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Antibodies to Coxsackie B viruses in congestive cardiomyopathy

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SUMMARY Fifty patients with congestive cardiomyopathy have been studied for evidence of previous Coxsackie B virus infection and compared with age- and sex-matched controls who had been admitted to hospital for investigation of other cardiac diseases.

High neutralisation titres ($\geqslant$ 1024) to Coxsackie B viruses were more common among the patients than among the controls. On subdividing the patients according to their length of symptomatic history before study, high titres were more common only in those with a short history ($\leqslant$ 1 year). High titres were more common when there had been a febrile illness at the onset of symptoms.

Endomyocardial biopsies of 18 patients disclosed no evidence of myocarditis, or, in 12 cases, of viral involvement.

Although the evidence remains circumstantial, these results support the theory that Coxsackie B viruses may cause congestive cardiomyopathy and encourage further research into the mechanisms of myocardial cell damage by these viruses.

The causal relation of Coxsackie B viruses to acute myopericarditis is now well established. Though the development of congestive cardiomyopathy occurring after acute viral myocarditis is not well documented, several cases of prolonged cardiac enlargement or dysfunction have been reported (Sainani *et al.*, 1968; Smith, 1970; Levi *et al.*, 1977).

The development of a cardiomyopathy several months after experimental Coxsackie B myocarditis has been observed in mice (Wilson *et al.*, 1969). Microscopically, there was persistent myocardial fibrosis, with small foci of inflammatory cells. After similar infection in hamsters, abnormalities of left ventricular muscle mechanics were still present at a time when histological examination disclosed only scanty focal myocytolysis (Abelmann *et al.*, 1975).

Coxsackie B virus infections are often subclinical, and only a small proportion results in obvious cardiac involvement (Grist *et al.*, 1975). It is therefore possible that chronic congestive cardiomyopathy may develop after an asymptomatic infection. As neutralising antibody titres against Coxsackie B viruses tend to remain raised for many months after acute infection (Grist and Bell, 1974), we have investigated these titres in a group of patients with congestive cardiomyopathy.

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Patients and methods

Fifty patients with congestive cardiomyopathy seen at Hammersmith Hospital between January 1975 and June 1977 were studied. Forty-five of the patients were men and 5 were women. Congestive cardiomyopathy was defined as a chronic disorder of heart muscle of unknown cause or association, characterised by poor systolic function (Goodwin and Oakley, 1972). Cases of specific heart muscle disease or secondary cardiomyopathy were excluded. Though it is recognised that alcohol has an acute depressant effect on left ventricular function, there is no satisfactory evidence that it causes any permanent or irreversible impairment (Goodwin and Oakley, 1972). Patients were not, therefore, excluded from this study if they admitted to abnormally high alcohol consumption. This was the case in 4 patients and the consumption was moderate in a further 4.

The diagnosis in 48 cases was based on haemodynamic evidence at cardiac catheterisation of impaired left ventricular function, together with generalised left ventricular hypokinesia on angio-

graphy. In the remaining 2 cases a clinical diagnosis was confirmed *post mortem*. Coronary arteriograms were performed in 15 cases, and were normal in 14. In 1 patient an isolated lesion in the right coronary artery was detected. This was not considered likely to be responsible for diffuse ventricular hypokinesia (Gau *et al.*, 1972; Dash *et al.*, 1977).

Twelve patients gave a history of an acute febrile illness between 1 and 15 months before initial presentation with congestive cardiomyopathy and between 1 month and 13 years before this study. Pericarditis had been reliably diagnosed 2 months previously in 1 such case. There were symptoms suggestive of pericarditis during the acute illness, occurring 9 and 14 months before this investigation in only 2 other cases. In all cases there was an interval of at least 1 month between the acute febrile illness and the apparent onset of cardiac failure.

