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Editorial

Dilated Cardiomyopathy: Chronic Viral Infection or Autoimmune Disease? A Critical view of the Viral and The Autoimmune Hypotheses

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Dilated cardiomyopathy has been defined as a heart muscle disease of unknown aetiology with dilation of the ventricles.[1] It occurs throughout the world although its true incidence is not known. In the United States its prevalence is around 20 per 100000 a year and there is an incidence of 6 new cases per 100000 a year.[2] Similar findings have been reported for England, with a higher incidence in third world countries. These figures are probably an underestimate, as asymptomatic cases or patients with fewer symptoms may not have been recognised.

Dilated cardiomyopathy is probably a heterogenous condition, and different aetiologies may be operating in patients with a similar clinical syndrome. The usual presentation is with heart failure, dysrhythmia, or thromboembolism, often at a late stage, and is associated with a poor long term prognosis. The diagnosis is made by exclusion and relies on showing the absence of coronary heart disease, valvular or pericardial disorders, and specific heart muscle diseases. Treatment, other than with angiotensin converting enzyme inhibitor therapy or cardiac transplantation, makes little difference to survival and has made no impact on the progression of the disease.[3] Clearly we will not be able to improve our treatment until we understand more about the disease and its pathogenesis. The two major hypotheses for explaining the progression of the condition are 1) persistent viral infection; 2) autoimmunity.

Indirect evidence for an aetiological link between viruses and human disease

Difficulties in establishing the specific diagnosis of viral heart disease have always been found to be a major problem in clinical cardiology. Evidence of causation is based on the successful isolation of infectious virus from the heart tissue of such patients; this has been accomplished only in rare cases of acute fulminant myocarditis. Considerable evidence has accrued that enteroviruses and in particular coxsackie B viruses are associated with acute myo-pericarditis, whereas the prevalence of subclinical cardiac infection with these viruses is a matter of debate.[4-7] In 1964 Burch first put forward the hypothesis that dilated cardiomyopathy was a long term sequela of myocarditis.[8]

Animal models. Inferential evidence to implicate enteroviruses as etiological agents came from animal models and in particular the murine model, which bears a close relationship to the disease seen in humans.[4] Following infection a diffuse myocarditis develops within days and is associated with an appreciable mortality. This is accompanied by high titres of virus within the heart following which virus can no longer be recovered. Host susceptibility varies considerably between mice strains with neonatal, male animals being most susceptible. Viral elimination relies largely on the synergistic activity of neutralising antibody and activated macrophages and virulence can be enhanced by exercise and interference with the immune defence mechanism. Studies by Wilson *et al* provided the first experimental evidence that acute murine myocarditis following coxsackie B3 virus infection is followed by permanent heart muscle disease.[9] Serial studies on myocardial tissue have demonstrated that this model closely resembles the histological findings seen in human dilated cardiomyopathy. However all attempts to see the virus by electron microscopy, isolate the virus by conventional techniques, or detect viral antigen by immuno-histochemistry from the tissue have failed.

Recently Kandolf has applied recombinant DNA technology to this problem with interesting results.[10] Using athymic mice, coxsackie B3 virus and *in situ* hybridisation, evidence for a persistence of viral genomic material has been found in myocytes up to six months after the acute infection. The concept of a persistent coxsackie B virus infection within the heart was surprising as enteroviruses are generally associated with acute lytic infections, although persistent picornavirus infections have rarely been described in both animals[11] and in humans.[12] The presence of viral RNA in chronic myocardial disease does not prove, however, that the virus is still cytopathic or that it is responsible for chronic myocardial damage; furthermore, the relevance of these experimental observations to human cardiac disease is not known.[13]

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Retrospective serology. A second line of evidence for viruses as causative agents is derived from observations of raised antibody titres to Coxsackie virus among patients with dilated cardiomyopathy.[14–18] The development and refinement of an enzyme-linked immunosorbant assay (ELISA) to detect virus specific IgM, has recently rekindled interest in retrospective serology as a means for substantiating the viral hypothesis in dilated cardiomyopathy.[19] In this study 33 percent of patients with end-stage dilated cardiomyopathy were positive for virus specific IgM compared to 12 percent seen in the control population of healthy blood donors.[19]

Although these data are suggestive of viral etiology in some cases, there are several limitations: 1) enteroviruses are common human pathogens and retrospective serology or history of infection is not enough to prove causation[13]; 2) infection with one serotype can also stimulate production of antibodies to other serotypes in a manner which is variable among individuals, therefore infections with non cardiotropic serotypes can raise antibody titers to cardiotropic types, even in the absence of cardiac infection[13]; 3) since enteroviruses are common agents, controls should share the patient's environment, being ideally the patient's unaffected relatives. To our knowledge, no studies have yet employed such controls.

