of mononuclear inflammatory cells in the tissue play important roles in suppressing virus growth. The reactions responsible for viral clearance are not T-cell–dependent. In contrast, T-cell–mediated immunity appears to be involved in the pathogenesis of the lesions. Thus the severity of myocarditis is reduced in animals depleted of T cells, and *in vitro* studies have shown that infection stimulated production of cytotoxic T cells capable of lysing viral infected myocardial cells *in vitro*. The nature of the viral antigen recognized by the effector cells is not known, but it is presumed to be a component of the plasma membrane of myofibers, because T cell cytotoxicity involve recognition of surface glycoproteins controlled by the major histocompatibility complex. It is unlikely that the surface change is caused only by infectious virus or absorbed particles, since neutralizing antibody does not block T cell killing. The infection also elicits production of autoreactive cells capable of lysing uninfected myofibers *in vitro*; at least one population of the autoreactive cells belongs to the "natural killer" group. Such autoreactive cells would be endowed with properties enabling them to cause tissue destruction after infectious virus has been cleared from the heart.

Experimental evidence points to at least two other mechanisms for cardiac injury during coxsackieviral infection. Virus replication can induce myofiber necrosis directly, and this mode of injury is likely to predominate when animals are immunosuppressed and viral replication is unchecked. In addition, infection results in abnormalities in myofiber contractility, and this effect persists for several weeks after virus replication has been terminated.

Finally, we would predict on the basis of the work done on coxsackieviral myocarditis that in other picornavirus-induced diseases T- and NK-cell–mediated reactions would also play a role in pathogenesis. In particular, it is likely that this is the case in coxsackievirus-induced diabetes mellitus, where it is known that islet cell lesions contain a lymphocytic infiltrate.[195,298,306]

ADDENDUM

A Memorial Tribute

Dr. Jack Fletcher Woodruff died of a cardiac arrhythmia on April 17, 1980, at the age of 44, one day following the submission of this review article. His loss will be deeply felt by his family, friends, and colleagues. He was a gifted and dedicated pathologist.

Dr. Woodruff, born in Bridgeport, Connecticut, attended the University of Massachusetts at Amherst and was graduated cum laude. After obtaining an MD degree from Temple University School of Medicine in 1962, he came to Cornell University Medical College, where he participated in the intern and residency programs and was an instructor, assistant professor, and associate professor in the Department of Pathology.

It was also at Cornell that Dr. Woodruff began to investigate the immunopathology of Coxsackie B-3 virus-induced myocarditis as a Research Fellow with Dr. Edwin D. Kilbourne. Initially, Dr. Woodruff showed that postweaning malnutrition greatly enhanced the severity of coxsackievirus disease in mice. Later, he and his wife, Dr. Judith Woodruff, were the first to demonstrate that the lymphocytic infiltration and myofiber destruction observed in Coxsackie B-3 virus-induced myocarditis correlated with the presence of immune T lymphocytes in the host, and that immune T lymphocytes could specifically lyse virus-infected fibroblasts and myofibers *in vitro*. His work has been instrumental in furthering our understanding of the mechanisms of this disease.

Dr. Woodruff was a man of sincerity, warmth, and humor who was never too busy to care for or help others. He had unceasing drive, curiosity about life, and absolute integrity. He will be missed.

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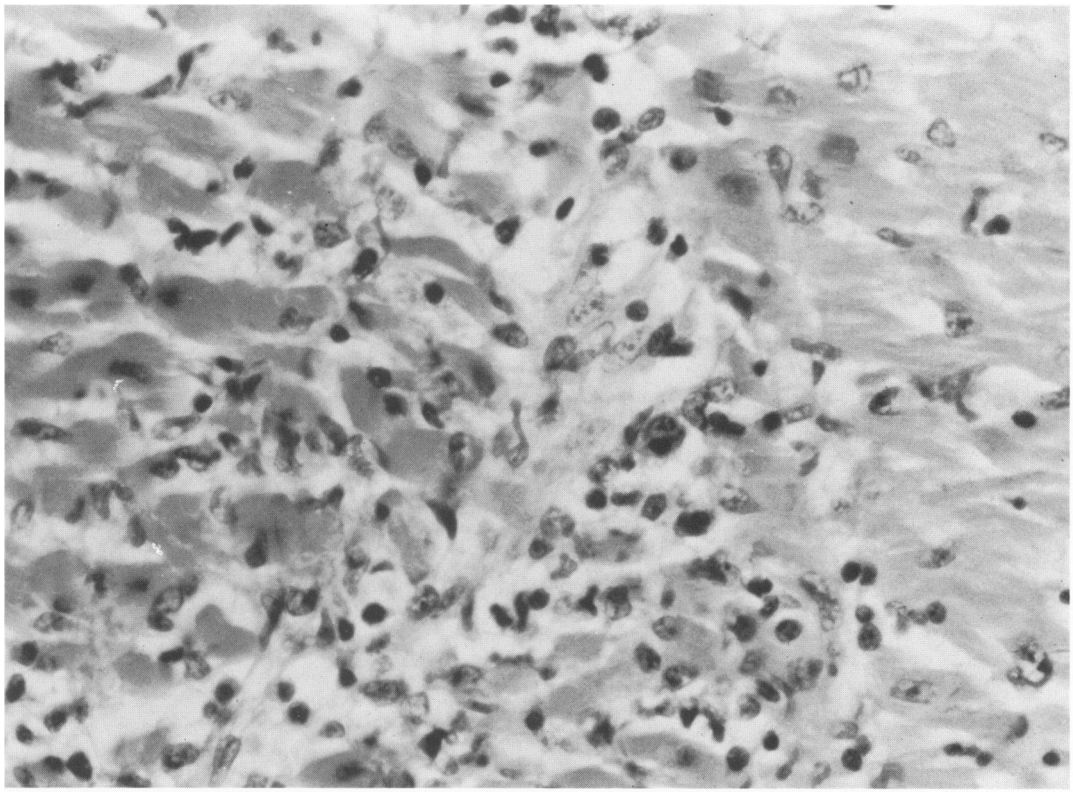
Figure 1—Acute pancarditis in a 10-year-old white girl who developed a influenzalike illness during an enterovirus epidemic. The patient convalesced for a few days and then resumed full physical activity. She died suddenly 10 days after initial symptoms. The entire myocardium was infiltrated by an extensive mononuclear inflammatory cell infiltrate, which often surrounded partially necrotic myofibers. (H&E, ×500) (With a photographic reduction of 8%)

