Prospects for adenovirus-mediated gene therapy of inherited diseases of the myocardium

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1. Introduction

Many genetic loci linked to a variety of diseases have been identified, and disease-causing mutations characterized. These have included diseases of the myocardium, such as X-linked dilated cardiomyopathy [1–3], hypertrophic cardiomyopathy [4–6], and Long QT syndrome [7–9]. The elucidation of gene defects has allowed different therapeutic strategies to be proposed, including the use of pharmaceutical agents to replace or to antagonize the mutated protein, and replacement of the defective gene with a functional one (gene therapy). There have been many publications describing the use of vectors to transduce target cells for the correction of gene defects or for anti-viral therapy (for reviews see [10–14]). Such vectors have included recombinant retroviruses, adenoviruses, adeno-associated viruses, and herpes viruses, as well as non-viral vectors. Each vector has inherent advantages and disadvantages. At this time the adenoviruses are most commonly used and represent the most likely vector for efficient transduction of the myocardium. While many groups have reported the use of recombinant adenoviruses to transduce cells in vitro and in vivo, and even their use in clinical trials for the correction of the cystic fibrosis gene defect [15,16], there have been few reports describing the targeted expression of gene products for the correction of myocardial diseases. In this review we will discuss the potential of gene therapeutic approaches for the treatment of myocardial disease, as well as consider some of the limitations and risks associated with the use of the adenovirus-based vectors.

2. Recombinant adenoviruses as gene therapy vectors

Recombinant adenoviruses have been the most commonly utilized vectors for the transduction of cells both in vitro and in vivo. This has primarily been due to their ability to be propagated and purified to high titers, their ability to transduce non-dividing cells, and their broad spectrum of target tissues. Most studies have used so-called first-generation adenovirus vectors (Fig. 1). The functions of the adenoviral early (E) and late (L) proteins have been reviewed elsewhere [17]. In brief, the E1 region encodes proteins essential for cell transformation, as well as for transactivation of other viral genes, host cell shutoff, and control of the lytic cycle. The E2 region encodes the DNA polymerase, a DNA binding protein involved in the control of viral gene transcription, and a terminal protein involved in viral assembly. The E3 region is non-essential for in vitro replication and down-regulates major histocompatibility complex (MHC) at the cell surface, decreasing the target for recognition by cytotoxic T-cells. The E4 region encodes proteins involved in the regulation of L gene transcript splicing, and most of the L proteins are viral structural proteins.

For the preparation of recombinant adenoviruses, the foreign gene, together with a suitable promoter, are cloned into a plasmid containing the left end of an adenovirus genome, replacing the E1 region [18]. Since E1 controls
the expression of the other adenoviral proteins, deletion of this region essentially shuts off virus transcription in recombinant viruses. This plasmid is transfected into 293 cells, which carry a complementary copy of the E1 region, together with adenoviral genomic DNA lacking the E1 region. The recombinant genome, derived by homologous recombination between the plasmid and the adenoviral DNA, is packaged into virion and released into the culture media following cell lysis. The infectious virus is purified away from empty virion by density gradient centrifugation. One major problem associated with this preparation method is that a small amount of contaminating wild type virus is also generated, albeit usually less than 0.01% of the titer of the recombinant virus.

Adenovirus enters cells by receptor-mediated endocytosis and since the receptor is widely expressed, a variety of tissues can be transduced. The viral genome usually persists as an episome, although integration into the host genome has been reported, especially for adenovirus type 12 [19]. Most commonly the virus is delivered systemically: it was shown by Huard et al. [20] that, depending upon the route of virus administration, different tissues could be transduced.