Progression to dilated cardiomyopathy in patients with myocarditis. Several longitudinal studies provide support for the concept that dilated cardiomyopathy in some patients may result from prior viral myocarditis. Quigley *et al*[20] followed up 23 patients with biopsy proven myocarditis of unspecified aetiologies for a period of 5 years using non-invasive assessment with repeat biopsy if indicated. Although all the patients studied had significant haemodynamic disturbance at initial diagnosis, as early as 6 months, two distinct groups became apparent. Group 1 (9) recovered and had normal studies at rest, while Group 2(14) still had a marked degree of cardiac impairment. At late follow up 7 patients from Group 1 had an isolated abnormality in exercise induced ejection fraction response. However, in Group 2,12 had developed dilated cardiomyopathy and 4 of these had died. Follow up studies after coxsackie B myo-pericarditis have provided similar results.[21,22] From these clinical observations, as well as from experimental models it is clear, however, that myocarditis often has a benign course, and that only a minority of individuals, with a predisposing genetic and immunogenetic background progress to a syndrome resembling dilated cardiomyopathy.

Direct evidence for an aetiological link between viruses and human disease

Recombinant DNA technology. One major prerequisite for the introduction of nucleic acid hybridisation as a diagnostic tool in suspected enteroviral infections was the molecular cloning and characterisation of the coxsackie B virus genome. They were shown to possess a high degree of nucleic acid homology between the serotypes, and amongst the enterovirus group as a whole. This together with their ease of cloning has enabled rapid progress in this area using several different enterovirus group specific probes and conventional hybridisation techniques. Bowles produced the first report of enteroviral RNA persisting in the myocardial biopsies of patients with dilated cardiomyopathy.[23] Using a group specific probe in a slot blot hybridisation assay, he found 9 of the 17 patients with biopsy confirmed inflammatory heart disease (myocarditis/healing/healed myocarditis) to be probe positive, while none of the 4 control patients with other aetiologies (alcohol, puerperal, amyloid and muscular dystrophy) were probe positive. Similarly Archard *et al*[24] have studied 50 patients with myocarditis/dilated cardiomyopathy using the technique of slot blotting with a different enterovirus probe. Of these, 28 have proved to be probe positive with an equal distribution between the histological categories of active myocarditis, healing myocarditis and healed myocarditis/dilated cardiomyopathy. No control case gave a positive result in this study. Kandolf using *in-situ* hybridisation techniques has confirmed that a persistent coxsackie B virus infection can be produced, not in myocytes but in cultured human fibroblasts.[10] In this study, Kandolf has found evidence of persistent viral infection in 3/15 patients with dilated cardiomyopathy compared to 0/10 control patients with coronary heart disease.

Although detection of enterovirus RNA in a tissue provides direct proof of the presence of virus in cells of the host, which represents a remarkable progress compared to the inferential evidence given by serology, the implications of these new findings are far from conclusive. Results obtained with the slot bloot technique are difficult to interpret, because they may reflect hybridisation artifacts.[13] More refined approaches, in particular in situ hybridisation and the polymerase chain reaction have provided the first direct evidence for persistence of enteroviral genome, at least in some human hearts with myocarditis and cardiomyopathy.[10,13] However, wide discrepancies exist in relation to the incidence of 'virus-positive' hearts among different studies. These may be related to the technique used, to differences in clinical diagnosis as well as to the random nature of inflammatory heart disease and of endomyocardial biopsy; therefore the real incidence of enterovirus persistence in the human heart is not known.[13] Most importantly, detection of enterovirus RNA in a tissue does not imply active infection, since RNA viruses can generate defective interfering particles that inhibit replication of infectious virus and thus allow low level virus persistence in the absence of active infection.[25] Persistence of defective, non-infectious virus would be in good agreement with the fact that isolation of infectious virus from the myocardium has been achieved only in few cases of acute fulminant myocarditis, but not

in dilated cardiomyopathy. If infectious virus cannot be recovered, a causative role of persistent subclinical viral infection in mediating chronic myocardial damage cannot be inferred and the significance of persistent defective viruses in the myocardium remains uncertain.[13]

