

CHAPTER

# 23

## Childhood Myocarditis and Dilated Cardiomyopathy

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Myocarditis, particularly in children, remains a major cause of morbidity and mortality worldwide.<sup>1,2</sup> Dilated cardiomyopathy (DCM) is the major reason for cardiac transplantation in the United States and Europe, with an annual incidence of 2 to 8 cases per 100,000 and an estimated prevalence of 36 per 100,000.<sup>3</sup> The idiopathic form of DCM accounts for approximately 50% of the patients undergoing transplantation. Each year in the United States more than 750,000 cases of heart failure are reported,<sup>4</sup> with approximately 250,000 deaths, and myocarditis or DCM probably accounts for 25% of these cases.<sup>5</sup> At present, the treatment of these conditions is limited to management of the symptoms or transplantation, and the cost is thought to be \$3 billion to \$4 billion annually. Therefore, understanding the basis for this disorder and developing preventive and disease-specific therapies would have a major impact on health care in the United States. In this review, we describe some of the progress toward understanding the etiologies of these disorders in children and clarification of the mechanisms of pathogenesis.

## **DIAGNOSIS OF VIRAL INFECTION**

### **HISTORICAL PERSPECTIVES**

Viral infections of the heart are important causes of morbidity and mortality in children and adults. A patient who has acute myocarditis, the best studied of these infections, typically presents with severe clinical manifestations, especially in the newborn period.<sup>6</sup> Idiopathic DCM appears to occur as a late sequela of acute or chronic viral myocarditis,<sup>1,7-10</sup> due to persistence of virus<sup>7</sup> or an autoimmune phenomenon due to previous exposure to the inciting virus.<sup>11</sup> The affected individual may require long-term medical therapy for congestive heart failure and, in many cases, heart transplantation may be required. In some cases, sudden cardiac death occurs,<sup>8</sup> particularly in athletes.<sup>12</sup>

Endomyocardial biopsy (EMB) and histopathologic study demonstrating cellular infiltrates (particularly lymphocytes), edema, myocyte necrosis, and myocardial scarring were developed to improve diagnostic capabilities, but results were inconsistent among pathologists. The so-called Dallas criteria,<sup>13</sup> described in 1987, were developed in an attempt to improve the high rate of diagnostic disagreement among pathologists by using uniform criteria. However, because of insensitivity<sup>14</sup> and possible risks involved in biopsies, particularly in small or critically ill children, many centers abandoned EMB as a diagnostic tool.

An initial association between virus infection and the development of myocardial disease was made several decades ago. Grist and Bell<sup>15</sup> presented comprehensive serologic data correlating enterovirus infection with myocarditis. However, the role of these viruses in

DCM was less well established and based mainly on the observation of high titers of neutralizing antibody in cases of sudden-onset disease.<sup>16</sup> This led to the proposal that DCM is a progression from an enteroviral myocarditis.

Enteroviruses, and particularly the coxsackievirus B (CVB) group, have a major positive tropism for skeletal and cardiac muscle. However, isolation of infectious virus from patients with heart muscle disease is rare.<sup>17</sup> For example, in a study of EMB samples from 70 patients with myocarditis or DCM, no enterovirus was isolated from or virus-specific antigens detected in any of these samples,<sup>18</sup> despite evidence of virus association from retrospective serologic study.

Detection of virus-specific IgM is more significant, in that it usually reflects recent or persisting infection. CVB-specific IgM was detected in nearly 40% of patients with myocarditis compared with none of the controls.<sup>19</sup> Such IgM responses have been shown to persist for up to 6 months.<sup>20</sup> CVB-specific IgM responses have also been reported in patients with end-stage DCM undergoing cardiac transplantation, with the IgM responses persisting for up to 19 months before transplantation.<sup>21</sup>

The concept of an enteroviral origin of heart muscle disease is reinforced by animal models of myocarditis and DCM. A cardiotropic strain of coxsackievirus B3 (CVB3) induces inflammatory heart muscle disease in mice. Infectious virus cannot be isolated from myocardium after the first 2 to 3 weeks,<sup>22,23</sup> although many of the animals progress to left ventricular disease reminiscent of DCM,<sup>24,25</sup> supporting the hypothesis that DCM can be a sequela of a viral myocarditis.

## MOLECULAR DIAGNOSTIC TECHNIQUES

The failure to isolate virus or to detect viral antigens in patient EMB samples, despite the serologic demonstration of persistent infection, prompted the development of virus-specific molecular hybridization probes. These were designed to detect the presence of enteroviral RNA sequences in myocardial or other tissue samples. The studies by Bowles and coworkers<sup>26,27</sup> and by Kandolf et al.<sup>28,29</sup> led to the direct demonstration of persisting enteroviral infection of the myocardium in myocarditis patients and supported the hypothesis that DCM was caused by enteroviral persistence and is a late sequela of viral myocarditis. Polymerase chain reaction (PCR) has been used in the rapid detection of viral sequences in many tissues and body fluids, including the myocardium of patients with suspected myocarditis or DCM.<sup>30-36</sup> Evidence from our laboratory suggested that adenovirus often is found in hearts of affected children and could be an important cause of myocarditis and DCM.<sup>37,38</sup>

We (unpublished data) have studied more than 750 myocardial samples from patients with myocarditis or DCM (or both) by using PCR to detect a range of viruses, including the enteroviruses, adenoviruses, cytomegalovirus (CMV), herpes simplex virus, Epstein-Barr

virus, parvovirus, influenza virus, and respiratory syncytial virus. The patients were divided into groups by age: neonates (age between 1 day and 1 month); infants (age between 1 month and 1 year); toddlers (age between 1 year and 5 years); children (age between 5 years and 13 years); adolescents (age between 13 years and 18 years); and adults (age greater than 18 years). More than 65% of the samples came from patients between the ages of 1 day and 13 years; more than 600 of the patients had a diagnosis of myocarditis, and the remainder had DCM. More than 200 samples from individuals with medical histories inconsistent with these criteria were included as unaffected, age-matched controls.

The overall prognosis of the patients with acute myocarditis was poor, with an overall mortality of more than 50%. Approximately 40% of the DCM patients underwent heart transplantation. The majority of patients with myocarditis had poor recovery of their cardiac function, while the remaining patients had mild recovery with persistence of depressed cardiac function or complete recovery or underwent transplantation.

Serologic findings consistent with viral infection were seen in 38% of patients studied, primarily enterovirus and CMV, from acute and convalescent titers. Only 7 patients had positive postmortem viral cultures from multiple organs, including the heart. Four of these patients had postmortem cultures positive for enterovirus from heart, brain, liver, and kidney, and 3 patients grew adenovirus from specimens of the lungs and heart. Two patients grew CMV from specimens of the heart and lungs (1 in a patient whose sample grew enterovirus, 1 in a patient whose sample grew adenovirus). One other child had adenoviral particles in the heart by electron microscopy but had negative viral cultures.

