Coxsackie viral myocarditis

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Summary: Coxsackie viral myocarditis is a common disease, yet idiopathic dilated cardiomyopathy is a less common consequence. Insights gained from studying the Coxsackie virus B-3 murine model of myocarditis has led to the hypothesis that an acute Coxsackie viral myocarditis can result in persistent, non-viral mediated cellular responses that result in a chronic inflammatory state leading to progressive myocyte loss and ultimate development of dilated cardiomyopathy. Although the evidence linking myocarditis to dilated cardiomyopathy is circumstantial in man, the identification of defects in immunoregulation may provide the impetus to further research into the pathogenesis and ultimately the development of more rational therapies directed at modulating immune responses to alter the natural history of clinical dilated cardiomyopathy.

Introduction

Although viruses have been implicated as a common aetiology of human cardiac disease, a causative role has been difficult to prove. One-half of patients with acute myocarditis and one-third with acute pericarditis have had a concomitant Coxsackie B virus infection (Grist & Bell, 1974). Since clinical infection with Coxsackie B virus is common and most cases of acute myocarditis are subclinical with uneventful recovery, the epidemiological significance of linking this viral infection to acute myocarditis is open to serious question.

The recent postulate that viral myocarditis may lead to chronic dilated cardiomyopathy (DC) in some individuals underscores the importance of the need for a more complete understanding of the role of initial viral infections in inciting the complex immunological responses that ultimately lead to persistent chronic myocardial inflammation and DC (Robinson & O’Connell, 1983). Moreover, since no specific therapy for primary myocardial disease exists, a further understanding of the pathogenesis of viral-initiated myocardial perturbations may lead to the development of therapies designed to treat the cause rather than the effect (congestive heart failure) of DC.

Since the initial myocarditis may be subclinical and undetected before the onset of chronic cardiac disease, full understanding of the progressive pathogenesis of myocardial damage may be impossible in man. Insights gained from studying Coxsackie virus B-3

(CVB-3)-induced myocardial dysfunction in animal systems may result in the development of diagnostic and therapeutic modalities that can be directly applied to humans.

Murine Coxsackie B-3 myocarditis

The murine CVB-3 myocarditis model is ideally suited for the study of the pathogenesis and modification of immune responses to prevent a chronic cardiomyopathy that may follow the initial viral myocarditis. Serial studies have documented the evolution of murine CVB-3 myocarditis from an acute viral syndrome to chronic DC, a scenario that seems to parallel the pathophysiology in human disease. When weanling mice are infected with CVB-3, an acute myocarditis results (Table I) (Woodruff, 1980). The viraemic phase is limited to 3 or 4 days and the virus is cultured from the myocardium only up to the tenth day. A generalized, nonspecific cellular response to viral

Table I Characteristics of the acute phase of murine Coxsackie B-3 myocarditis

– Myocardial viral replication for 7–9 d
– Viral clearance mediated by B-lymphocytes and macrophages
– Fatal when pretreated with cyclophosphamide or corticosteroids
– Unaffected by prior bone marrow irradiation/reconstitution with thymectomy or anti-thymocyte serum

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injury has been noted in other tissues during the acute viral infection (Burch & Harb, 1981). The myocardial necrosis that occurs early is patchy, affecting only isolated myofibres and leaving adjacent myofibres intact. Neutralizing virus-specific antibodies are detected in the serum on the seventh day and remain elevated for several months (Kitaura, 1981b). Despite the absence of cultural virus, a lymphomononuclear inflammatory infiltrate is present in the hearts of Swiss ICR mice at 6 months after the initial infection (Table II). Histopathology in these animals with persistent inflammation includes fibrosis, dystrophic mineralization, and myocardial hypertrophy (Wilson *et al.*, 1969). By 15 months, there is hypertrophy, mural thrombi, myocardial disintegration, and fibrous scarring (Reyes *et al.*, 1981). These findings are exacerbated if the mice are subjected to daily periods of strenuous exercise (swimming) during the acute phase. At 15 months, the lymphomononuclear infiltration is no longer present. The pathology in Swiss ICR mice is identical to that described at autopsy in patients with idiopathic DC. Even the electrocardiographic abnormalities, which include AV block, ventricular and atrial premature contractions, and left bundle branch block are similar to electrocardiographic changes in human dilated cardiomyopathy (Kawai *et al.*, 1982). Identification of the biphasic (acute-viral and chronic-nonviral mediated) nature of murine myocarditis has led to conjecture about the pathogenesis of immune-mediated potential of myocardial damage. When weanling and adult mice are infected with CVB-3, the virus is no longer detectable in the myocardium after the eighth day (Woodruff & Woodruff, 1974). When mice are pretreated with anti-thymocyte serum or given sublethal bone marrow irradiation and thymectomy, thus diminishing cell-mediated immune responses, the acute viral-mediated phase of the illness remains unchanged. The virus is cleared normally and viral antibody titres are normal. Survival is dramatically improved and inflammation is markedly attenuated

