Cellular immunity in congestive cardiomyopathy

Hypersensitivity to cardiac antigens

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SUMMARY The presence of type IV hypersensitivity to cardiac antigens in 26 patients with congestive cardiomyopathy was sought by two in vitro techniques. Neither test showed a significant group abnormality, but 10 patients did have hypersensitivity to heart antigen, in particular to congestive cardiomyopathic heart antigen. These patients were characterised by worse haemodynamic data and a more rapid and malignant course of the disease than in the rest of the group.

Immunological abnormalities are known to occur in congestive cardiomyopathy, but their role and importance in aetiology have not been determined.^{1,2} The possibility that an insult to the heart such as a viral infection might initiate damage which immunological mechanisms then perpetuate has been suggested.^{3,4} Raised virus titres have been reported in congestive cardiomyopathy without the isolation of virus particles in the myocardium.^{4,5} Animal experiments have shown that acute viral infections of the heart can progress to chronic myocardial damage where evidence of virus is absent after the first few days of infection⁶; T cell function appears to be important in determining the long term outcome of these infections.^{7,8} A viral antigen or an abnormal cardiac antigen exposed by a virus may be responsible for setting up a hypersensitivity reaction which would continue after the infection had disappeared. Type IV hypersensitivity to heart antigens has been reported in a proportion of patients with congestive cardiomyopathy,^{9,10} but whether the abnormality is primary or secondary, or whether those with such apparent autoimmunity comprise only a subset of patients with congestive cardiomyopathy, or whether the tests used to detect hypersensitivity are not sensitive enough is not known.

This study examined the possibility that type IV hypersensitivity to cardiac antigens is an underlying mechanism of damage in congestive cardiomyopathy.

In vitro hypersensitivity to antigen preparations made from a normal heart, two different congestive cardiomyopathic hearts, and a hypertrophic cardiomyopathic heart was assessed in two groups of patients with congestive cardiomyopathy or ischaemic heart disease and in normal healthy controls.

Patients and methods

Twenty six patients with congestive cardiomyopathy diagnosed according to the classification of Goodwin and Oakley¹¹ were studied. All but two patients had undergone cardiac catheterisation, which showed normal coronary arteries and histological compatibility with the diagnosis on endomyocardial biopsy¹²; in the two exceptions, the diagnosis was confirmed at necropsy. For comparison, a group of 26 patients with ischaemic heart disease matched for symptoms using the New York Heart Association classification were studied (Table 1); mean haemodynamic data are also shown in Table 1 for the two patient groups, and there were no significant differences between them (Student's unpaired *t* test). A third group of 18 normal healthy controls were age and sex matched with the cardiomyopathy group.

Type IV hypersensitivity is a cell mediated (or delayed) type of hypersensitivity usually involving T lymphocytes and non-specific inflammatory cells. It was studied by two in vitro techniques: leucocyte migration inhibition and lymphocyte transformation using cardiac muscle preparations as the antigens. Details of the methods are previously described by Lowry *et al*,¹³ and the procedures in this study were identical except for the use of different antigens.

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Table 1 Details of patients

Patient group	No of patients	Mean (SD) age (yr)	Sex (M/F)	NYHA class					Cardiac catheterisation data			
				I	IIa	IIb	III	IV	No of patients	Mean (SD) LVEDP (mm Hg)	Mean (SD) LVESI (ml/m ²)	Ejection fraction (%)
Congestive cardiomyopathy	26	50 (11)	22/4	1	7	6	6	6	24	17 (8)	147 (88)	34 (21)
Ischaemic heart disease	26	55 (8)	22/4	3	6	4	10	3	22	26 (10)	126 (61)	28 (23)
Normal	18	46 (12)	15/3									

NYHA, New York Heart Association; LVEDP, left ventricular end diastolic pressure; LVESI, left ventricular end systolic index.

