The Detection of Cardiotropic Viruses in the Myocardium of Patients With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy

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OBJECTIVES
We sought to investigate the role of cardiotropic viruses, including adenovirus, cytomegalovirus (CMV), enterovirus and parvovirus, in arrhythmogenic right ventricular dysplasia/cardiomopathy (ARVD/C).

BACKGROUND
Arrhythmogenic right ventricular dysplasia/cardiomyopathy is characterized by a gradual loss of myocytes, inflammatory infiltrates and replacement by fatty and fibrous tissue. It has been speculated that ARVD/C is a sequela of viral myocarditis in some patients, and the role of the coxsackievirus B3 has been debated.

METHODS
Myocardial samples from 12 patients with ARVD/C were analyzed by polymerase chain reaction for the presence of cardiotropic viruses.

RESULTS
Enteroviral sequences were detected in seven patients and adenovirus type 5 in another two patients. During the same period, 215 control samples were analyzed in which only CMV (n = 2) and enterovirus (n = 1) were detected. This suggests a link between ARVD/C and the presence of viral genome (enterovirus or adenovirus) in the myocardium.

CONCLUSIONS
We report that cardiotropic viruses are more frequently identified in patients with ARVD/C than in control subjects. However, the role of these viruses in ARVD/C pathogenesis remains unknown. (J Am Coll Cardiol 2002;39:892–5) © 2002 by the American College of Cardiology Foundation

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) is a complex arrhythmogenic disorder associated with cardiomyopathy and characterized by a gradual loss of myocytes and replacement by fatty and fibrous tissue (1). It leads to dilation of the right ventricle (RV) and impaired cardiac function. The clinical course is characterized by ventricular arrhythmias (ventricular tachycardia), heart failure, syncope and sudden death. In Italy, the prevalence has been reported to be 1 in 5,000 people, accounting for 20% of sudden deaths in young adults (2) and 25% of sudden cardiac deaths among athletes (3). However, the incidence and prevalence are unknown in the U.S. Goodin et al. (4) reported a frequency at autopsy of 0.55% among young adults with sudden cardiac death in Maryland.

ARVD/C was first reported as the partial replacement of the RV myocardium by fat or fibrous tissue. In 1965, Dalla Volta et al. (5) described the disease as “auricularization of the RV curve due to loss of the contractile power of the RV.” Later, Frank et al. (6) referred to this new entity as RV dysplasia, and Fontaine et al. (7) added the term “arrhythmogenic.” In 1996, ARVD/C was added to the World Health Organization classification of cardiomyopathies (8).

In familial cases of ARVD/C, autosomal-dominant inheritance with reduced penetrance has been reported and accounts for ~30% of cases. In the remaining sporadic cases, it is believed to be due to an acquired etiology or to unidentified inheritance. The first gene for autosomal-dominant ARVD/C was recently identified, encoding the cardiac ryanodine receptor 2 on chromosome 1q42-43 (9). In addition, two genes responsible for Naxos disease, a complex autosomal-recessive form of ARVD/C with associated palmoplantar keratoderma and woolly hair, have been identified as plakoglobin and desmoplakin—important proteins in the cardiac adherens junctions (10,11).

The role of infectious agents in sporadic cases of ARVD/C has been proposed because of the common finding of inflammatory infiltrates in the myocardium (1,12), suggesting that ARVD/C is a sequela of myocarditis (like dilated cardiomyopathy [DCM]). Therefore, viruses associated with myocarditis have been proposed as potential etiologic agents. In 1998, Grumbach et al. (13) reported the detection of coxsackievirus B3 in the myocardium of 3 of 8 patients with ARVD/C, although Calabrese et al. (14) did not detect enteroviral ribonucleic acid (RNA) in any of 20 patient samples analyzed. Thus, the role of enteroviruses has remained controversial, and involvement of other cardiotropic viruses (including adenovirus, cytomegalovirus [CMV] and parvovirus) has not been reported. Here we describe the detection of viruses (enterovirus or adenovirus) in 7 of 12 patients with sporadic ARVD/C.
Clinical diagnostic criteria for ARVD/C. All human studies were performed in accordance with local Institutional Review Board regulations. Diagnostic criteria were those according to McKenna et al. (15). All patients were required to have clinical ventricular tachycardia (VT) with a left bundle branch block pattern, echocardiographic evidence of RV dilation, and/or without evidence of RV thinning and/or outflow tract dilation by echocardiography or magnetic resonance imaging, and histopathologic evidence of RV fibrofatty infiltration. All patients were evaluated by electrocardiography, Holter monitoring, echocardiography, magnetic resonance imaging and either endomyocardial biopsy, autopsy or explant histologic analysis. Biopsy samples were obtained only in patients in whom the clinical diagnosis was uncertain. A family history was obtained for all patients, and pedigrees were constructed. Nuclear family members (i.e., parents and siblings) were clinically evaluated for evidence of ARVD/C, using identical criteria. Any other family members (e.g., grandparents, aunts, uncles, cousins) considered at risk due to a medical history were also fully evaluated noninvasively. Control samples were obtained at the time of explantation for patients undergoing transplantation for end-stage congenital heart disease, or autopsies in trauma victims, and for those dying suddenly due to hypertrophic cardiomyopathy.