PCR amplified a viral product in approximately 40% of the samples obtained from patients with myocarditis compared with 1.5% of control samples. Of these positive myocarditis samples, adenovirus was detected in more than 50% (80% adenovirus type 2, 20% type 5; Fig. 23-1 and 23-2; see color plate 41) and enterovirus in 33%, whereas the remainder were mainly CMV but also included a few herpes simplex virus type 1, Epstein-Barr virus, parvovirus, influenza, and respiratory syncytial virus positives. Compared with the positive peripheral cultures obtained, 80% amplified viral genome, with 76% agreement in the results obtained by PCR. PCR analysis of blood drawn from 300 patients at the same time that tissue was obtained demonstrated only 3 of 300 blood samples analyzed by PCR amplified viral genome (CMV in 2, enterovirus in 1).

In the patients with DCM, 20% were positive for viral genome: adenovirus in 60% of the PCR-positive samples and enterovirus in the remaining 40%. None of the blood samples from these patients were PCR positive.

These data show that adenovirus is detected at least as often as the enteroviruses in the hearts of children and adult patients.<sup>37-39</sup> Further, no significant differences were observed among age groups with respect to the relative frequencies of detection of adenovirus and enterovirus.

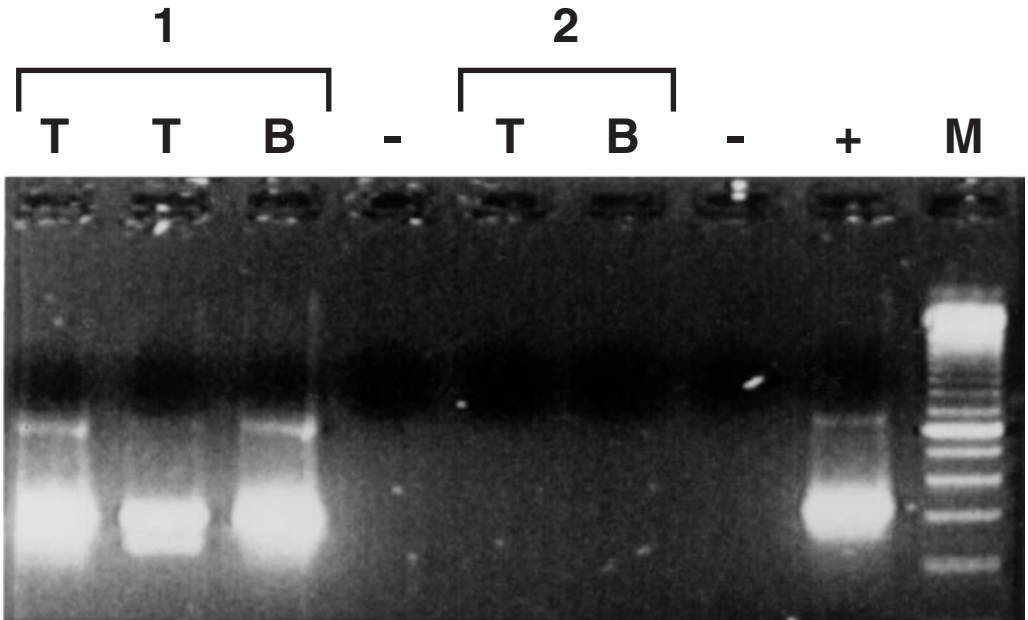


Fig. 23-1. The detection of adenovirus DNA by nested polymerase chain reaction in tracheal aspirate (*T*) and endomyocardial biopsy (*B*) samples in 2 patients: one positive for adenovirus type 5 (patient 1) and the other negative (patient 2). Lanes - are water controls and + is adenovirus type 2 positive control DNA. Lane M is a 100-bp DNA ladder (Life Technologies). The adenovirus identified in each of the samples from patient 1 was determined to be type 5 by DNA sequencing of the polymerase chain reaction product.

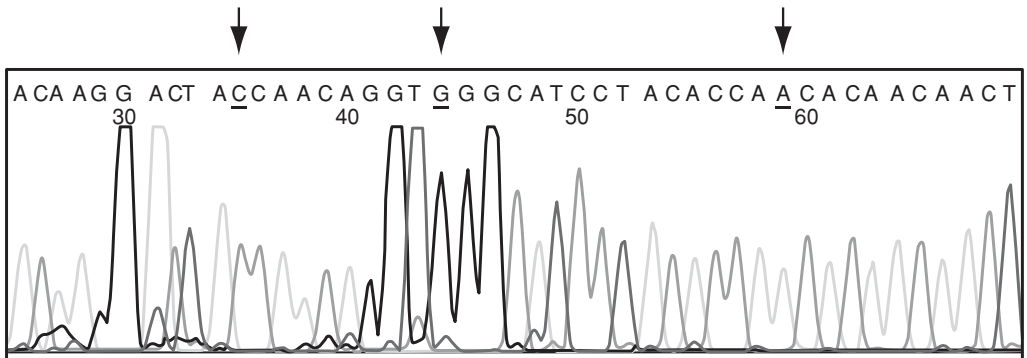


Fig. 23-2. DNA sequence analysis of an adenovirus-specific polymerase chain reaction product. The region shown is highly divergent between adenovirus serotypes, allowing rapid identification of the virus amplified—in this case adenovirus type 5. Analysis of the 3 nucleotides indicated is sufficient to differentiate all adenovirus serotypes sequenced to date. See color plate 41.

Different isolates of CVB3 vary in their cardiovirulence. Tu et al.<sup>40</sup> reported that a single nucleotide difference, at position 234 of the CVB3 genome, determined the phenotype of the virus. If this nucleotide was a cytidine, the virus was attenuated compared to another strain of CVB3 with uridine at this location. Subsequently it was reported that in natural isolates of CVB3, regardless of cardiovirulence, this position was invariably uridine, suggesting that other nucleotides are important in determining the viral phenotype.<sup>41</sup> By construction of chimeras from cardiovirulent and noncardiovirulent strains of CVB3, critical regions were identified within the 5' untranslated region, including within stem-loop motifs associated with the internal ribosome entry site.<sup>42</sup> In addition, 2 amino acid changes within the VP2 and VP3 structural proteins had some additive effects on cardiovirulence. DNA sequencing of the genome of adenovirus variants detected by PCR could potentially distinguish between cardiovirulent and noncardiovirulent adenovirus subtypes, although the size of the adenoviral genome and the number of adenoviral types (more than 40 have been identified) may make such an analysis impractical and likely uninformative. To date, it appears that the group C adenoviruses are primarily associated with heart muscle disease.

## HEART DISEASE IN CHILDREN INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) infection is increasingly recognized as an important cause of heart disease, particularly myocarditis and DCM. However, the pathogenesis of the heart-muscle disease in the acquired immunodeficiency syndrome is unclear. CMV sequences have been detected in myocardial samples. For example, Wu et al.<sup>43</sup> reported a study of the role of CMV infection in the development of HIV-associated cardiomyopathy. Using probes derived from the CMV immediate-early and delayed-early genes, they analyzed by *in situ* hybridization EMB samples from 12 HIV-infected patients with global left ventricular hypokinesis demonstrated on 2-dimensional echocardiography and 8 autopsy cardiac samples from HIV-infected patients without cardiac disease during life. Of the 12 EMB specimens, 6 had hybridization for transcripts of the CMV immediate-early gene, consistent with nonpermissive or latent infection. Similar patterns were not found in any of the 8 autopsy control samples. All 6 patients presented with unexplained congestive heart failure and had biopsy samples with immunohistochemical evidence of increased myocardial major histocompatibility complex (MHC) class I expression, a finding typical of non-HIV myocarditis. None of the EMB samples had characteristic CMV inclusions and no specific hybridization was noted with the delayed-early gene probe, suggesting that no active viral DNA replication was present. Only 2 of the 6 patients with myocyte hybridization with the immediate-early probe had clinical evidence of solid organ infection with CMV at presentation with cardiovascular complaints.