There have been few reports describing the delivery of a transgene to the myocardium. Transduction by direct injection into the myocardium has been reported in rat [21–23], mouse [24], dog [25] and pig [26], as well as by intracoronary infusion in rabbits [27], perfusion of the donor heart [23], and systemic injection [19, 28]. Virus transduction mainly occurred in the region of the myocardium directly surrounding the site of injection [26], or the coronary artery injected [27], while the liver was also transduced in some cases [21, 27]. In hearts that were infected by perfusion, the distribution of transgene expression was more widespread within the myocardium than after direct injection. However, perfusion is only a viable option if the target organ is to be transplanted in the host. Therefore, improved methods of delivery to the myocardium will need to be developed to achieve sufficient levels of transgene expression, particularly since for many diseases most, if not all, myocytes will be required to be transduced to achieve a therapeutic effect. Further, in most cases, transgene expression peaked approximately one week after virus injection and declined rapidly thereafter [21, 23, 26]. This has been observed in most in vivo studies using adenovirus vectors and is probably due to the host immune response against virus-encoded proteins [29], or in many model systems, against the transgene product being used as an indicator of virus transduction [22, 30]. For the first-generation recombinant adenovirus vectors the synthesis of virus proteins results either from leaky expression from the vector sequences or from the contamination of vector stocks with wild-type virus.

Two approaches have been used to improve the persistence of transgene expression: (1) the development of the so-called second- and third-generation vectors, and (2) immunosuppression of the host. The first second-generation vectors had a temperature-sensitive mutation in the E2a region, further ablating virus replication [31]. However, the use of this vector produced only small increases in the duration of transgene expression in the cotton rat [31], mice and dogs [30], although Engelhardt et al. [31] reported a reduction in the cellular immune response. Other possibilities are to develop vectors constitutively expressing E3 [32], which could reduce viral antigen presentation and, thus, T-cell killing of transduced cells, and vectors lacking E4 as well as E1 [33]. Lee et al. [32] reported that transduction with an adenoviral vector expressing both β-galactosidase and E3 failed to stimulate the proliferation of anti-adenovirus or anti-transgene-specific antibodies. Gao et al. [33] reported that transduction of mouse liver with an E1/E4-deleted vector resulted in reduced virus protein expression and a blunted immune response against the virus, as well as a reduction in virus-induced apoptosis in the target organ. Recently recombinant adenovirus vectors lacking all virus-encoded genes have been described [34–36], but the ability of these vectors to achieve persistent transgene expression has not been reported.

Barr et al. [37] reported that following systemic recombinant adenovirus administration into immunodeficient mice, transgene expression persisted indefinitely within the liver. Further, tracheal administration in neonatal cotton rats, having a relatively naïve immune system, resulted in persistent transgene expression, for at least 6 months [38]. Based upon the observations that, in the absence of an immune response against the viral-encoded proteins or the transgene product, persistent expression was obtained, host immunosuppression has been studied. Cyclosporin A administration in a canine hemophilia B model resulted in a prolongation of therapeutic levels of the blood clotting factor IX, from 3 weeks to 6 months, following adenovirus administration [39]. The co-administration of soluble CT-LA Ig (which blocks co-stimulatory signals between T-cells and antigen presenting cells) with recombinant adenovirus in mice, resulted in persistent transgene expression, but without long-term immunosuppression [40].
An important consideration for the correction of myocardial disease using adenoviral vectors is the limitation of the expression of the transgene to within the myocardium. The widespread expression of the adenovirus receptor probably means that such control will have to be exerted at the level of gene expression, by using a cardiac muscle-specific promoter. Lee et al. [41] used the myosin light chain-2 promoter to direct expression of the luciferase reporter gene in transgenic mice, while Rothmann et al. [42] obtained cardiac muscle-specific expression of a luciferase gene under the control of the same promoter, following direct injection of a recombinant adenovirus into the cardiac cavity. Virus was detected by polymerase chain reaction (PCR) in many tissues, but gene expression was limited, almost exclusively, to the heart [42]. Thus, it should be possible to direct the expression of a gene to a specific tissue but many problems remain in controlling the stoichiometry and timing of gene expression, although the ability to control gene expression by drug or hormone treatment has been proposed [43–45].

3. Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is the most common form of cardiomyopathy, responsible for approximately 60% of cases. This disorder, a primary myocardial disease that causes ventricular dilation and dysfunction, primarily of the left ventricle, has many etiologies [46–49]. It is believed that approximately 30% of cases are familial in nature [50,51] and of these, the transmission may be autosomal dominant or recessive, X-linked, or mitochondrial. The most common form of disease appears to be the autosomal dominant type, however.

Patients with DCM are treated symptomatically with anticoagulant measures, antiarrhythmic medications (when necessary) and, in some cases, β-blockers. Failure of medical therapy usually leads to consideration for cardiac transplantation.

Two X-linked cardiomyopathies have been described, X-linked dilated cardiomyopathy (XLCM) and Barth syndrome. XLCM typically presents in teenage boys or males in their twenties and rapidly leads to severe symptoms of congestive heart failure with associated ventricular arrhythmias, usually resulting in death or transplantation within 1 year of presentation. Towbin et al. [52] first identified linkage in XLCM to the dystrophin gene on Xp21, the gene responsible for Duchenne and Becker muscular dystrophy. Muntoni et al. [1] described mutations in the muscle promoter and muscle-expressed exon 1 in XLCM and more recently, other mutations have been described [3]. Barth syndrome, or X-linked cardio-skeletal myopathy with neutropenia, abnormal mitochondria and 3-methylglutaconic aciduria typically presents with severe life-threatening heart failure in infancy. The gene responsible, G4.5 [2], is located on Xq28.

Multiple loci have been linked to autosomal dominant inherited DCM, including genes for pure DCM on 1q32 [53], 9q13–q22 [54], and 10q21–23 [55], and genes for DCM with conduction disease on 1p1–1q1 [56] and 3p25–p22 [57]. None of these genes have been identified at this time.

While the correction of the dystrophin defects associated with XLCM has not been reported, a number of groups have reported approaches for the correction of Duchenne muscular dystrophy (DMD). This is a potentially lethal disorder of skeletal muscle resulting from mutations in the dystrophin gene [58], distinct from those identified in XLCM [1,3]. The mdx mouse lacks dystrophin, and has been used by several groups as a model for therapeutic approaches for DMD [59]. Due to their limited capacity, the first-generation adenovirus vectors were unable to accommodate the 14kb dystrophin cDNA and promoter. Therefore, so-called dystrophin mini genes, obtained by cloning the truncated cDNA from Becker muscular dystrophy patients [60], have been used as the therapeutic gene. The transduction of skeletal muscle of mdx mice by recombinant adenoviruses encoding such dystrophin constructs, with transient correction of the dystrophin defect, has been reported [61–63]. Recently, two groups described the use of third-generation adenovirus vectors lacking all viral genes and capable of packaging the entire dystrophin cDNA under the control of either the muscle-specific creatine kinase promoter [64] or the Rous sarcoma virus long terminal repeat promoter [35]. In both cases, efficient transduction of mdx muscle fibers were obtained following intramuscular injection and in one of the studies restoration of the dystrophin-associated proteins to the muscle membrane and a decrease in centrally located nuclei, a characteristic of dystrophin-deficient muscle, were observed [64].

These vectors offer the ability to package up to 30 kb of promoter/gene construct, a size that should be sufficient for most genes, and the absence of virus genes should reduce the problems of the host immune response against the vector, as long as the vector is sufficiently purified from helper virus. Neither study reported on the persistence of transgene expression, although Haecker et al. [35] noted some decrease in dystrophin expression was observed between the second and fourth weeks. This, however, could be due to an immune response against the transgene product itself.