After cardiac catheterization and endomyocardial biopsy, the biopsy specimens were snap-frozen in liquid nitrogen or formalin-fixed for polymerase chain reaction (PCR) analysis. All explanted hearts were sampled (RV and left ventricle [LV]) and snap-frozen within 10 min of explantation. All autopsies were performed in standard fashion; LV and RV wedges were frozen, formalin-fixed or paraffin-embedded. In all cases, histologic analysis included hematoxylin-eosin, Masson trichrome and period acid-Schiff staining. Histologic results were based on the McKenna criteria for ARVD/C (15) and the Dallas criteria for myocarditis (16).

Patient samples and PCR analysis. Total RNA and deoxyribonucleic acid (DNA) were isolated simultaneously from tissue samples, as previously described (17–19). For detection of the enteroviruses, RSV and influenza viruses (i.e., RNA viruses), reverse transcriptase-PCR was employed, whereas for adenoviruses, CMV, herpes simplex virus (HSV), parvovirus and Epstein-Barr virus (EBV) (i.e., DNA viruses), PCR was utilized (17–19). Primers were designed to the 5’ untranslated region of the enteroviruses, the hexon gene of the adenoviruses, the phosphoprotein 120 gene of CMV, DNA polymerase of HSV (types 1 and 2), the EcoR I fragment of EBV, the VP1 gene of parvovirus B19, the nonstructural protein region of influenza A and the F-glycoprotein of RSV (17–19). For all analyses, negative and positive control reactions were performed in an identical manner to the test reactions, using water and viral nucleic acid, respectively, as the template.

All samples were analyzed without knowledge of the clinical, culture or serologic data and were performed in duplicate. Verification of the presence of amplifiable nucleic acid extracted from each sample was performed by amplification of cellular nucleic acid (K-ras or beta-actin).

Statistical analysis. Frequencies of virus detection between the ARVD/C group and the control group were compared using chi-square analysis.

RESULTS

During the seven-year period (1993 to 2000), samples from 1,000 individuals were evaluated by viral PCR, including 12 with ARVD/C (mean age 19 years; range 16 to 23 years) and 215 control subjects. Of the control subjects, 123 had congenital heart disease (mean age 14 years; range 3 months to 31 years), 42 had hypertrophic cardiomyopathy (mean age 21 years; range 3 to 29 years) and 50 were trauma victims (mean age 20 years; range 13 to 30 years). None of the control subjects had a history of recent viral illness or histologic evidence of myocarditis.

All cases of ARVD/C were considered sporadic based on pedigree analysis, indicating no other affected individuals or sudden deaths in the extended family by history and noninvasive testing. Clinical screening of family members of all patients was negative for evidence of ARVD/C. No patient had previous episodes of flu-like or other viral illnesses in the three months before diagnosis.

Of the 12 ARVD/C patient samples, viruses were detected in 7 (58%) (Table 1). Of these seven patients, enteroviruses were detected in five patients and adenovirus type 5 in two patient (Fig. 1 and Table 1). In contrast, only
TABLE 2. Characteristics of the Patients With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Who Were Positive for Virus

<table>
<thead>
<tr>
<th>Patient Initials</th>
<th>Age of Onset (yrs)</th>
<th>Gender</th>
<th>Origin of Sample</th>
<th>PCR Result</th>
<th>PCR Date</th>
<th>Pathology</th>
<th>Clinical Presentation</th>
<th>Disease Duration</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. P.</td>
<td>16/M</td>
<td></td>
<td>RV biopsy</td>
<td>Enterovirus</td>
<td>4/93</td>
<td>Fibrofatty infiltration of RV</td>
<td>Syncope, VT</td>
<td>26 mo</td>
<td>ICD</td>
</tr>
<tr>
<td>R. P.</td>
<td>22/M</td>
<td></td>
<td>RV, LV explant</td>
<td>Adenovirus</td>
<td>5/93</td>
<td>Fibrofatty infiltration of RV, lymphocytes</td>
<td>CHF, biventricular failure</td>
<td>16 mo</td>
<td>Transplant</td>
</tr>
<tr>
<td>M. G.</td>
<td>17/F</td>
<td></td>
<td>RV biopsy</td>
<td>Enterovirus</td>
<td>6/94</td>
<td>Fibrofatty infiltration of RV, lymphocytes</td>
<td>Syncope</td>
<td>3 yrs</td>
<td>Stable</td>
</tr>
<tr>
<td>C. B.</td>
<td>23/M</td>
<td></td>
<td>RV, LV explant</td>
<td>Enterovirus</td>
<td>11/94</td>
<td>Fibrofatty infiltration of RV/LV</td>
<td>Polymorphic VT</td>
<td>2 yrs</td>
<td>ICD, transplant</td>
</tr>
<tr>
<td>D. P.</td>
<td>19/F</td>
<td></td>
<td>RV, LV autopsy</td>
<td>Enterovirus</td>
<td>1/95</td>
<td>Fibrofatty infiltration of RV</td>
<td>Syncope, SCD</td>
<td>Autopsy diagnosis</td>
<td>SCD</td>
</tr>
<tr>
<td>J. L.</td>
<td>19/F</td>
<td></td>
<td>RV biopsy</td>
<td>Enterovirus</td>
<td>4/96</td>
<td>Fibrofatty infiltration of RV, lymphocytes</td>
<td>VT</td>
<td>30 mo</td>
<td>ICD</td>
</tr>
<tr>
<td>A. M.</td>
<td>16/M</td>
<td></td>
<td>RV biopsy</td>
<td>Adenovirus</td>
<td>10/99</td>
<td>Fibrofatty infiltration of RV/LV</td>
<td>Syncope, VT</td>
<td>6 mo</td>
<td>ICD</td>
</tr>
</tbody>
</table>