The first comprehensive study of the etiologic basis of heart disease was reported by Barbaro et al.<sup>44</sup> They performed a prospective, long-term clinical and echocardiographic follow-up study of 952 asymptomatic HIV-positive patients to assess the incidence of DCM. All patients with a diagnosis of DCM underwent EMB for histologic, immunohistologic, and virologic assessment. During a mean follow-up period of 60 months, an echocardiographic diagnosis of DCM was made in 76 patients (8%). The incidence of DCM was higher in patients with a CD4 count of less than 400 cells/ $\mu$ L and in those who received therapy with zidovudine. A histologic diagnosis of myocarditis was made in 63 of the patients with DCM (83%). Inflammatory infiltrates were predominantly composed of CD3 and CD8 lymphocytes, with staining for MHC class I antigens in 71% of the patients. In the myocytes of 58 patients, HIV nucleic acid sequences were detected by *in situ* hybridization, and active myocarditis was documented in 36 of the 58. Among these 36 patients, 6 were also infected with CVB (17%), 2 with CMV (6%), and 1 with Epstein-Barr virus (3%). They concluded that DCM might be related to a direct action of HIV on the myocardial tissue or to an autoimmune process induced by HIV, possibly in association with other cardiotropic viruses. Although these data indicate a similar origin for myocarditis and DCM in HIV-infected adults and non-HIV-infected adults, the frequency of detection of CMV was somewhat lower than in previous studies.<sup>43</sup>

In 1999 we<sup>45</sup> reported a similar study in 32 pediatric patients with advanced HIV disease. In 13 of the 32 samples (41%) from HIV-infected children, 1 or more virus types were detected. The virus identified most often was adenovirus (10 of 32 = 31%), followed by CMV (7 of 32 = 22%).

DNA sequence analysis of the adenoviruses amplified from the HIV-infected patient samples demonstrated only adenovirus type 5. This is in contrast to the apparent predominance of adenovirus type 2 in non-HIV-infected children with myocarditis or DCM (see previous section). This difference may reflect a different spectrum of adenoviral susceptibility in HIV-infected and non-HIV-infected children or a difference in viral pathogenesis in immunocompromised children. However, it does appear that the group C adenoviruses are identified most often in myocardial samples.

Active myocarditis was observed in 11 of the 32 HIV-infected patient myocardial samples (34%), and infiltrates borderline for myocarditis were observed in another 13 cases—a frequency of myocarditis considerably higher than in the study by Barbaro et al.<sup>44</sup> However, the pediatric patients studied were those with advanced, end-stage disease, whereas the patients studied by Barbaro and colleagues were initially asymptomatic. Our results may indicate that children with HIV are more prone to the development of myocarditis, perhaps because of a greater susceptibility to infection with cardiotropic viruses. Adenovirus was detected in 4 of the 11 samples with myocarditis, in 3 samples with borderline infiltrates, in 1 patient with infiltrates confined to the epicardium, and in 2 with

no histologic evidence of inflammation. Of the 2 patients with adenovirus but no inflammation, 1 was reported to have died of congestive heart failure and the other of adenoviral pneumonia. Adenovirus was detected in 3 of the 6 patients with congestive heart failure; only 1 had myocardial infiltrates, and these were confined to the epicardium. Among the 3 patients with DCM, 1 was positive for adenovirus. Seven of the 18 patients (39%) with postmortem cardiomegaly were positive for adenovirus by PCR. Two patients were reported to have adenoviral pneumonia at the time of death; both patients were positive for adenoviral DNA by PCR, including 1 with disseminated infection and positive myocardial culture.

Interestingly, 6 of 10 patients positive for adenovirus had other organisms identified in the heart. All 6 had myocardial inflammation; however, only 1 had clinical cardiac symptoms. This contrasts sharply with the findings in 4 patients in whom adenovirus was the sole myocardial isolate; all 4 were symptomatic and only 2 of 4 had myocardial infiltrates. The frequency of postmortem cardiomegaly was similar in both groups of patients. These clinical and pathologic features in patients with PCR evidence of adenovirus support a pathogenic role for this virus in the development of heart disease in HIV-infected pediatric patients.

CMV was detected in 3 myocarditis samples and in 4 samples with borderline lymphocytic infiltrates. Extracardiac systemic infection with the virus was detected by culture or by histologic study (or both) in 6 of the 7 patients, considerably more often than detected in adult patients by Wu and colleagues.<sup>43</sup> Two patients had clinical cardiac symptoms, including 1 who had terminal acute congestive heart failure and myocardial infiltrates borderline for myocarditis. In the other, borderline myocarditis and disseminated systemic CMV infection were identified. Clinically, the heart was enlarged on chest radiograph and the patient was hypotensive. Another patient positive for CMV by myocardial culture and PCR was clinically asymptomatic but had myocarditis and mildly decreased left ventricular function assessed 1 week before death.

The relatively mild inflammatory infiltrates in most of the virus-positive samples could result from several things, including the fact that these HIV-infected patients were immunocompromised, precluding a significant cellular immune response against infected cells. Indeed, in 26 of 29 patients with CD4 lymphocyte counts available to permit Centers for Disease Control and Prevention classification, class C3 reflected severe immunosuppression. Additionally, we have observed in non-HIV-infected myocarditis patients that the level of inflammatory infiltration is less in adenovirus-infected samples than in, for example, enterovirus-infected samples.<sup>37</sup>

These data indicate that in HIV-infected children and adults, myocarditis and DCM can develop as a result of infection of the myocardium by the same viruses that infect non-immunocompromised individuals (ie, adenovirus, enteroviruses, and CMV).

## **ALTERNATIVE DIAGNOSTIC APPROACHES**

Other important causes of morbidity and mortality in children are infectious disorders of the respiratory tract.<sup>46</sup> Rapid respiratory and metabolic deterioration may occur, requiring intubation and mechanical ventilation. Respiratory decompensation is often accompanied by cardiac dysfunction due to myocarditis.<sup>10,47,48</sup>

To determine whether the analysis of tracheal aspirate samples would be informative for the diagnosis of viral myocarditis, Akhtar et al.<sup>49</sup> analyzed tracheal aspirate samples and EMB samples from 10 patients presenting with myocarditis or DCM, with or without presumed pneumonia by PCR, for evidence of viral infection. Of the 7 patients with PCR-positive tracheal aspirate samples, 4 were also positive by aspirate culture (enterovirus). In all cases, PCR performed on EMB specimens identified the same virus as detected in the tracheal aspirate samples. In the case of the child diagnosed by tracheal aspirate PCR to have EBV, EMB PCR also identified this relatively uncommon cause of pneumonitis and myocarditis. Confirmation of this diagnosis was later provided by serologic test during convalescence. Another patient who presented clinically with myocarditis and pneumonitis was positive by PCR for adenovirus from 2 consecutive tracheal aspirate samples (Fig. 23-1) and also was positive by PCR for adenovirus from EMB samples. In another case of myocarditis with pneumonia, the PCR, in addition to amplifying the same agent as isolated by culture (enterovirus), also amplified the adenovirus genome. Adenovirus respiratory tract infections are common in children, and in this case it may have contributed to myocardial injury.