A bovine model of inherited dilated cardiomyopathy has been studied [65] and appears to resemble features of the human disease, particularly with relation to changes in the β-adrenoreceptor-G protein-adenyl cyclase pathway. The genetic defect underlying the disease in this model is unknown at present, and thus, its relevance to any of the known human loci. In addition, no animal models exist for Barth syndrome or XLCM at present, precluding the types of study reported in the mdx mouse. However, such developments in gene delivery techniques should encour-
age efforts to use somatic gene therapy as an approach for the treatment of these diseases.

4. Hypertrophic cardiomyopathy

Familial hypertrophic cardiomyopathy (FHC) is a cardiac disorder that is inherited in an autosomal dominant fashion and causes sudden death. It is manifested as ventricular hypertrophy predominantly affecting the interventricular septum and associated with myocardial and myofibrillar disarray. To date seven different genetic loci have been mapped for FHC, including chromosome 1q3, 3p, 7q3, 11p11, 12q23, 14q11, and 15q2, and six genes identified. A significant number of families are not linked to the known loci, indicating that other FHC genes exist.

Currently, FHC is primarily managed medically, using either β-blocker therapy (i.e., propranolol, atenolol) or calcium channel-blocker therapy (i.e., verapamil). These agents are directed at the pathophysiology of the disease (i.e., diastolic dysfunction) and its resultant symptoms, but do not reduce the hypertrophic response. In patients with left (or right) ventricular outflow tract obstruction, surgical options are also considered. Of these, myectomy is the preferred approach: excessive myocardium is removed, thereby increasing the size of the left ventricular cavity and allowing for better filling, as well as removing the obstruction to outflow. Another option in patients with obstruction may be dual chamber pacing. This method, which is somewhat controversial, may decrease the level of obstruction and has also been reported to decrease the amount of hypertrophy in some patients [66].

In 1990, Geisterfer-Lowrance et al. [5] found that the gene for β-cardiac myosin heavy chain (MyHC) is responsible for chromosome 14-linked FHC. Mutations in the β-cardiac MyHC gene are responsible for the disease phenotype in about 30% of FHC families. Several lines of evidence support that the MHC mutations act through a dominant-negative mechanism, i.e., the mutated protein interferes with the function of the wild type.

In 1994, Thierfelder et al. [67] reported that missense mutations in the α-tropomyosin gene cause chromosome 15q2-linked FHC. Tropomyosins are ubiquitous 35–45 kD proteins associated with the actin filaments of myofibrils and stress fibers. They also established that the cardiac troponin T gene is responsible for chromosome 1-linked FHC. Troponin T is a component of the troponin complex that is located on the thin filament. Mutations in the cardiac troponin T gene cause about 15% of FHC cases, while less than 3% of FHC cases had mutations in α-tropomyosin. It is not clear by which mechanism mutations in the cardiac troponin T and α-tropomyosin act.

In 1995, Bonne et al. [68] and Watkins et al. [69] independently established that mutations in the cardiac myosin binding protein-C (MyBP-C) gene cause chromosome 11-linked FHC. Cardiac MyBP-C is arrayed transversely in sarcomere A-bands and binds myosin heavy chain in thick filaments and titin in elastic filaments. Phosphorylation of MyBP-C appears to modulate contraction. More recently, Poetter et al. [70] identified families with FHC due to mutations in the myosin regulatory light chain (located at 12q23) and myosin essential light chain (3p). Since α-tropomyosin, cardiac troponin T, β-MyHC, cardiac MyBP-C, myosin regulatory and essential light chain mutations cause the same phenotype, FHC is a disease of the sarcomere.

Watkins et al. [69] initially reported phenotype-genotype correlation analysis in patients with FHC and β-MHC mutations. They noted that certain mutations acted in malignant fashion, causing early death in affected individuals, while other mutations were benign with respect to long-term survival. Similar studies have been reported confirming these findings for β-MHC, as well as identifying similar patterns for mutations in troponin T [71]. Hence, certain subgroups of patients would certainly benefit from advances in genetic-based therapies.