Patients with other forms of heart disease, admitted for cardiac catheterisation during the same period, were also studied. Those with known infective or inflammatory disease or with abnormal serum proteins were excluded. The closest age- and sex-matched subject was then selected from this group of patients as a control for each patient with congestive cardiomyopathy. The mean age of the cardiomyopathy patients was 43·2 years and of the controls 42·3 years. The geographical origin of the 2 groups was similarly distributed. Among the cardiomyopathy patients 43 were European, 4 were Asian, and 3 were Negro. Of the controls 42 were European, 6 were Asian, and 2 were Negro. Cardiac failure requiring treatment with diuretics was present in 14 of the controls.

COXSACKIE B NEUTRALISATION TESTS

A micro-neutralisation test system was used for the Coxsackie B antibody studies, with a standard volume of 25 microlitres (Bell and Grist, 1970). The stock Coxsackie B1-6 viruses were grown from strains obtained from the Coxsackie reference laboratory (Public Health Laboratory Service, Epsom) and used at a concentration of 100 tissue culture infective doses per microplate well. A continuous line of green monkey kidney cells (VERO) was used as the indicator cell line at a concentration of 25 000 cells per well. Sera were titrated in doubling dilutions from 1:16 to 1:1024. Cell controls and virus back titrations were performed. Each serum was tested for cytotoxicity. The identity of the virus serotypes was confirmed at weekly intervals. Titres to individual B1-6 viruses were determined. In each subject the highest titre to any of the Coxsackie B viruses was also used for analysis. Serial titres were examined in 17 patients in the congestive cardiomyopathy group. In these cases the titres on the second occasion were used for comparison with the control group. Either the χ^2 test with Yates' correction or the Fisher exact test were used for statistical evaluation of the results.

CARDIAC BIOPSIES

In 18 of the patients with congestive cardiomyopathy, transvenous endomyocardial biopsies (Konno and Sakakibara, 1963) were obtained from the right ventricle by one of us (C.M.O.) during cardiac catheterisation. These were examined histologically. In 12 cases standard isolation techniques in tissue culture of primary monkey kidney cells were used to detect infectious virus in the biopsy material and serial sections taken from the biopsies were examined by electron microscopy for the presence of virus particles.

HLA ANTIGENS

HLA typing was performed on the lymphocytes of 20 of the cardiomyopathy patients (Sachs, 1976).

Results

VIRAL ANTIBODIES

Titres $\geqslant$ 1024 to all the individual Coxsackie B viruses except B_6 were more common in the congestive cardiomyopathy patients than controls; but for no type were numbers sufficiently great to reach statistical significance. High titres to Coxsackie B_6 were not encountered in either group. When the Coxsackie B group was taken as a whole, titres $\geqslant$ 1024 were found in 15 patients with congestive cardiomyopathy compared with 1 control (χ^2 = 12·57, P < 0·0005, Fig. 1).

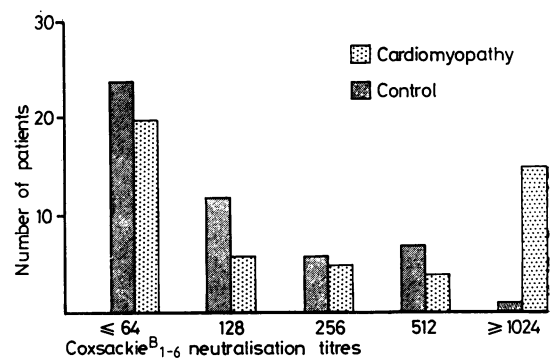

Fig. 1 *The frequency of neutralisation titres to Coxsackie B viruses for the patients with primary congestive cardiomyopathy and for the control patients. For each patient the highest titre to any Coxsackie B virus is taken. Titres $\geqslant$ 1024 are more common in the cardiomyopathy patients (χ^2 = 12·57, P < 0·0005).*

