Viral hypothesis

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Introduction

Arrhythmogenic right ventricular dysplasia (ARVD/C) is a complex arrhythmogenic disorder associated with cardiomyopathy and characterized by 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 as 1:5000 people, accounting for 20% of sudden deaths in young adults (2) and 25% of cardiac sudden deaths among athletes (3). However, the incidence and prevalence are unknown in the United States: Goodin and colleagues reported a frequency at autopsy of 0.55% among young adults with sudden cardiac death in Maryland (4).

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

The role of infectious agents in sporadic cases of ARVD/C has been proposed due to the common finding of inflammatory infiltrates in the myocardium,(1, 9) suggesting that ARVD/C is a sequela of myocarditis (like dilated cardiomyopathy or DCM). Therefore, viruses associated with myocarditis have been proposed as potential etiologic agents. In 1998, Grumbach *et al*. reported the detection of Coxsackievirus B3 in the myocardium of 3 of 8 patients with ARVD/C (10), although Calabrese and

colleagues did not detect enteroviral RNA in any of 20 patient samples analyzed (11).

Viral Heart Disease

Viral infections of the heart are important causes of morbidity and mortality in children and adults. Acute myocarditis, the best studied of these infections, typically presents with severe clinical manifestations, especially in the newborn period (12). Idiopathic DCM appears to occur as a late sequela of acute or chronic viral myocarditis (13-17), either due to persistence of virus (13) or to an autoimmune phenomenon occurring secondary to previous exposure to the inciting virus (18). The affected individual may require long-term medical therapy for congestive heart failure (CHF) and, in many cases, heart transplantation (OHT) may be required. In some cases, sudden cardiac death occurs (17), particularly in athletes (19).

Endomyocardial biopsy (EMB) and histopathology demonstrating cellular infiltrates (particularly lymphocytes), edema, myocyte necrosis, and myocardial scarring was developed to improve diagnostic capabilities, but was inconsistent amongst pathologists. The so-called "Dallas" criteria (20), described in 1987, were developed in an attempt to improve the high rate of diagnostic disagreement between pathologists by utilizing uniform criteria. However, due to insensitivity (21) 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 (22) presented comprehensive serological 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 (23). 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 tropism for skeletal and cardiac muscle. However, isolation of infectious virus from patients with heart muscle disease is rare (24). 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 (25), despite evidence of virus association from retrospective serology.

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 in one study (26). Such IgM responses have been shown to persist for up to 6 months (27). CVB-specific IgM responses in patients with end-stage dilated cardiomyopathy undergoing cardiac transplantation have also been reported, with the IgM responses persisting for up to 19 months prior to transplantation (28).

The concept of an enteroviral etiology of heart muscle disease is reinforced by animal models of both myocarditis and DCM. A cardiotropic strain of Coxsackievirus B3 (CVB3) induces inflammatory heart muscle disease in mice where infectious virus cannot be isolated from myocardium after the first 2-3 weeks (29, 30), although many of the animals progress to left ventricular disease reminiscent of DCM (31, 32), supporting the hypothesis that DCM can be a sequela of a viral myocarditis.

The failure to isolate virus or detect viral antigens in patient EMB samples, despite the serological 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 us (33, 34) and later by Kandolf *et al.* (35, 36) 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. More recently, polymerase chain reaction (PCR) has been employed in the rapid detection

of viral sequences in many tissues and body fluids, including the myocardium of patients with suspected myocarditis or DCM (Figure 1) (37-43). More recent evidence suggests that adenovirus is a commonly found in hearts of affected children and could be an important cause of myocarditis and DCM (44, 45).

In 2003 we reported a comprehensive study of more than 750 myocardial samples from patients with myocarditis and/or DCM using PCR to detect a range of viruses, including the enteroviruses, adenoviruses, cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), parvovirus, influenza and respiratory syncytial virus (RSV) (46). The patients were divided into groups based on 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 above 18 years). Greater than 65% of the samples came from patients between the ages of 1 day and 13 years, while more than 600 of the patients had a diagnosis of myocarditis, with the remainder DCM.