Geisterfer-Lowrance et al. [72] have recently described a murine model of FHC resulting from a mutation of the α-cardiac MyHC gene. In heterozygotic animals the cardiac histopathology and dysfunction resembled the human condition, while animals homozygous for the mutation died within a few days of birth. Such animal models will facilitate the testing of the efficacy of gene therapy protocols.

An adenovirus vector encoding the β-MyHC under the control of a cytomegalovirus (CMV) promoter, has been described [73]. This construct efficiently transduces feline cardiac myocytes but little other data have been reported. The efficacy and simplicity of gene therapy approaches will depend upon the mechanism of pathogenesis of the various mutations. For example, dominant-negative mutations will require that the expression of the endogenous gene be down regulated or inhibited, possibly by the co-expression of mutation-specific ribozymes [74,75] together with the functional gene. Lieber and Kay [76] reported that transduction of the liver of transgenic mice that produce human growth hormone (hGH) with a recombinant adenovirus encoding a ribozyme against hGH resulted in greater than 95% reduction in hGH production. Feng et al. [77] have shown that transduction with a recombinant adenovirus encoding a ribozyme designed to cleave the mutant form of the H-ras oncogene transcript, resulted in reversion of the neoplastic phenotype in H-ras transformed cells.

5. Long QT syndrome

Long QT syndrome (LQT) is a cardiac disorder that causes syncope, seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes, and is characterized by elongated QT intervals on electrocardio-
There are two forms of inherited LQT, an autosomal dominant and an autosomal recessive form. Autosomal recessive LQT, known as Jervell and Lange-Nielsen syndrome, is associated with congenital sensori-neural deafness. No genetic locus has been found for autosomal recessive LQT. Autosomal dominant LQT, known as Romano-Ward syndrome, is more common and is not associated with any other phenotypic abnormalities. In 1991, Keating et al. [78] mapped the first gene for autosomal dominant LQT to chromosome 11p15.5 (LQT1). Subsequently, Towbin et al. [79] demonstrated genetic heterogeneity and Jiang et al. [80] mapped the second LQT locus to chromosome 7q35–36 (LQT2) and the third to 3p21–24 (LQT3). Recently, Schott et al. [81] mapped the fourth LQT locus to 4q25–27 (LQT4).

Genes for LQT1, LQT2, and LQT3 have been identified. In 1995, Curran et al. [7] reported the identification of the gene for LQT2 as HERG, a cardiac potassium channel gene with six transmembrane segments, and Wang et al. [8] reported the finding that LQT3 is the cardiac sodium channel gene, SCN5A. Electrophysiological and biophysical characterization of HERG expressed in Xenopus oocytes established that it encodes the rapidly activating delayed rectifier potassium current I\textsubscript{K\textsubscript{r}}. Paradoxically, increases in potassium concentration were shown to increase outward HERG current. LQT-associated HERG mutants were also characterized in Xenopus oocytes and it was found that they act through either a loss-of-function or a dominant-negative mechanism. Thus, interventions that increase outward HERG current are likely to be effective treatments for LQT2 patients.

SCN5A has a putative structure that consists of four homologous domains, each of which contains six transmembrane segments. LQT-causing mutations in SCN5A generate a late phase of inactivation-resistant inward sodium current by either dispersed reopening, long-lasting bursts, or both. Thus, SCN5A mutations act through a gain-of-function mechanism (i.e., mutant channels function normally, but with altered properties of inactivation). Drugs that inhibit the persistent inactivation-resistant sodium current associated with LQT mutations could potentially be effective in treating LQT3 patients.

Wang et al. [9] reported the cloning of a novel gene named KVLQT1 for LQT1. KVLQT1 is highly expressed in the human heart and encodes a protein homologous to potassium channels with a conserved potassium-selective pore-signature sequence flanked by six membrane-spanning segments. KVLQT1 is a voltage-gated potassium channel protein which, when co-expressed with MinK, a potassium channel subunit with only one transmembrane spanning segment, generates the slowly activating potassium current I\textsubscript{Ks} in cardiac myocytes. Similar to HERG, KVLQT1 mutations probably act through either a loss-of-function mechanism or a dominant-negative mechanism.