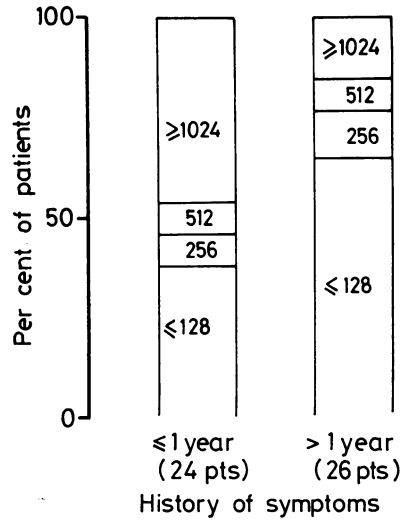

Fig. 2 *The frequencies of the highest titre to any Coxsackie B virus in 50 patients with a primary congestive cardiomyopathy depending on the length of symptomatic history before the virological study. Titres ≥ 1024 are more common when there is a short history of symptoms ($\chi^2 = 4{\cdot}42, P < 0{\cdot}05$). It is only in this group that high titres are more common than in the control patients ($\chi^2 = 11{\cdot}79, P < 0{\cdot}001$).*

The cardiomyopathy patients were divided into 2 groups, depending on whether the study was performed within one year of the onset of symptoms or later. There was a greater proportion with titres ≥ 1024 in the group with the shorter history ($\chi^2 = 4{\cdot}42$, P < 0·05, Fig. 2). Furthermore, it was only in this group that titres ≥ 1024 were significantly more common than in the control group ($\chi^2 = 11{\cdot}79$, P < 0·001). Of the 17 patients in whom serial titres were measured, 3 showed a fourfold or greater rise for a Coxsackie B virus, which in 2 cases rose to ≥ 1024. In these 2 cases, however, there was no history of a febrile illness and the symptoms had lasted for 2 and 3 years, respectively, before study.

A titre of ≥ 1024 was found in 6 of the 12 patients with a history of an acute febrile illness. This was a greater frequency than among the other cardiomyopathy patients but did not reach statistical significance. Serial titres to Coxsackie B viruses were measured in 5 of these patients. None showed a fourfold or greater rise, but in 1 case falling titres from a peak of ≥ 1024 were shown. Only in those patients with a symptomatic history of duration less than 6 months could a statistically significant association of a titre ≥ 1024 with a previous febrile illness be demonstrated (P < 0·02, Table).

Table *Relation between length of history of illness, a previous febrile illness, and high titres (≥ 1024) to Coxsackie B viruses*

Length of history	*Febrile illness*		*No febrile illness*	
	Total	*High titre*	*Total*	*High titre*
All patients	12	6 (50%)	38	9 (24%)
< 1 year	7	5 (71%)	17	6 (35%)
< 6 months*	5	4 (80%)	10	1 (10%)

*P < 0·02, Fisher exact test.

Among the 8 patients who admitted to more than social drinking, a titre of ≥ 1024 was found in 1 and a titre of 512 in a further 1. There was no tendency for these patients to have a longer history of symptoms before study than the other patients with cardiomyopathy.

Antibody titres to the organisms responsible for influenza, measles, psittacosis, and Q fever were also measured using standard techniques. They were infrequently raised and were similar in cardiomyopathy and control patients. In no patient or control was hepatitis B antigen detected.

CARDIAC BIOPSIES

There was no histological evidence of myocarditis or necrosis in the endomyocardial biopsy specimens. No virus was cultured, and electron microscopy of ultrathin sections disclosed no structures identifiable as viral particles.

HLA ANTIGENS

The distribution of HLA antigens determined in 20 cardiomyopathy patients did not differ from that found in a local normal population. Antigens AW19 (A29 and AW30/31) occurred in 4 patients; none of whom had a high titre to a Coxsackie B virus.

Discussion

Reliable diagnosis of cardiac infection by Coxsackie B viruses is often difficult even during an acute illness. It depends largely on showing the presence of virus within the heart or pericardium and simultaneously rising serological titres (Lerner and Wilson, 1973). Neither electron microscopy nor isolation techniques gave any evidence of Coxsackie B virus in our myocardial biopsies. This is consistent with previous immunofluorescence studies both of human biopsies (Kawai, 1971) and of mouse myocardium after Coxsackie infection (Wong *et al.*, 1977a). It contrasts with the high incidence of positive immunofluorescent staining in a series of routine necropsies by Burch *et al.* (1967), whose findings have never been independently confirmed.