Figure 2—Chronic Coxsackie B-3 viral myocarditis in a 17-year-old white male who was treated for over a year with corticosteroids. Clinical course lasted for 1½ years; death was caused by an arrhythmia. Myocardium showed marked myofiber dropout with replacement fibrosis. Small foci of acute and chronic inflammatory cells were also noted (not shown). (H&E, ×500) (With a photographic reduction of 8%)



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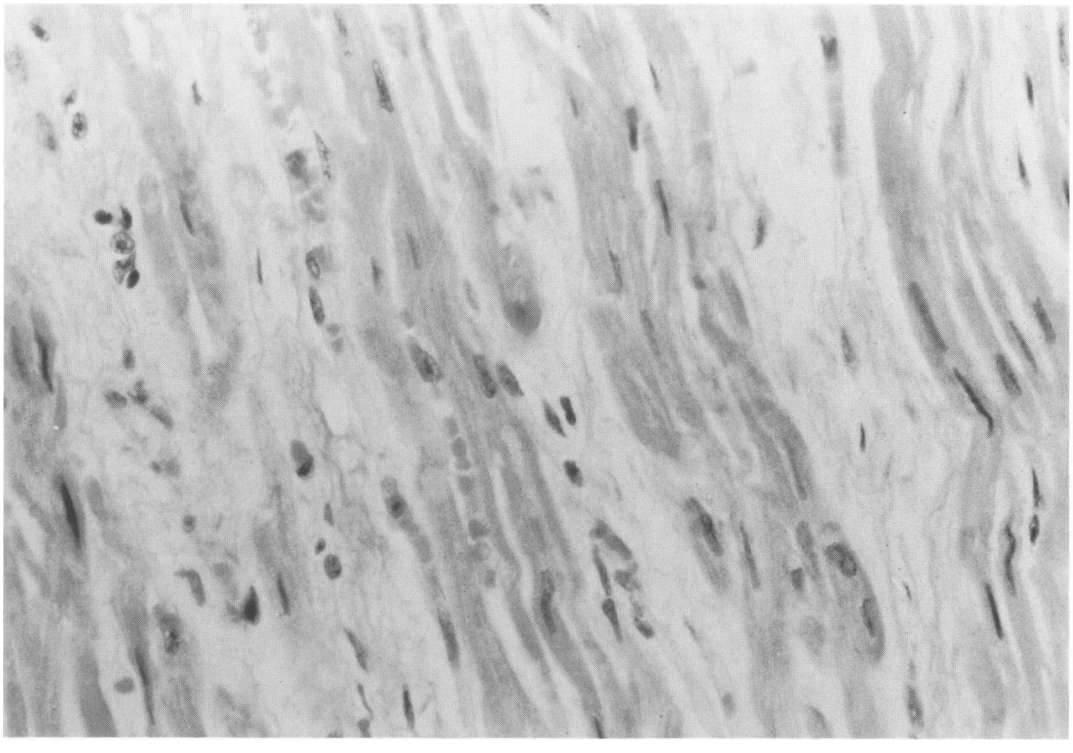