CHF = congestive heart failure; ICD = implantable cardioverter defibrillator; LV = left ventricle; PCR = polymerase chain reaction; RV = right ventricle; SCD = sudden cardiac death; VT = ventricular tachycardia.

DISCUSSION

Detection of viruses in the myocardium. Here we report the detection of virus sequences in myocardial samples of patients with sporadic ARVD/C. The viruses detected are the same as those identified in the myocardium of patients with myocarditis or DCM, supporting the hypothesis that ARVD/C is a sequela of viral myocarditis in some patients.

Sequence analysis was not performed on the enterovirus-positive samples, but in patients with myocarditis or DCM, coxsackievirus B3 is the most commonly detected enterovirus, and Grumbach et al. (13) reported the detection of coxsackievirus B3 in patients with ARVD/C. In both patients in whom adenovirus was detected, the virus was serotyped by DNA sequencing as adenovirus type 5, a group C adenovirus commonly detected in patients with myocarditis or DCM (19).

The role of viruses in ARVD/C pathogenesis. The cause and effect association between the detection of virus and the etiology of myocarditis and DCM has been widely accepted. In a recent study of heart transplant patients undergoing routine follow-up for rejection, we reported a significant association between the detection of virus in the myocardium and the onset of disease (20). However, it is not clear whether the detection of viruses in patients with ARVD/C represents a cause and effect relationship. The similarities between ARVD/C and myocarditis/DCM in terms of histopathologic findings and ventricular dilation could support an etiologic role for these viruses. However, the distinct pathologic differences between ARVD/C and myocarditis/DCM after sudden death.

A comparison of the histologic findings of myocardial samples, as well as the clinical characteristics at clinical presentation, did not distinguish patients positive for either enterovirus or adenovirus (Table 2) from those negative for viral genome.

All of the patients with ARVD/C presented with syncope or VT, or both, except for one young man (R. P.) (Table 2) who presented with heart failure. The primary presentation of syncope and VT is an uncommon event in pediatric patients, and in many centers, these individuals undergo electrophysiologic testing and endomyocardial biopsy during the diagnostic evaluation, unless an etiologic diagnosis (such as long QT syndrome or Brugada syndrome) is made by noninvasive testing. In addition, the noninvasive evaluation of ARVD/C is commonly nondiagnostic, leading to invasive evaluation. Hence, in all patients presenting with syncope or VT, biopsies were obtained (n = 6) at five different medical centers. Of the remaining patients, three underwent transplantation and three underwent autopsy after sudden death.

Table 2. Characteristics of the Patients With Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy Who Were Positive for Virus

Figure 1. The detection of adenoviral deoxyribonucleic acid (DNA) in arrhythmogenic right ventricular dysplasia cardiomyopathy patient samples. Lane M = 100 base pair (bp) DNA ladder; RP = patient R.P.; AM = patient A.M.; – = negative control (water); AD2 = adenovirus type 2 positive control DNA.
DCM, with respect to fatty infiltrates, primary RV disease versus LV disease, as well as the high frequency of ventricular arrhythmias, suggest that in patients with ARVD/C, other pathologic processes are occurring, which could arguably result in increased susceptibility to infection with cardiotropic viruses. In this case, the detection of viruses may simply represent a marker of the pathologic changes occurring in the myocardium. Interestingly, Diong and Knowlton (21) recently reported that dystrophin-deficient mice, which have increased disruption of the sarcolemma, are more susceptible to enterovirus infection of the myocardium and the development of virus-induced cardiomyopathy, suggesting these two hypotheses are not mutually exclusive.

Assuming an etiologic role for these viruses, rather than being a marker of disease, it is possible that infection of the myocardium results in activation of inflammatory mediators and adipose deposition cascades that ultimately disrupt the cardiac adherens junctions and the T-tubule system. These changes could secondarily cause ventricular irritability or ion channel disruption, or both (e.g., through ryanodine receptor changes), resulting in the signs and symptoms of ARVD/C.

Conclusions. Cardiotropic viruses (e.g., enterovirus and adenovirus) are identified more frequently in patients with ARVD/C than in control subjects. However, the role of these viruses in disease pathogenesis remains unknown, particularly whether these viruses contribute to disease or whether the diseased myocardium is more prone to virus infection.

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