According to accepted criteria (Lerner and Wilson 1973) a single high serum antibody titre is an investigation with a low order association for establishing a viral aetiology. The ubiquity of Coxsackie B viruses and the tendency for titres to be boosted heterotypically make interpretation difficult. However, titres of 512 and over are thought to be strongly suggestive of recent infection (Grist and Bell, 1974). The significantly higher titres found in this study in the patients with congestive cardiomyopathy confirm a previous, smaller study (Kawai, 1971). There is no reason to believe that Coxsackie B antibodies are stimulated by the presence of cardiac failure. However, to investigate this possible artefact, we divided the control patients into two groups according to whether they were taking regular diuretics. There was no tendency for controls with cardiac failure by this definition to have higher titres to the Coxsackie B viruses.

The relation to recent infection is supported by the finding that high titres are significantly more common than in the controls only in patients giving a short clinical history. This also explains the failure to show raised viral antibodies in previous studies of patients with congestive cardiomyopathy, in whom the clinical history was usually longer than one year before study (Sanders, 1963; Fletcher *et al.*, 1968). Accurate definition of the length of history in congestive cardiomyopathy is difficult. Clinical presentation is commonly at the onset of cardiac failure, which may, as in one of our cases, occur several months after subclinical cardiomegaly has already developed. However, it is likely that grouping of a large number of patients by length of clinical history will separate with some accuracy those with a more prolonged disease.

It might be argued that some of the cases with a short clinical history before study should be considered as myocarditis rather than chronic cardiomyopathy. The relation of high titres to the history of a previous febrile illness supports such a contention. This is not, however, strong evidence since it is only significant for the small number of patients in whom the febrile illness occurred within 6 months of the virological study. Chronic congestive cardiomyopathy was diagnosed in all cases, since there was evidence of persistent cardiac failure in the absence of any clinical features of recurrent or relapsing myopericarditis. Furthermore, endomyocardial biopsies of 18 of the patients, including 3 of the 5 with a recent febrile illness, did not show any features of myocarditis.

The evidence that Coxsackie B viruses are involved in initiating congestive cardiomyopathy in man remains circumstantial; but our findings encourage research into the mechanisms of myocardial cell damage by these viruses. There is no evidence that serum antibodies to Coxsackie B viruses are themselves of pathogenetic importance. Recent experiments in mice (Wong *et al.*, 1977b) have shown that after Coxsackie B_3 infection cytotoxic 'T' lymphocytes can be raised which will damage myocardial cells *in vitro*. As yet, there is no direct evidence that these cells can mediate chronic myocardial damage.

The major histocompatibility complex in mice has been associated with sensitivity to viral infections (*British Medical Journal*, 1976). In man, the neurotropism of poliomyelitis appears to be partly dependent on the presence of HLA antigens A3 or A7 (Morris and Pietsch, 1973). Antigens of the AW19 group have been associated with 'chronic rheumatic heart disease' in the absence of a history of rheumatic fever; and it has been suggested that these foci might act as conditioning factors converting a viral myocarditis into a severe pancarditis (Ward *et al.*, 1976). We have found no specific focus to be associated with congestive cardiomyopathy and cannot therefore give support to this theory.

The aetiological significance of Coxsackie B group viruses in human congestive cardiomyopathy may lie in their role as a trigger for immunologically mediated damage to the myocardium. Predisposing or circumstantial factors at the time of infection may act synergistically in the initiation and continuation of the pathological changes. There is good evidence in mice that factors such as exercise, age, steroids, and malnutrition conspicuously affect the severity of myocarditis after Coxsackie B infection. The suggestion that some specific HLA foci might act as such a predisposing factor in man is not substantiated in this study. It is possible, however, that there are changes in cell-mediated immune responsiveness in patients with congestive cardiomyopathy and that investigation of this aspect may shed further light on the aetiology of this disease.

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