Several different therapeutic options exist for LQT patients, including \(\beta\)-adrenergic blockade, left cardiac sym pathetic denervation, pacing, or implantation of a cardioverter-defibrillator. None of these therapies, however, shorten the QTc or prevent ventricular arrhythmias in all patients. With identification and molecular characterization of several LQT genes and their disease-causing mutations, new therapeutic strategies have become possible. Mexiletine, a sodium channel blocking agent, has been shown to markedly shorten the QTc of chromosome 3-linked LQT patients and to have only a modest effect on chromosome 7 and 11-linked LQT patients [82]. Raising the serum potassium concentration was effective in shortening the QTc for patients with chromosome 7-linked LQT [83]. It is important to point out that, although the therapies described above are effective in reducing QTc on electrocardiograms, it is still uncertain whether these therapies can eliminate ventricular arrhythmias and its associated clinical features, including sudden death. An effective treatment for patients with chromosome 11-linked LQT is currently unknown.

The in vitro transduction of canine myocytes by a recombinant adenosine, encoding an inactivation-defective \(Drosophila\) Shaker B (ShK) potassium channel [84] under the control of the RSV-LTR promoter, has been reported. Myocytes were isolated from normal dog hearts, as well as hearts from dogs with congestive heart failure, resulting from pacemaker implantation [85,86], and transduced with the recombinant adenosine. In isolated failing myocytes there is a deficiency of voltage-dependent potassium channels, resulting in prolonged action potentials. Introduction of the ShK gene into the failing myocytes reversed the action potential prolongation [84], and the observed phenotype change was dependent upon the level of transgene expression.

The viability of gene therapy for the treatment of LQT will depend greatly upon the number of defective ion channels identified in these patients, the ability to distinguish between these differences and the ability to accurately regulate transgene expression. Since some of the mutations may act through dominant-negative or gain-of-function mechanisms, it is likely that a gene therapy-based approach will involve, at least in part, interference of the expression of the mutated protein, as described above for FHIC mutations. At present the development of pharmaceuticals as a means of controlling this condition appears to be the more viable option [82,83].

6. Adenovirus-mediated cardiac disease

Viral myocarditis typically presents in children as an acute, fulminating disease and is associated with high morbidity, while adults more commonly present with the less fulminant chronic form of disease. Over the past decade a number of studies have provided evidence for persistent viral infection of the myocardium in adult patients with myocarditis or idiopathic dilated cardiomyopa-
thy (IDCM) by the detection of viral genomic nucleic acid sequences [87–90]. These data support the concept that IDCM is a sequela of a viral myocarditis.

Most of the studies demonstrating viral genomic sequences in the myocardium of patients with myocarditis or IDCM concentrated upon the enteroviruses and CMV as potentiating agents. However, little data is available establishing a direct pathological role for these viruses, the role of other viruses and whether disease in children is similarly associated with persistent viral infection.

Martin et al. [91] and Griffin et al. [92] studied myocardial samples of children presenting with either myocarditis or IDCM, by PCR to detect enteroviruses and CMV, as well as adenovirus, Epstein–Barr virus (EBV), Herpes Simplex virus (HSV), parvovirus and influenza A. Amplification of viral genome was seen in 34 of 58 (59%) cases of myocarditis and in 6 of 28 (21%) cases of IDCM but in none of the 22 controls [91]. In contrast, virus was isolated (by culture techniques) from only one cardiac sample and nine peripheral samples, in all cases from patients with myocarditis. In 24 of the virus positive cases by PCR, adenovirus was amplified (Fig. 2); these included all of the cases of IDCM. In comparison, 12 cases were PCR positive for enterovirus and 2 each for CMV and HSV. Since that time we have analyzed nearly 400 such samples, which have confirmed these initial studies. These data are summarized in Table 1. Primers specific for the hexon region of adenovirus (encoded within the L region: Fig. 1) were used: DNA sequence analysis of a number of the hexon amplimers generated indicates that in most cases the patient was infected by adenovirus type 2 (our unpublished data). With respect to the observation that the adenoviruses and enteroviruses are the most commonly detected viruses in the myocardium, it is interesting to note that adenovirus types 2 and 5 and Coxsackie B virus share a common receptor [93].