These results suggest that tracheal aspirate samples are a useful substrate for PCR analysis in intubated pediatric patients with suspected viral pneumonitis, with or without myocarditis. Tracheal aspirate sample PCR may provide a safer means than EMB to arrive at an etiologic diagnosis in viral myocarditis, especially when the right ventricular free wall and outflow tracts are pathologically thinned. However, these results should not be generalized to include, for example, any unselected patient with intubated respiratory disease or children with known cardiac dysfunction and recurrent cardiac decompensation. Confirmation of these findings is needed before changes in diagnostic methodology are embraced.

## **TRANSPLANT REJECTION AND MYOCARDITIS**

Cardiac transplantation in children is a lifesaving procedure aimed at sustaining long-term, productive survival in recipients. The major short-term and long-term risks preventing extended survival include allograft rejection, coronary artery disease in the transplanted organ, and lymphoproliferative disease, but the underlying causes of these disorders are not completely understood. The diagnosis of allograft rejection relies on

histopathologic criteria but these criteria are known to mimic myocarditis in patients who have not received a transplant.<sup>50,51</sup>

The association between viral genome in the myocardium and concomitant rejection is known. Schowengerdt et al.<sup>50,51</sup> reported results of the analysis by PCR of 40 patients who underwent serial right ventricular EMB for rejection surveillance after heart transplantation, with viruses identified in 41 samples from 21 patients. Viral genomes amplified included CMV in 16 samples, adenovirus in 14, enterovirus in 6, parvovirus in 3, and HSV in 2. In 13 of the 21 patients positive for viral genome, EMB histologic scores were consistent with multifocal moderate-to-severe rejection (Internal Society for Heart and Lung Transplantation scores of 3A or greater). However, the longer-term implications of the detection of virus by PCR are unclear.

Adenovirus infection in the transplanted lung is significantly associated with graft failure, histologic obliterative bronchiolitis, and death. Bridges et al.<sup>52</sup> reported that of 16 patients undergoing lung or heart-lung transplantation, virus was identified in the transplanted lung during follow-up on 26 occasions; adenovirus was identified most frequently (8 of 16 patients) and had the greatest impact on outcome. In 2 patients with early fulminant infection, adenovirus was also identified in the donor. Adenovirus was significantly associated with respiratory failure leading to death or graft loss and with the histologic diagnosis of obliterative bronchiolitis.

In a study of 45 explanted hearts from patients who underwent heart transplantation, enteroviral genome was detectable in only 1 of 27 patients with DCM and in 1 patient with lymphocytic myocarditis.<sup>53</sup> The enterovirus-positive DCM patient showed a higher index of severe rejection (> 3A) in the first 6 months, compared with the other patients tested; the enterovirus-positive myocarditis patient died of disease recurrence 2 months after transplantation.

These findings suggest that the identification of virus, and particularly adenovirus and enterovirus, is predictive of a poor prognosis in organ transplant recipients, further confirming the similarity between myocarditis and rejection. They also indicate a need for the development of a rapid viral diagnostic technique to determine the suitability of a donor organ for transplantation.

## **ANIMAL MODELS OF MYOCARDITIS/DCM**

In many strains of mice, inoculation with CVB3 results in myocarditis.<sup>10</sup> The myocardium heals once infectious virus is cleared. However, in some strains of immunocompetent weanling mice, such as C3H/HeJ or A.SW, virus can be isolated from the myocardium during the first few days after inoculation. Histopathologic changes characteristic of

myocarditis develop only after infectious virus is no longer present. In such models myocardial damage is biphasic. The initial acute phase involves virus replication and cell lysis, with immune clearance of virus, followed by a chronic phase that involves infiltration of the myocardium by inflammatory cells and the production of cardiac-specific auto-antibodies. A murine model of DCM, after infection with encephalomyocarditis virus, has been described.<sup>54,55</sup> About 3 months after the development of myocarditis, cardiac dilatation, myocardial fibrosis, and hypertrophy of myocardial fibers occur, in the absence of cellular infiltration or myocardial necrosis. Despite the fact that infectious virus cannot be isolated after the first few days, viral genomic RNA sequences were detected in some samples at 3 months. A similar model using CVB3 in Swiss ICR mice has been described.<sup>56</sup>

To date there have been no animal models of adenovirus-induced heart disease reported. However, the cotton rat (*Sigmodon hispidus*) is susceptible to infection by some strains of human adenovirus,<sup>57</sup> and it was reported that the intranasal inoculation of cotton rats with Ad5 resulted in the development of pneumonitis.<sup>58</sup> Cellular infiltration of the interstitial and intra-alveolar areas and the peribronchiolar and perivascular regions was seen, with moderate damage occurring to the bronchiolar epithelium. The histologic changes could be divided into 2 phases. The first, probably due to the action of cytokines, involved the infiltration of primarily monocytes, macrophages, and neutrophils, but rarely lymphocytes, into the alveoli, bronchial epithelium, and peribronchiolar regions. The second phase, probably a cytotoxic T-cell response to the virus, involved a predominantly lymphocytic infiltrate into the peribronchiolar and perivascular areas. The degree of histopathologic change depended on the initial adenovirus dose, with doses of greater than  $10^8$  plaque-forming units (pfu) resulting in severe damage to the type II alveolar cells.

We<sup>59</sup> have begun to develop a model of adenovirus-induced myocarditis in the cotton rat. Adenovirus type 5 ( $10^7$  pfu) was administered to cotton rats by intranasal (IN), intraperitoneal (IP), or intracardiac (IC) injection. The animals were killed (2 per group) after 4, 14, or 28 days. In addition, 2 IC injected animals were killed after 3 months.

Adenoviral DNA was detected in the lungs of all animals at days 4 and 14, except for 1 animal receiving virus by IP injection that was negative at day 14. At day 28, only the animals administered virus by the IN or IC route were positive (3-month IC animals were not tested). Adenoviral DNA was detected in the hearts of all animals inoculated by the IC route, even at 3 months postinjection (Figure 23-3). Adenoviral DNA was detected in the hearts of only IN and IP animals at day 4 and 1 IP injected animal at day 14.

Animals inoculated IN were considered normal, whereas animals inoculated IP had borderline myocarditis at day 4 and myocarditis at days 14 and 28 (Fig. 23-4; see color plate 42). Even at day 14 there was evidence of fibrosis and myocyte necrosis. Animals inoculated IC had epicarditis, with subepicardial myocarditis at day 4 and myocarditis at 14 days, 28 days, and 3 months.