PCR amplified a viral product in approximately 40% of the samples obtained from patients with myocarditis compared with just 1.5% of “control" samples. Of these positive myocarditis samples, adenovirus was detected in more than 50%, enterovirus in 33%, while the remainder were mainly CMV but also included a few HSV Type 1, EBV, parvovirus, influenza, and RSV positives. 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 (47). Further, no significant differences were observed between age groups with respect to the relative frequencies of detection of adenovirus and enterovirus.

One interesting trend has been the increasing number of reports of myocarditis associated with parvovirus B19 infection (48-53), including studies where virus has been detected in both control

subjects with high frequency (54, 55). Whether this is simply of a result of improved diagnostics, or reflects an underlying change in the etiology is unclear at this point. In one recent study (56), parvovirus B19 was detected in endomyocardial biopsy samples using real time PCR, a method that not only allows viruses to be detected but also determine how much is present. Parvovirus genomic DNA was detected in nine of 80 patients (11.2%), comprising 4 of 31 (12.9%) with inflammatory infiltrates and 5 out of 49 (10.2%) patients with left ventricular dysfunction without myocardial inflammation. The copy numbers for the viral genomes ranged between 30 and 3900 per microgram of cellular DNA, with the 4 patients with myocarditis having 70, 740, 3400 and 3900 copies per microgram of DNA in the endomyocardial biopsy, while the 5 patients with idiopathic left ventricular dysfunction had copy numbers of 30, 38, 52, 58 and 90, respectively per microgram of DNA. To put these into meaningful numbers, 1 microgram of DNA represents the amount of DNA in approximately 300,000 cells. Thus, the detection of 30 copies means there is just one virus genome per 10,000 cells. Whether the presence of so little virus is important in the pathogenesis of these diseases is unclear, and presently there are no data to compare with the detection of the enteroviruses or adenoviruses. It should also be noted that in this study, no virus was detected in control samples. Quantitative studies of this type, correlated with patient outcome, may help clarify the role of parvovirus in heart muscle disease.

Viruses and ARVD/C

In addition to the patients described above with myocarditis or DCM, we have also reported the analysis of myocardial samples from 12 patients with ARVD/C: each of these were considered to be acquired or sporadic cases (57). During the 7 year period (1993-2000) samples from individuals were evaluated by viral PCR, including 12 with ARVD/C (mean age 19 years; range 16 to 23 years) and 215 controls. Of the control patients, 123 were individuals with congenital heart disease (CHD) (mean age 14

years; range 3 months to 31 years), 42 individuals with hypertrophic cardiomyopathy (mean age 21 years; range 3 to 29 years), and 50 trauma victims (mean age 20 years; range 13 to 30 years). None of the controls had a history of recent viral illness or histologic evidence of myocarditis.

All patients with ARVD/C were considered sporadic based on pedigree analysis indicating no other affected individuals or sudden death in the extended family by history and non-invasive testing; clinical screening of family members in all cases was negative for evidence of ARVD/C. No patient had prior episodes of flu-like or other viral illness in the three months prior to diagnosis.

Of the 12 ARVD/C patient samples, viruses were detected in 7 (58%). Of these 7, enteroviruses were detected in 5 and adenovirus type 5 in 2 (Figure 1). In contrast, only 3 (2%) of the control patient samples were virus positive, CMV in 2 and enterovirus in the third, indicating a significantly increased frequency of virus identification in ARVD/C patients than in controls ($P<0.01$). The 12 ARVD/C patients were diagnosed in 11 different centers in the United States (Table 1): analysis of patient demographics showed that the control group included individuals which matched the ARVD/C patients for age, sex, race, ethnicity, and geographic region.