during the chronic phase. These studies imply that T-lymphocytes (T-Ly), the primary effectors of cell-mediated cytotoxic responses, do not participate in the acute phase and are not responsible for viral clearance, yet play a major role in the chronic phase. This biphasic nature of immunological responses allows a treatment 'window' to be established.

Immunosuppressive agents that affect cell-mediated immune responses selectively when given after viral replication may successfully attenuate the chronic inflammatory response. Although T-Ly play little role in the acute phase, the actual effects on viral clearance are as yet undefined (Rager-Zisman & Allison, 1973a,b; Chaturvedi *et al.*, 1978; Woodruff & Woodruff, 1971; Woodruff, 1979). Viral clearance may actually result from the complex interaction of interferon, humoral antibodies, B-lymphocytes, and macrophages (Norris & Loh, 1973). Attenuation at any of these levels of host immune response during the early phase may result in overwhelming viral dissemination. The study of viral clearance in the murine CVB-3 model may not, however, be relevant to studies that assess treatment in human disease. Several factors, including the delay in seeking medical attention and the subclinical nature of human viral myocarditis, may make identification of the illness during the phase of acute viral replication an unusual occurrence.

The immunopathogenesis of the chronic non-infectious phase is theoretically vulnerable to specific modulation of immune responses which could inhibit or reverse a similar inflammatory reaction in man. The role of T-Ly in the chronic phase was implicated when splenic lymphocytes were harvested from previously infected mice (Wong *et al.*, 1977). These lymphocytes were specifically toxic to target cells previously infected with CVB-3, but did not lyse uninfected myocardium, liver, or kidney; this implies that T-Ly may be responsible for the myocardial necrosis seen in chronic myocarditis. Myocardial necrosis without a lymphomononuclear infiltrate was not prominent in the hearts of athymic nude mice (absent T-Ly) infected with CVB-3 in a previously published study (Hashimoto & Komatsu, 1978). However, we have documented the development of the chronic inflammatory reaction in athymic nude mice (Robinson *et al.*, 1981). When CVB-3 myocarditis was induced in athymic mice (nu/nu on a BALB/c background), viral clearance was similar to BALB/c controls. Both developed inflammatory infiltrates during the chronic phase. The infiltrates in the athymic nudes consisted of lymphomononuclear cells, which were alpha-naphthyl acetate esterase positive implying a T-cell lineage. These cells histologically resemble natural killer (NK) cells. Athymic nude mice have no T-Ly, but have increased numbers of NK cells, and in some circumstances, NK cells are known to be the effectors of chronic cell-mediated immune responses in these

Table II Characteristics of the chronic phase of murine Coxsackie B-3 myocarditis

- Absence of culturable virus
- Mediated by T-lymphocytes (and/or NK cells)
- Attenuated by bone marrow irradiation/reconstitution and thymectomy
- Pathology
6 months
- hypertrophy, fibrosis, and inflammation
1 year
- hypertrophy, fibrosis, and mural thrombi without inflammation

animals. The role of NK cells and T-Ly cells in the chronic phase needs further clarification. Therapies designed may ultimately require efficacy in suppressing both NK and T-Ly responses.