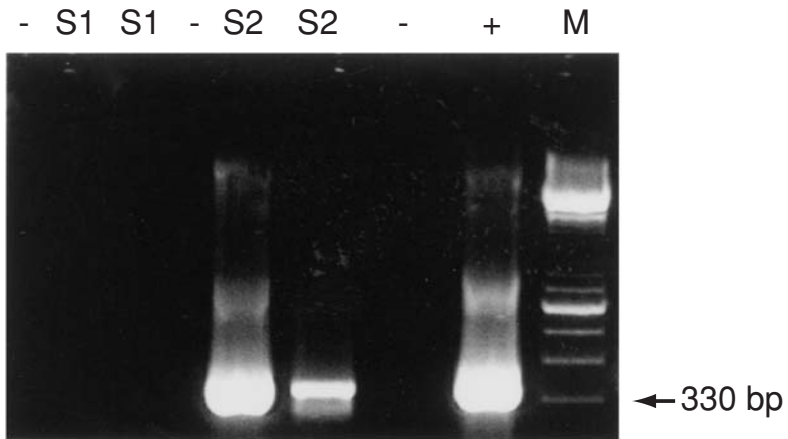


Fig. 23-3. The detection of adenoviral DNA by nested polymerase chain reaction in myocardial samples from 2 cotton rats injected with adenovirus, 3 months postinjection (lanes S2). Lanes S1 are myocardial samples from sham-infected animals. See Figure 23-1 for details.

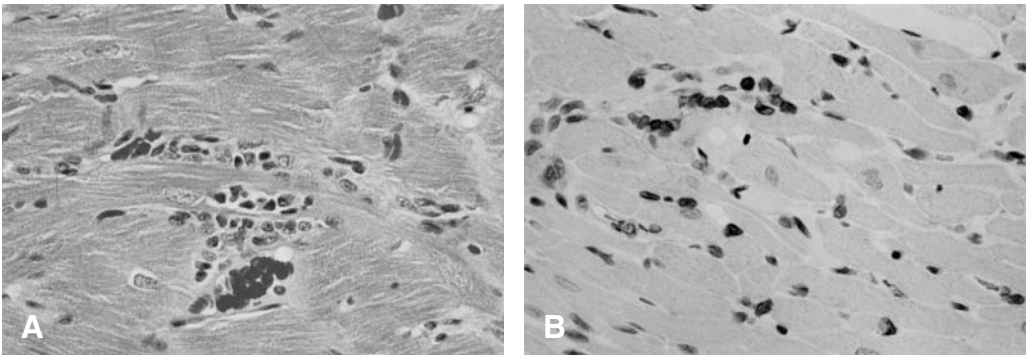


Fig. 23-4. Myocarditis in the cotton rat heart. *A*, Hematoxylin-eosin staining shows a discrete cluster of lymphocytes and macrophages adjacent to a degenerating myocyte. There is also focal loss of myocytes with early fibrous scarring. *B*, T-cell immunostain demonstrates a cluster of cells surrounding myocytes. (x132.) See color plate 42.

From these preliminary data, it appears that the IP and IC administration of adenovirus result in the development of myocarditis in the cotton rat. The myocarditis demonstrated in these animals is histologically mild, similar to adenovirus myocarditis in humans. Further, wild-type adenovirus is capable of persisting in the myocardium of cotton rats for at least 3 months.

## **PATHOGENESIS OF HEART FAILURE IN MYOCARDITIS AND DCM**

### **VIRAL PERSISTENCE**

Although the evidence is compelling that enteroviruses are capable of persisting in the myocardium of patients with myocarditis or DCM in the absence of virus-antigen expression or the formation of infectious virions, few reports relate to the specific nature of the mechanism. During a normal lytic infection, enteroviral RNA replication is mediated by the virus-encoded RNA-dependent RNA polymerase via a replication intermediate, comprising the positive-sense genomic strand and a negative-template strand. The positive-strand RNA is normally present in 100-fold excess over the negative strand as a result of asymmetric synthesis. However, in the myocardium of patients with myocarditis or DCM infected with enterovirus, approximately equimolar amounts of the positive and negative strands are synthesized.<sup>60,61</sup> It is possible that the synthesis of complementary RNA strands results in interference in translation of the genomic RNA because of the RNA-RNA hybridization: such double-stranded RNA is likely to be more stable than single-stranded RNA.

Most of the information relating to adenovirus latency or persistence has come from the study of infected tonsils or adenoids. Infectious virus can rarely be isolated directly from the tissue but is recovered after cultivation of the tissue<sup>62</sup> or from stimulated lymphocytes<sup>63</sup> *in vitro*. After propagation of tonsillar tissue *in vitro*, adenoviral DNA can be detected in high molecular weight DNA fractions, suggesting that the viral genome has been integrated into the host chromosomes.<sup>64</sup> Latent infection of lung by adenovirus can also cause chronic obstructive pulmonary disease,<sup>65</sup> with adenoviral DNA integrating in a linear fashion and subsequent rearrangement and amplification of the early regions, particularly E1A.<sup>65</sup> The E1A region has been implicated in the sensitization of the infected cells to destruction by cytokines<sup>66</sup> and in the induction of apoptosis.<sup>67</sup> This region could be an important component of the mechanism of inflammatory responses against chronically infected cells.

### **APOPTOSIS AND IMMUNE RESPONSE**

Little is understood about the pathogenesis underlying the development of heart failure associated with myocarditis or DCM. Although the pathologic features of acute myocarditis are well documented, hearts from patients with DCM display relatively nonspecific histologic changes. These include widespread myocardial fibrosis and associated hypertrophy of surviving cardiomyocytes. Apoptosis of cardiomyocytes may be responsible for these changes.<sup>68</sup> In a small number of cases apoptotic cells were detected in myocardial tissue samples from patients with DCM by an *in situ* labeling protocol (TUNEL), including adenovirus-infected samples.<sup>69,70</sup>

Thus, it is possible that in adenovirus-infected cardiomyocytes the dissociated expression of E1A and E1B could result in the induction of apoptosis by overexpression of E1A, by underexpression of E1B, or by expression of mutated forms of the E1B gene products. Alternatively, other adenoviral gene products may influence the apoptotic pathway in, as yet, uncharacterized ways.

Another effect of the expression of E1A is to shut down the expression of  $\alpha$ -myosin heavy chain by transcriptional repression.<sup>71</sup> The long-term effect of this on the myocardium could be to impair cardiac myocyte function, potentially leading to congestive heart failure.