While there are concerns about the effects of the acute inflammatory response to recombinant adenovirus vectors and of the ability of these vectors to replicate in the transduced tissue due to rescue by wild type virus, it appears that the role of adenoviruses, and potentially adenoviral vectors, in chronic diseases of humans has not been fully appreciated. The pathological role of adenoviruses in myocarditis and IDCM is unclear, although studies in cotton rats suggest that systemic delivery of adenovirus results in histopathological changes compatible with a diagnosis of pneumonitis and myocarditis ([94]: our unpublished data, respectively).

Considerable effort is being employed to develop adenoviral vectors lacking large portions of the genome in order to prevent virus gene expression and replication in the target tissue, the elimination of wild type virion from recombinant adenoviral preparations and the use of immunosuppressive drug therapy to block the immune response at the time of delivery. However, it should be noted that, despite the presence of adenovirus sequences within the myocardium of myocarditis and IDCM patients, virus is rarely isolated and virus-specific antigens are not detected by immunohistochemistry, suggesting that the virus persists in a defective form, as has been shown for chronic enterovirus infection of muscle [95]. Further, the inflammatory infiltrate detected in the adenovirus-infected myocardium is considerably less than that detected in en-

Table 1
The frequency of virus detection by PCR in myocardial samples from patients with myocarditis or IDCM, or from control cases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th># samples</th>
<th>PCR positive (% positive)</th>
<th>Virus amplified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocarditis</td>
<td>199</td>
<td>90 (45%)</td>
<td>Adeno (46), Entero (32), HSV (4), CMV (5), Parvovirus (2), EBV (1)</td>
</tr>
<tr>
<td>IDCM</td>
<td>132</td>
<td>26 (20%)</td>
<td>Adeno (16), Entero (10)</td>
</tr>
<tr>
<td>Controls</td>
<td>65</td>
<td>1 (&lt; 2%)</td>
<td>Entero (1)</td>
</tr>
</tbody>
</table>
terovirus-infected samples [91], indicating that the absence of gross, acute pathological events do not preclude the development of chronic disease.

Taken together, these data suggest that the pathological changes associated with myocarditis and IDCM may be dependent upon the expression of a limited number of adenoviral genes in the myocardium. We suggest that the total elimination of wild type virus from recombinant adenovirus preparations should be considered a priority for the use of these vectors in clinical trials.

7. Summary

While there have been considerable improvements in the development of adenoviral vectors with respect to addressing the problems associated with persistence of transgene expression and the elimination of the immune response against virus-encoded proteins, many difficulties remain to be overcome before somatic gene therapy will be a viable option for the treatment of myocardial disease. These include (1) the ability to transduce sufficient numbers of myocytes to observe a therapeutic response. (2) If there is any correlation between the gene mutation and disease progression and prognosis, there will be a significant requirement for accurate and early diagnosis and mutation identification. (3) The regulation of gene expression, particularly in diseases such as LQT, where the stoichiometry of the expression of the ion channel is likely to be critical. (4) In many diseases where only a mutated protein is synthesized it is likely that the normal transgene product will elicit an immune response. (5) Consideration of the mechanisms of pathogenesis of the mutated gene will determine the efficacy of gene therapy approaches, since dominant negative and gain-of-function mutations will require that the endogenous gene is down regulated. (6) Finally, the role of adenovirus infection in chronic diseases of the myocardium needs to be further studied in order to understand the pathogenetic mechanism.

References