ANTIGEN PREPARATION

Four different human hearts were used to prepare the antigens: (a) a normal heart obtained within 14 hours of death from a woman who died after trauma; (b) a congestive cardiomyopathic heart (A) confirmed by cardiac catheterisation and biopsy and later at necropsy from a 16 year old boy with a rapidly progressive illness (two months) and no history of preceding viral illness; (c) a congestive cardiomyopathic heart (B) obtained at cardiac transplantation from a 42 year old man with a 15 year history of heart failure which began after a flu-like illness in 1966; and (d) a hypertrophic cardiomyopathic heart from an 18 year old man in whom the diagnosis was made by echocardiography two days before death and confirmed at necropsy (one of the patient's siblings and an uncle had died of hypertrophic cardiomyopathy). The hearts were stored at -70°C before antigen preparation.

A saline soluble extract of each heart was prepared in an identical manner. A piece of ventricle (approximately 100 g wet weight) was thawed and finely sliced and then homogenised in sterile physiological saline for 15 minutes. The mixture was centrifuged at 1600 g for 10 minutes and the supernatant retained. This was filtered through an $8.0\text{ }\mu\text{m}$, a $0.45\text{ }\mu\text{m}$, and finally through a $0.22\text{ }\mu\text{m}$ filter in a sterile 200 ml Millipore membrane filtration unit. Sterility was confirmed by standard culture procedures. Protein concentration of each preparation was measured using a modification of the technique described by Daughaday *et al.*¹⁴ The antigen preparations were stored in aliquots at -70°C until used. A dose-response curve was plotted for each antigen to determine the optimum range of antigen concentration that would detect sensitised cells without causing cytotoxicity. Cytotoxicity was assessed using a viability count: $>15\%$ cell death and clumping of cells was taken to represent cytotoxicity.

STATISTICAL ANALYSIS

The Kruskal-Wallis one way analysis of variance was used to test the significance of difference between the three groups for leucocyte migration inhibition and

lymphocyte transformation.¹⁵ The χ^2 test (or the Fisher exact probability test where numbers were small) was used as a test of significance when inhibited and non-inhibited groups were compared.¹⁵ The Mantel-Haenszel test was used to test the significance of differences between the groups for survival.¹⁶

Results

The leucocyte migration inhibition test did not show significant differences between the three groups when tested with normal heart antigen ($0.99 > p > 0.98$), congestive cardiomyopathic (A) antigen ($0.90 > p > 0.80$), congestive cardiomyopathic (B) antigen ($0.30 > p > 0.20$), or hypertrophic cardiomyopathic antigen ($0.50 > p > 0.30$). A migration inhibition index < 0.80 is usually considered to represent inhibition.¹⁷ Table 2 shows the percentage of patients showing such inhibition for each group and each antigen. Although more of the congestive cardiomyopathic group were inhibited by the normal and congestive cardiomyopathic (A) and (B) heart antigens, none of these differences reached statistical significance.

Not all the patients were tested successfully with all antigens as some died or had operations before testing was complete. The patients were initially tested with normal and congestive cardiomyopathic (A) hearts so the number of results are greater with these antigens. Using these antigens, 10 of 25 congestive cardiomyopathic patients, four of 22 ischaemic heart disease patients, and two of 18 normal controls were

Table 2 Percentage of patients inhibited with heart antigens

Patient group	Antigen			
	Normal heart	CCM (A)	CCM (B)	HCM
Congestive cardiomyopathy	27	28	37	16
Ischaemic heart disease	8	9	13	24
Normal	11	6	21	29

CCM (A) and CCM (B), from two different patients with congestive cardiomyopathy; HCM, hypertrophic cardiomyopathy.