The adenoviruses have strategies for modulating the immune response. Several adenoviral-encoded proteins are capable of interacting with host immune components.<sup>67</sup> These include proteins encoded by the E3 region that can protect cells from tumor necrosis factor (TNF)-mediated lysis<sup>72</sup> and down-regulation of MHC class I antigen expression.<sup>73</sup> The E1A proteins are capable of promoting the induction of apoptosis,<sup>67</sup> inhibiting interleukin (IL)-6 expression,<sup>74</sup> and interfering with IL-6 signal transduction pathways.<sup>75</sup> These functions of E1A may be particularly pertinent for explaining the myocardial abnormalities observed in DCM patients: IL-6 promotes lymphocyte activation, and this was reduced in the adenovirus-infected patient samples in the study by Pauschinger et al.<sup>39</sup>

The presence of mononuclear cell infiltrates within the heart is a characteristic of myocarditis. These mononuclear cells are a significant source of the cytokines IL-1 $\beta$  and TNF. Henke et al.<sup>76</sup> demonstrated the release of TNF- $\alpha$  and IL-1 $\beta$  by human monocytes exposed to CVB3. Both of these cytokines participate in leukocyte activation, which may promote a specific lymphocyte response during viral infection. However, these cytokines may also promote cardiac fibroblast activity.<sup>77</sup> Therefore, local secretion of cytokines in the myocardium may perpetuate the inflammatory process and lead to the fibrosis associated with cardiomyopathy and resultant deteriorating cardiac function. Evidence also implicates IL-1 $\beta$  and TNF- $\alpha$  as potential inhibitors of cardiac myocyte  $\beta$ -adrenergic responsiveness.<sup>78</sup> Further, TNF- $\alpha$  is capable of inducing apoptosis. Transgenic mice expressing TNF- $\alpha$  in the myocardium have been described.<sup>79-81</sup> Severe cardiac dysfunction indicated by biventricular dysfunction and depressed ejection fraction was evident in these transgenic mice, and the mice died prematurely. At necropsy globular dilated hearts were observed, and on histologic examination there was evidence of myocyte apoptosis and severe inflammatory infiltration of the walls of all chambers, indicative of an acute myocarditis. There was also significant ventricular fibrosis. These data support a role for TNF- $\alpha$  in the pathogenesis of myocarditis and idiopathic DCM. The prolonged expression of inflammatory cytokines and immunomodulators, such as TNF- $\alpha$  and IL-1 $\beta$ , has been reported in patients with chronic myocarditis or DCM.

Another possible effect of cytokine expression is the induction of inducible nitric oxide synthase. Increased expression of nitric oxide synthase has been proposed to account for some of the dilation associated with DCM<sup>82</sup> and has been demonstrated in a murine CVB3-induced myocarditis model.<sup>83</sup> In a study of a cardiac myosin-induced myocarditis model in mice, it was shown that nitric oxide synthase expression is induced in both macrophages and cardiomyocytes.<sup>84</sup> However, nitric oxide synthesis did not appear to be essential for the development of pathologic conditions because myocarditis developed in mice lacking interferon regulatory transcription factor-1, a transcription factor that controls expression of inducible nitric oxide synthase. Despite the failure to synthesize nitric oxide synthase in the myocardium, the prevalence and severity of disease in interferon regulatory transcription factor-1-deficient animals were similar to control animals. In addition, no difference was detected in animals lacking the interferon regulatory transcription factor-2 gene, a negative regulator of interferon regulatory transcription factor-1-induced transcription.

### CYTOSKELETON DYSFUNCTION IN DCM: THE COMMON FINAL PATHWAY HYPOTHESIS

In addition to the acquired form of DCM, inherited forms of the disease are described frequently. During the past several years, clues have emerged to the underlying cause of familial DCM, and the underlying basis for other inherited cardiovascular diseases.<sup>85,86</sup> For instance, the basis for familial hypertrophic cardiomyopathy, a primary heart muscle disease in which ventricular wall thickening (hypertrophy) and diastolic dysfunction occur, has been demonstrated to be mutations in genes encoding sarcomeric proteins such as  $\beta$ -myosin heavy chain,  $\alpha$ -tropomyosin, cardiac troponin T, cardiac troponin I, myosin-binding protein-C, cardiac actin, and the essential and regulatory myosin light chains.<sup>87</sup> In addition, the inherited long QT syndromes have been shown to be due to mutations in genes encoding ion channels, such as the potassium channel genes *KVLQT1*, *KCNE1*, *KCNE2*, and *HERG* and the cardiac sodium channel gene *SCN5A*.<sup>88</sup> Because of the consistent protein classes mutated in phenotypically similar patients (ie, sarcomeric proteins in familial hypertrophic cardiomyopathy; ion channels in long QT syndromes), we hypothesized that a common final pathway is disturbed in individual cardiovascular disorders and that similar protein types would also be mutated in DCM.<sup>85,86</sup>

Currently, only 5 genes have been identified and characterized in cases of familial DCM. In Barth syndrome, the gene *G4.5*, which encodes a novel protein family called taffazins, is mutated.<sup>89</sup> Although well characterized at the molecular level, the function of the encoded protein is not known. In contrast, dystrophin is the gene responsible for X-linked DCM and is well defined.<sup>90-92</sup> This gene, which also causes Duchenne and Becker muscular dystrophy when mutated, encodes a large (427 kDa) cytoskeletal protein that resides at the inner face of the sarcolemma (Fig. 23-5; see color plate 43),<sup>93</sup> colocalizing

with  $\beta$ -spectrin and vinculin. Dystrophin protein is thought to assume a rod-shaped structure with an actin-binding domain at the amino terminus. The carboxy-terminal domain is associated with a large transmembrane glycoprotein complex, the dystrophin-associated glycoprotein complex, which is thought to mechanically stabilize the plasma membrane of muscle cells (Fig. 23-5). This complex is formed by the dystroglycan subcomplex ( $\alpha$ -dystroglycan and  $\beta$ -dystroglycan), sarcoglycan subcomplex ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -sarcoglycan), caveolin-3, neuronal nitric oxide synthase, syntrophin,  $\alpha$ -dystrobrevin, and sarcospan and serves as a link among cytoplasmic actin, the membrane, and the extracellular matrix of muscle (Fig. 23-5). Mutations in dystrophin or dystrophin-associated glycoprotein complex subcomplexes result in a wide spectrum of skeletal myopathy or cardiomyopathy (or both) in humans and animal models such as the mouse or hamster.<sup>94-102</sup>

The third mutant gene thus far identified, *cardiac actin*, has been identified as the gene responsible for 15q14-linked autosomal dominant familial DCM.<sup>103</sup> It has also been shown to cause familial hypertrophic cardiomyopathy.<sup>104</sup> This mutant gene appears to cause a DCM phenotype when mutated near the dystrophin-binding domain, whereas mutations that result in disruption of the protein at its interaction with the sarcomere result in familial hypertrophic cardiomyopathy. The actin-dystrophin link, when disrupted, dissociates the actin cytoskeleton from the muscle membrane and extracellular matrix, leading to cellular degeneration and necrosis and a DCM phenotype. Disruption of the sarcomere instead leads to familial hypertrophic cardiomyopathy.

The other 2 genes identified in familial DCM include *desmin* (2q35)<sup>105</sup> and *lamin A/C* (1p1-1q21),<sup>106</sup> both of which are thought to cause abnormalities of structural support when mutated. Desmin is a component of the intermediate filaments while lamin A/C makes up part of the inner nuclear envelope (Fig. 23-5). Interestingly, these genes are associated with skeletal myopathy and, in some cases, with conduction system disease.<sup>107-109</sup> We<sup>85,86</sup> hypothesized that DCM is a disease of the cytoarchitecture—the cytoskeleton and dystrophin-associated glycoprotein complex in particular.