Evidence for an autoimmune pathogenesis

The other major hypothesis involved in the pathogenesis of dilated cardiomyopathy is autoimmune mediated damage to myocytes. Over the years, many animal experiments have been performed mainly using the murine model. Humoral immunity has been closely investigated by Matsumori[26] using the murine encephalomyocarditis virus model. In this study he was able to isolate virus (and antigen) from the heart only within the first few days of infection, after which it was absent. However, following the myocarditis a cardiac autoantibody could be detected whose activity seemed to parallel the development of the dilated cardiomyopathy. Furthermore vaccination against the virus appeared to offer protection not only from infection with the virus, but also from the development of the dilated cardiomyopathy. Other studies have concerned themselves with different aspects of the cell mediated immune response.[4,27] These have noted a marked cellular myocardial infiltration during viral myocarditis and myofibre lysis at a stage when virus cannot be recovered from the tissue. Later on, as the dilated cardiomyopathy develops, cytotoxic T cells have been demonstrated within the myocardium and have been shown to be both major histocompatibility complex restricted, and not restricted. In addition, the cytotoxicity of these cells can be blocked by pre-treating the mice with antiviral serum and thus profoundly altering the natural course of myocarditis and its long term sequelae. Most of these studies point out that after an initial phase of virus replication within the myocardium the virus is eliminated by physiologic immune mechanisms, however virus infection is able to trigger in genetically susceptible animals, an autoimmune response which is responsible for chronic myocardial damage. More recently, direct experimental evidence of autoimmunity to heart antigens has been provided, with the demonstration that mice immunised with the cardiac myosin isoform develop myocarditis and heart failure.[28]

There is also considerable amount of evidence in man for the role of both cell mediated immunity and humoral immunity in dilated cardiomyopathy. Retrospective studies have shown defects in lymphocyte transformation, T cell numbers and helper/suppressor cell ratios, and leucocyte migration.[29] Perhaps the most interesting recent development in this areas has been the discovery of a novel organ-specific cardiac antibody in patients with dilated cardiomyopathy (26%) as compared to the various control populations (all $<3.5\%$).[30] The presence of this antibody, in addition to providing a serologic marker for this condition, also brings us a step nearer to being able to define it as a distinct organ-specific autoimmune disease. Further evidence for an autoimmune contribution to the pathogenesis of the disease comes from the discovery of the abnormal expression of major histocompatibility complex class II antigens on endocardium and cardiac endothelium.[31] The reported association with HLA-DR4 antigen provides another interesting analogy between dilated cardiomyopathy and other autoimmune disease[32] and suggests that disease predisposition is genetically determined.

Conclusion

A number of hypotheses have been proposed to explain the involvement of viruses and the immune system in the pathogenesis of dilated cardiomyopathy. One suggests that enterovirus persists within the heart without significant antigen expression, its replication being ''defective'' in some way. Whether or not the presence of this virus within the myocardium is sufficient to impair cellular function and accounts for the pathological changes seen in dilated cardiomyopathy is uncertain.[13,33] An alternative view proposes that dilated cardiomyopathy is an organ-specific automimmune disease and has likened the disease to type 1 insulin dependent diabetes mellitus.[30]

The viral and autoimmune hypotheses are not mutually exclusive and it is quite conceivable that a viral infection within the heart can initiate a self perpetuating autoimmune response. The theoretical mechanisms by which this could occur are by no means new, and are generally accepted in the field of immunology. One such mechanism involves the expression of a cardiac neoantigen generated through the interaction of viral antigens and MHC class II antigens from the host. Another mechanism uses the concept of ''molecular mimicry'' between viral and cellular epitopes and has been reviewed recently by Rose.[28]

In experimental murine myocarditis infectious virus can no longer be recovered from the myocardium after two weeks[10,27,34] although nucleic acid sequences of the viral genome are still detectable.[10,34] The development of chronic destruction of myocytes and myocardial fibrosis is associated with the development of circulating lymphocytes.[28] In this experimental model chronic inflammation seems to be autoimmune in nature but initiated by viral infection, and this could harmonise with the clinical evidence.

In conclusion, we will not be able to improve the treatment of this condition until we understand more about it. For instance, the results of a recent trial of immunosuppression in patients with dilated cardiomyopathy, in which no benefit was shown,[35] are not surprising in view of the probable heterogeneity of both the etiology and pathogenesis of this condition. Different mechanisms of disease may be operating in patients with similar clinical presentation; only the indentification of these mechanisms and the immunologic, virologic and genetic characterisation of the individual patient may help to develop rational treatment.

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