The antigen responsible for stimulating cell-mediated immune responses during the chronic phase in murine myocarditis is unknown. Serial studies in murine CVB-3 infection demonstrate rapid disappearance of viral antigens during the acute phase (Feinstone *et al.*, 1973; Roesing *et al.*, 1979). T-Ly from previously infected CVB-3 mice do not lyse uninfected myofibres, implying that viral antigens do not cross-react with myocardial antigens (Huber *et al.*, 1980). Antigens extracted with hypertonic salt (KCl) extracts from the hearts of CVB-3 infected mice are not neutralized by viral antibodies, yet are capable of inhibiting cell migration of specifically immune peritoneal exudate cells obtained from previously immunized mice (Paque *et al.*, 1978). These results indicate that a novel antigen developed from myocardial tissue as a result of CVB-3 infection, and may serve as the stimulus to further cell-mediated immune reaction. An identical antigen has been detected in baboon hearts following CVB-3 infection (Paque *et al.*, 1981). When virus-specific cytotoxic lymphocytes and autoreactive cytotoxic lymphocytes (a population which preferentially absorb to and lyse uninfected myocytes) are transferred to T-Ly deficient mice, myocarditis could be induced by both cell populations, although the lesions caused by the autoreactive cytotoxic lymphocytes were more necrotizing than those from virus-specific cells (Huber & Lodge, 1984). In this system, it appears that CVB-3-induced myocarditis may also result from autoimmunity to myocyte antigens.

In summary, the acute phase of murine CVB-3 myocarditis is characterized by active viral replication and humoral and monocytic immune responses directed to viral clearance. During this phase, cytotoxic T-Ly and possibly NK cells directed toward a neoantigen or possibly pre-existent myocyte antigens act as the effectors of the chronic myocardial damage. The chronic inflammatory response ultimately leads to a clinical and pathological picture reminiscent of human dilated cardiomyopathy.

Human Coxsackie myocarditis

Evidence that Coxsackie virus causes human myocarditis is limited to isolation of the virus from myocardium as reported in only two patients (Sutton *et al.*, 1967; Longson *et al.*, 1969). A 'moderate order association' (Lerner *et al.*, 1975) of Coxsackie virus B with myocarditis has been made by the correlation of neutralizing antibody titres or isolation of the virus from rectal or nasopharyngeal swabs with acute

myocarditis (Bell & Grist, 1968; Freij *et al.*, 1970; Smith, 1970; Koontz & Ray, 1971; Schmidt *et al.*, 1973; Toshima *et al.*, 1979). The incidence of elevated titres to enteroviruses is greater in patients with chronic congestive heart failure due to primary myocardial disease than in the general population of patients with heart disease of other aetiology (Table III) (Cambridge *et al.*, 1979; Falase *et al.*, 1979; Kitaura, 1981a; Lau, 1982), and the progression from acute CVB myocarditis to a chronic dilated cardiomyopathy has only been described in isolated patients (Burch & Colcolough, 1969; Frenkel, 1972; Rose, 1973; Barson *et al.*, 1981; Morita *et al.*, 1983). Also, in patients without clinical myocarditis, from whom Coxsackie B virus was isolated in the nasopharynx, a high incidence of subsequent chronic congestive heart failure has been documented (Orinius, 1968). Lastly, 25% of patients with serological evidence of Coxsackie viral infection during acute myocarditis develop dilated cardiomyopathy (Sainani *et al.*, 1968).

Table III Evidence linking acute viral myocarditis to dilated cardiomyopathy in man

1.	Elevated enteroviral antibody titres in dilated cardiomyopathy.
2.	Progressive clinical syndrome initiated with viral myocarditis culminating in dilated cardiomyopathy.
3.	High incidence of heart failure on follow-up of patients with nasopharyngeal isolation of Coxsackie B virus in the absence of clinical acute myocarditis.
4.	High incidence of dilated cardiomyopathy on long term follow-up of patients who recovered from acute viral myocarditis.

Since the incidence of myocarditis during Coxsackie B infection is high, and DC low, inferences from the animal model suggest that identification of patients likely to develop a chronic cardiomyopathy lies in the understanding of the regulation of cytotoxic responses. Cell-mediated immune responses in patients with predominantly Coxsackie B myocarditis are augmented (Hori *et al.*, 1982; Maisch *et al.*, 1982). Lymphocytic cytotoxicity against myocardial cells *in vitro* can be detected in 30% of patients with DC (Jacobs *et al.*, 1979). Subtle defects in immunoregulation may identify that subset of patients likely to develop chronic cytotoxic responses following the initial viral infection. In this regard, a defect in suppressor lymphocyte function has been identified in a subset of patients with dilated cardiomyopathy and myocarditis (Eckstein *et al.*, 1982; Fowles *et al.*, 1979). Furthermore, deficient natural killer cell activity has been shown in a similar population (Anderson *et al.*,

1982). Although HLA haplotypes have been associated with idiopathic dilated cardiomyopathy (Anderson *et al.*, 1984), these studies need verification before firm conclusions can be drawn.

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