Other supportive data for this hypothesis exist. Maeda et al.<sup>110</sup> identified absence of the metavinculin transcript in the cardiac tissue from a patient with idiopathic DCM and confirmed the metavinculin abnormality by immunoblot, which demonstrated the absence of metavinculin protein in the heart. Metavinculin has a role in attaching the sarcomere to the cardiomyocyte membrane by complexing with nonsarcomeric actin microfilaments complexed with other cytoskeletal proteins (talins,  $\alpha$ -actinin, vinculin), which are linked to cadherin or to the integrin receptor. Arber et al.<sup>94</sup> showed that deficiency of muscle LIM protein in a mouse model results in DCM, heart failure, and disruption of cardiac myocyte cytoskeletal architecture. Muscle LIM protein is a structural protein that appears to link the actin cytoskeleton to the contractile apparatus and, although no mutations have been identified in humans, fits well with the Common Final Pathway hypothesis for DCM.

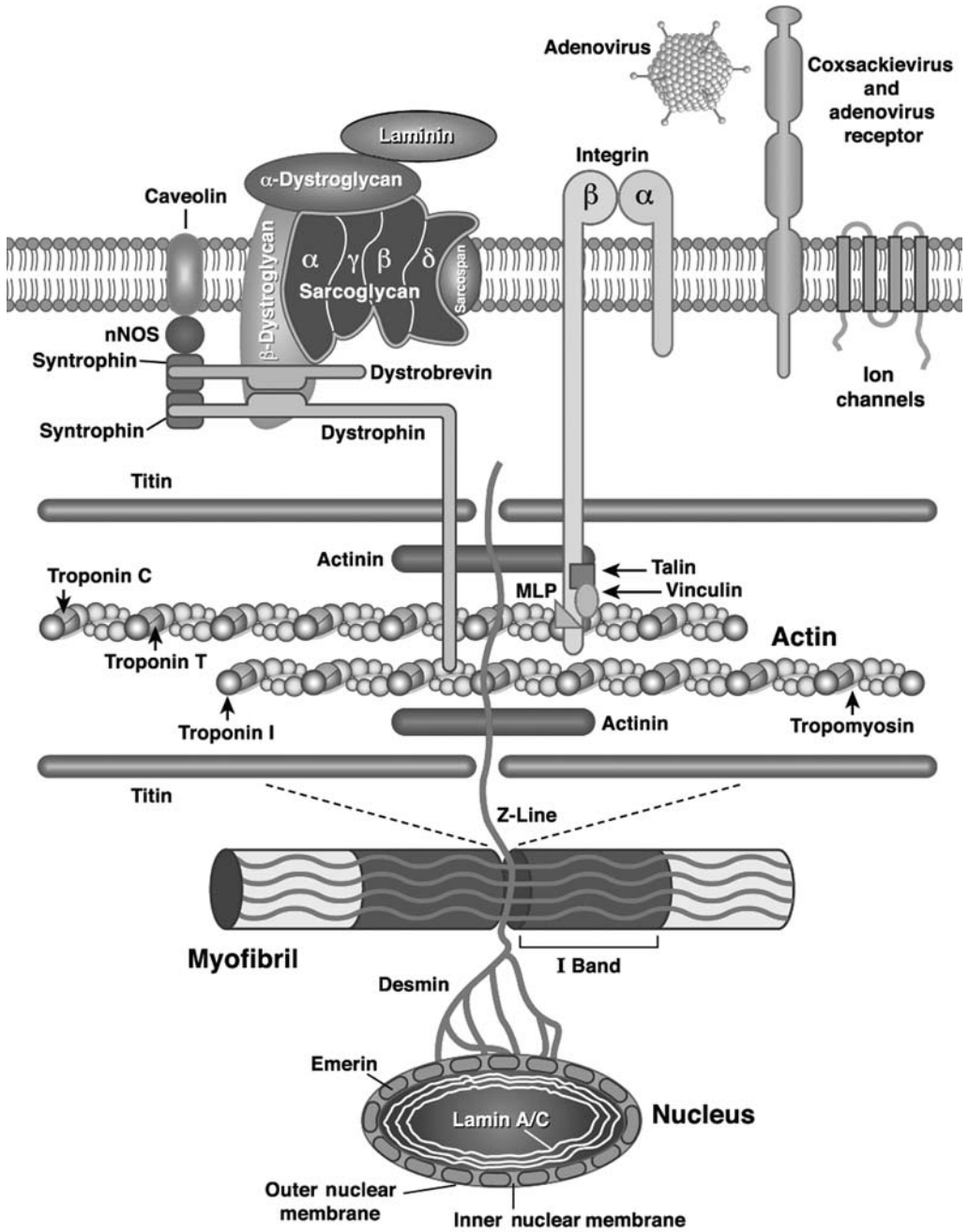


Fig. 23-5. Schematic representation of the cytoarchitecture of the cardiomyocyte, including components of the cytoskeleton, intermediate filaments, nuclear envelope, and dystrophin-associated glycoprotein complex. *MLP*, muscle LIM protein; *nNOS*, neuronal nitric oxide synthase. See color plate 43.

It is possible that vinculin, which maps to the 10q21-q23 region, and caveolin-3, which maps to 3p25, could be responsible for the familial DCM linked to these regions of the human genome.<sup>111,112</sup>

Badorff et al.<sup>113</sup> reported that the CVB3-encoded 2A protease cleaves dystrophin in cultured myocytes and in infected mouse hearts. This leads to disruption of dystrophin and the dystrophin-associated glycoprotein complex. Thus, it appears likely that one of the effects of the infection of the heart by the enteroviruses is the disruption of the sarcolemma. In addition, both TNF- $\alpha$  and IL-1 $\beta$  activate the GTPase Cdc42.<sup>114,115</sup> Constitutively active forms of this protein induce actin polymerization.<sup>115,116</sup> Thus, continuous stimulation of this signaling pathway could affect the integrity of the cytoskeleton. Whether viruses act directly on the cytoarchitecture or indirectly through inflammatory mediators, it appears that the Common Final Pathway hypothesis may be relevant to the pathogenesis of acquired and inherited forms of DCM in children and adults.

## NOVEL THERAPEUTICS

Conventional treatments for myocarditis include bed rest, diuretics, digitalis, angiotensin-converting enzyme inhibitors,  $\beta$ -adrenergic blockade, and antiarrhythmic medication. Because of the idea that myocarditis involves, at least in part, autoreactive immunologic damage, trials of immunosuppressive agents have been undertaken. The results have varied. For example, in one multicenter myocarditis trial, patients were studied during the acute phase of disease and no difference was observed between patients receiving immunosuppressive or conventional therapy.<sup>117</sup> However, studies in patients with chronic myocarditis suggest that immunosuppression may be efficacious in these patients,<sup>118</sup> with significant improvement in ejection fraction and New York Heart Association classification.