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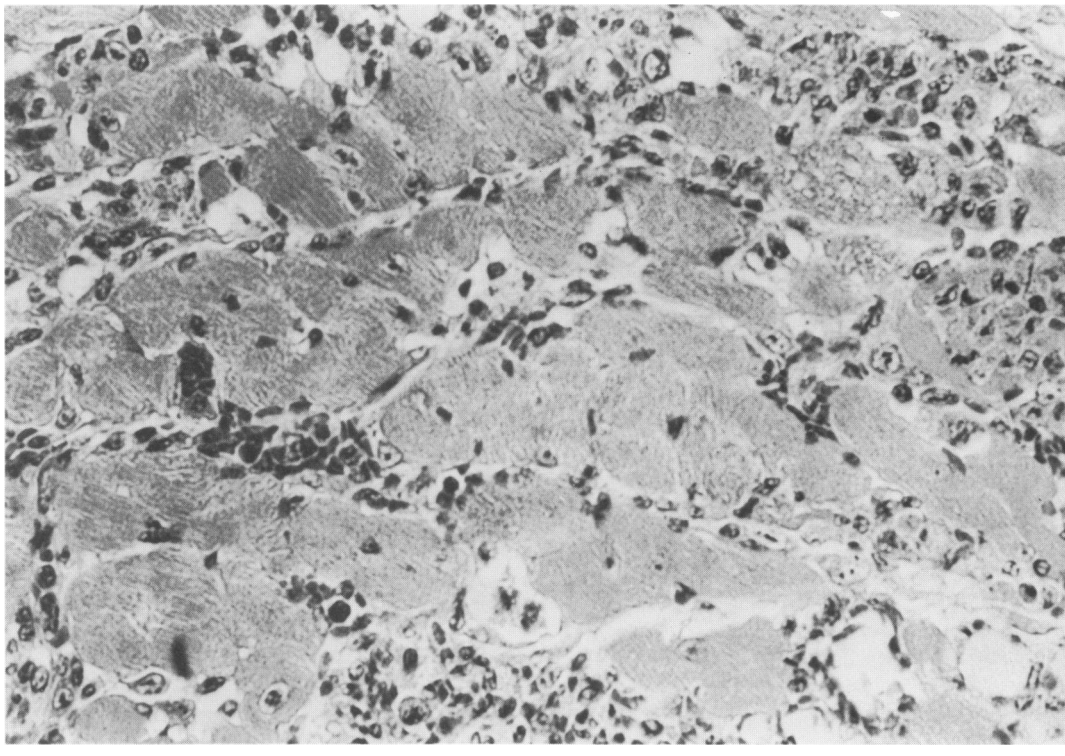
Figure 3—Myocardium of NRS-treated 6–8-week-old CD-1 mouse 6 days after coxsackievirus B-3 infection. Collections of mononuclear inflammatory cells surround partially nectotic myofibers. (H&E, ×380) (With a photographic reduction of 8%)

Figure 4—Myocardium of ATS-treated 6–8-week-old CD-1 mouse 6 days after coxsackievirus B-3 infection. Essentially no inflammation or necrosis is evident in this section (H&E, ×190) (With a photographic reduction of 8%)



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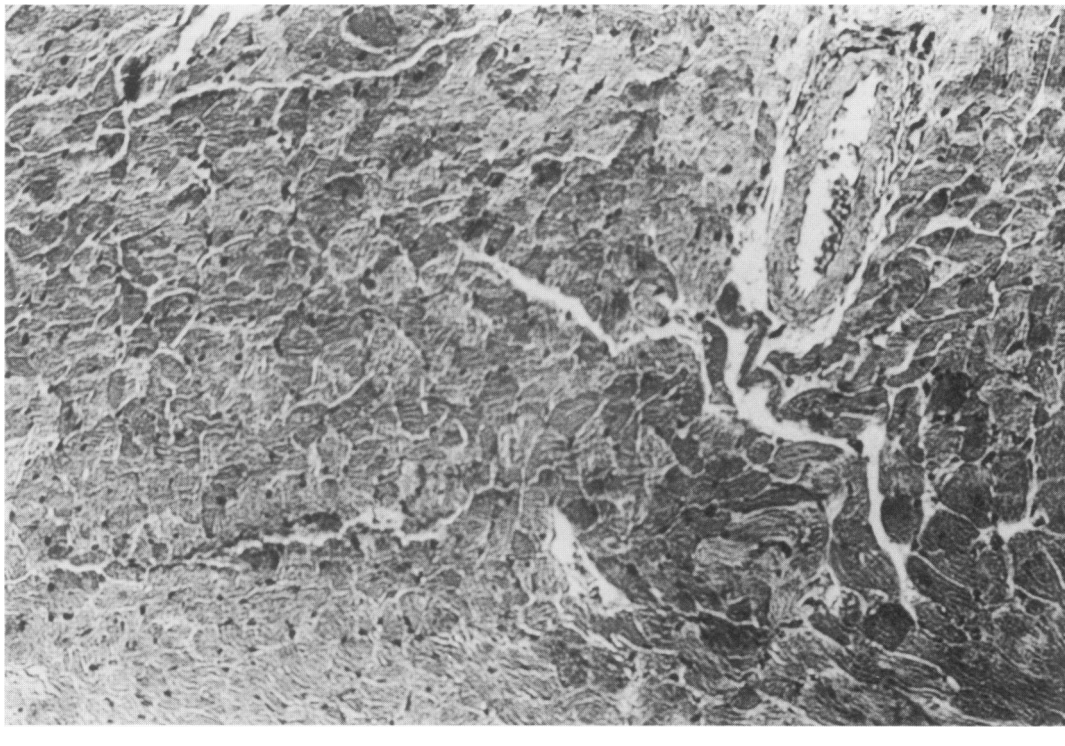

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