Comparison of the histology of myocardial samples, as well as clinical characteristics at clinical presentation did not distinguish patients positive for either enterovirus or adenovirus or from those negative for viral genome.

All of the patients with ARVD/C presented with syncope and/or VT except one young man who presented with heart failure. The primary presentation of syncope and VT is an uncommon event in pediatrics 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, Brugada syndrome) is made on non-invasive testing. In addition, the non-invasive evaluation of ARVD/C is commonly non-diagnostic leading to invasive evaluation. Hence in all cases presenting with syncope

or VT, biopsies were performed (n=6) at 5 different medical centers. In the remaining patients, 3 underwent transplantation and 3 underwent autopsy after sudden death.

Sequence analysis was not performed on the enterovirus positive samples, but in myocarditis and DCM patients Coxsackievirus B3 is the most commonly detected enterovirus, and Grumbach *et al*. reported the detection of Coxsackievirus B3 in ARVD/C patients (10). In both cases where adenovirus was detected, the virus was serotyped by DNA sequencing as adenovirus type 5, a group C adenovirus commonly detected in myocarditis or DCM patients (47).

The Role of Viruses in ARVD/C Pathogenesis

While the cause and effect association between the detection of virus and etiology of myocarditis and DCM has been accepted by many. In a recent study of heart transplant patients undergoing routine surveillance for rejection, we reported a significant association between the detection of virus in the myocardium and the onset of pathology (58). However, it is not clear whether the detection of viruses in ARVD/C patients represents a cause and effect relationship. The similarities between ARVD/C and myocarditis/DCM in terms of histopathology and ventricular dilation could support an etiologic role for these viruses. However, the distinct pathological differences between ARVD/C and myocarditis/DCM with respect to fatty infiltrates, primary right ventricular disease versus left ventricular disease, as well as the high frequency of ventricular arrhythmias, suggests that in ARVD/C patients other pathological 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 pathological changes occurring in the myocardium. Interestingly, Diong and Knowlton 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 (59), suggesting these two hypotheses are not mutually exclusive.

Assuming an etiologic role for these viruses rather than being a marker of pathology, it is possible that infection of the myocardium results in the 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 and/or ion channel disruption (e.g. via ryanodine receptor changes), resulting in the signs and symptoms of ARVD/C.

Conclusion

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

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TABLE 1. Characteristics of the ARVD/C patients positive for virus

Patient	Age of Onset/ Sex	Origin of Sample	PCR Result	PCR Date	Pathology	Clinical presentation	Disease Duration	Clinical Outcome
FP	16 yrs Male	RV Biopsy	Enterovirus	4/93	Fibrofatty infiltration of RV	Syncope, VT	26 mo	ICD
RP	22 yrs Male	RV, LV Explant	Adenovirus	5/93	Fibrofatty infiltration of RV, lymphocytes	CHF, Biventricular Failure	16 mo	Transplant
MG	17 yrs Female	RV Biopsy	Enterovirus	6/94	Fibrofatty infiltration of RV, lymphocytes	Syncope	3 yrs	Stable
CB	23 yrs Male	RV, LV Explant	Enterovirus	11/94	Fibrofatty infiltration of RV/LV	Polymorphic VT	2 yrs	ICD Transplant
DP	19 yrs Female	RV, LV Autopsy	Enterovirus	1/95	Fibrofatty infiltration of RV	Syncope, SCD	Autopsy Diagnosis	SCD
JL	19 yrs Female	RV Biopsy	Enterovirus	4/96	Fibrofatty infiltration of RV, lymphocytes	VT	30 mo	ICD
AM	16 yrs Male	RV Biopsy	Adenovirus	10/99	Fibrofatty infiltration of RV/LV	Syncope, VT	6 mo	ICD

Key: CHF: congestive heart failure. LV: left ventricle. RV: right ventricle. ICD: Implantable Cardioverter Defibrillator. SCD: sudden cardiac death.VT: ventricular tachycardia.

Figure 1.