Intravenous administration of immunoglobulin has been used for the treatment of autoimmune diseases,<sup>119-121</sup> including Kawasaki disease. Trials of intravenously administered immunoglobulin in acute myocarditis patients suggested that this treatment may improve left ventricular function, with patients experiencing better survival during the first year than the control group.<sup>122</sup> Further, in the CVB3-induced myocarditis model in the mouse, intravenously administered immunoglobulin therapy during the acute phase resulted in reduced inflammation and improved survival.<sup>120</sup> The successful treatment was reported of a patient with adenovirus-induced myocarditis with high-dose intravenously administered immunoglobulin.<sup>123</sup> However, a randomized trial of intravenously administered immunoglobulin for DCM in adults failed to demonstrate benefit.<sup>124</sup>

The observation that some patients succumb to idiopathic DCM, long after the healing of myocarditis, but with evidence that viral sequences persist in the myocardium, suggests

that other approaches may be beneficial for the treatment of this condition. Most antiviral therapies (eg, ganciclovir or zidovudine) rely on viral replication to be effective. In chronic myocarditis or idiopathic DCM, it is not obvious that viral replication is occurring or directly responsible for the pathogenic changes.

The possible role of CVB-encoded protease in the development of a pathologic cardiac condition by cleavage of dystrophin raises the possibility of an alternative approach to treating viral heart disease. Virus-specific protease inhibitors have been widely used for the treatment of HIV infection with considerable efficacy.<sup>125,126</sup> Enterovirus-specific protease inhibitors have been described, including one for poliovirus 2A and one for rhinovirus 3C.<sup>127,128</sup>

The identification of specific agents as causes of these conditions suggests that approaches directed toward the protection of humans from these viruses would be beneficial. The highly efficacious poliovirus vaccines<sup>129</sup> that have almost eliminated poliomyelitis suggest that the development of coxsackievirus B-specific vaccines is possible.<sup>130</sup> Support for such an approach comes from studies of endocardial fibroelastosis (EFE).

EFE is characterized by a diffuse thickening of the left ventricular endocardium. This results from proliferation of fibrous and elastic tissue and leads to decreased compliance and impaired diastolic function. Most patients have a dilated left ventricular chamber (dilated form), although some display ventricular hypoplasia. EFE usually occurs in infants and young children, who present with signs of congestive heart failure, and most cases are of unknown etiology. In the past, the incidence of EFE in the United States was relatively high—approximately 1 per 5,000 live births. In recent decades, however, the incidence has declined significantly for unknown reasons.

It was suggested that idiopathic cases of EFE result from increased endocardial mural tension produced by the left ventricular dilatation due to myocarditis.<sup>131</sup> Hutchins and Vie<sup>131</sup> studied 64 children with either myocarditis or primary EFE; of these, 5 had myocarditis only, 18 had idiopathic EFE, and the remaining 41 had evidence of both myocarditis and EFE. With longer survival, the severity of myocarditis decreased but was replaced by an increase in EFE. By 4 months, no patient had histologic evidence of myocarditis, which is reminiscent of the association between myocarditis and DCM.

The link between viral myocarditis and EFE, therefore, supported a role for chronic viral infection in the etiology of EFE.<sup>131</sup> However, as with myocarditis, there was little direct evidence for viral infection of the myocardium of patients with EFE by classical virologic techniques. Fruhling et al.<sup>132</sup> reported that a significant proportion of myocardial samples from EFE patients was culture-positive for coxsackievirus B. It also was proposed that EFE might develop in a particular subset of patients with viral myocarditis—those with mumps virus-induced disease. A link between mumps virus infection and EFE was established by positive skin reactivity tests.<sup>133</sup> In 1 case, the mother had a mumps infection

during the first trimester of pregnancy, whereas 2 other patients were exposed to mumps. It was suggested that intrauterine infection with the mumps virus may be involved in some cases of EFE.

It was first suggested in 1918 that myocarditis was a rare complication of mumps virus infection.<sup>134</sup> In 1984 a link was established among mumps, myocarditis, and subsequent cardiomyopathy.<sup>135</sup>

Ni et al.<sup>136</sup> identified mumps RNA by reverse transcription PCR in more than 70% of EFE samples, whereas 28% amplified adenovirus. These data support an etiologic role for viral infection in EFE and the hypothesis that EFE is a sequela of a viral myocarditis, particularly due to mumps virus. None of the samples obtained after 1980 were positive for mumps virus. Thus, it is possible that the remaining cases of EFE are caused by a different etiologic agent, such as adenovirus.

A mumps virus origin for EFE may also explain the dramatic decline in incidence in the last few decades. Since the introduction of the mumps vaccine, the prevalence of epidemic parotitis has decreased significantly.<sup>137</sup> Therefore, unlike the pattern of infection of the enteroviruses, which show periodic peaks in infection rates, the decline in incidence of EFE seems to reflect the decreased prevalence of mumps virus in the population. These data support the efficacy of a virus-specific vaccination (eg, adenovirus group C and CVB) in the prevention of an acquired form of heart disease.

Adenovirus-specific vaccines are already available for some serotypes, and the vaccine is provided for military personnel in the United States. However, this vaccine does not protect against the group C adenoviruses most commonly associated with heart disease. No data are available on the difference in the frequency of myocarditis in these individuals versus the general population.

## **THE COMMON COXSACKIEVIRUS B-ADENOVIRUS RECEPTOR**

It has remained something of a conundrum why 2 such divergent virus families as the human adenoviruses and coxsackievirus B cause these diseases. The description of the common human coxsackievirus B-adenovirus receptor (CAR) offers at least a partial explanation.<sup>138-140</sup>

CAR is a 46-kDa transmembrane glycoprotein with 2 extracellular immunoglobulin-like domains. Transfection of nonpermissive cells with a cDNA clone encoding this receptor allows both coxsackievirus B and adenovirus (through the fiber protein) attachment and infection.<sup>138</sup> In humans, this protein is expressed highly in the heart, pancreas, testes, and prostate and to some degree in many other tissues.<sup>140</sup> The human *CAR* gene consisting of 7 exons is encoded at 21q11.2,<sup>141</sup> and pseudogenes are located on chromosomes 15, 18, and 21.

It has been postulated that the physiologic function of CAR is as a cellular adhesion molecule, which in the developing brain is important in neural network formation.<sup>142</sup> However, the broad spectrum of tissues encoding this protein suggests that its function is more general in cell-to-cell contact and cardiomyocyte adhesion.

CAR is not limited to humans and mice. Ito et al.<sup>143</sup> reported that it is strongly expressed in the myocardium of newborn rats. Although in adult rats myocardial expression is reduced, in a rat model of myocarditis induced by immunization with cardiac myosin, CAR expression is enhanced during the active phase due to induction by inflammatory mediators. It is unknown whether such a phenomenon occurs in humans, but the increased expression of CAR should be considered as a host factor in the pathogenesis of viral myocarditis and DCM.

Adenovirus uses a second receptor for cell entry, the vitronectin receptor ( $\alpha_5$  integrin and  $\beta_3$  integrin). Although the interactions of CAR with components of the cytoskeleton are not yet identified, the vitronectin receptor interacts with vinculin and actin<sup>144,145</sup> (Fig. 23-5). Whether disturbances in these interactions contribute to the susceptibility or pathogenesis of myocarditis or DCM is under investigation.

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