Fig. 1 Individual migration index to congestive cardiomyopathic (A) antigen in patients with congestive cardiomyopathy (CCM) or ischaemic heart disease (IHD) and normal subjects. Mean (SEM) values are shown.

inhibited by one or both antigens (Fig. 1). There seemed to be a cluster of congestive cardiomyopathic patients showing inhibition compared with the other groups, but this did not reach statistical significance. Table 3 compares the mean age, haemodynamic data, and duration of symptoms of the 10 cardiomyopathic patients showing inhibition with normal heart and congestive cardiomyopathic (A) antigens with those of the rest of the group. There was a tendency for the patients showing inhibition to have worse haemodynamic data. Figure 2 shows the difference in survival between the 10 congestive cardiomyopathic patients who showed inhibition and the rest of the group. The difference in survival between the inhi-

bited and non-inhibited cardiomyopathic groups was not significant ($p > 0.1$), but the patient numbers were small.

Seven congestive cardiomyopathic patients were inhibited with congestive cardiomyopathic (A) antigen, and they also tended to have worse haemodynamic data than the rest of the group (Table 3). The mean duration of illness in these seven patients was 27 months compared with 48 months for the remainder of the group. Five of the seven died before the end of the study. The duration of illness for these five was 19 months, and all had been tested with congestive cardiomyopathic (A) antigen within three months of death.

One of the two normal controls who showed inhibition with normal heart antigen was the identical twin brother of one of the congestive cardiomyopathic patients who showed inhibition with both normal heart and congestive cardiomyopathic (A) antigens and who died. One normal control was inhibited with both normal and congestive cardiomyopathic (A) heart antigens. None of the ischaemic heart disease patients were inhibited with more than one antigen.

Testing with congestive cardiomyopathic (B) antigen did inhibit some different patients in the cardiomyopathic group, but there was some overlap with those already showing inhibition with other heart antigens. Finally, testing with hypertrophic cardiomyopathic antigen did not show inhibition predominantly in any one group, and there was overlap with patients showing inhibition with other antigens.

Figure 3 shows the lymphocyte transformation results; two different concentrations of normal heart antigen and congestive cardiomyopathic (A) antigen are represented. The variation in results was wide, but no stimulation occurred with either antigen, and there were no significant differences between the groups. In view of these results, lymphocyte transformation was not attempted with congestive cardiomyopathic (B) or hypertrophic cardiomyopathic antigens.

Table 3 Comparison of data between inhibited and non-inhibited groups

Patient group	Mean (SD) Age (yr)	Mean (SD) LVEDP (mm Hg)	Mean (SD) LEVSI (ml/m ²)	Mean (SD) ejection fraction (%)	Mean (SD) duration of symptoms (mth)	NYHA class				
						I	IIa	IIb	III	IV
Congestive cardiomyopathy:										
Inhibited with normal heart or CCM (A) antigens or both	50 (14)	20 (6)	189 (93)	29 (21)	37 (29)	—	4	—	3	3
Inhibited with CCM (A) antigen only	48 (16)	20 (7)	190 (116)	35 (24)	27 (23)	—	3	—	2	2
Not inhibited with either normal heart or CCM (A) antigens	51 (8)	16 (9)	127 (81)	36 (21)	46 (52)	1	3	6	3	2
Ischaemic heart disease:										
Inhibited with normal heart or CCM (A) antigens	55 (7)	17 (10)	89 (115)	56 (43)		1	1	1	1	—

LVEDP, left ventricular end diastolic pressure; LVESI, left ventricular end systolic index; NYHA, New York Heart Association classification; CCM (A) congestive cardiomyopathic heart A antigen.

Fig. 2 Survival curves for congestive cardiomyopathy patients seen during the past seven years (all), for the 10 patients inhibited with normal heart antigen or congestive cardiomyopathic (A) antigen, or both, and for the rest of the group not inhibited.

Discussion

Lymphocyte transformation and leucocyte migration inhibition have been successfully used as tests of cellular hypersensitivity to various antigens.¹⁸ Inhibition of leucocytes by lymphokines in the presence of tissue antigen suggests presensitisation of the cells, a response to heart tissue that would not be expected in a normal individual. Nevertheless, no test of cellular mechanisms proves that T cells are primarily responsible for tissue damage.¹⁹

Leucocyte migration inhibition has been widely used as a test of hypersensitivity and shown to correlate well with delayed type skin testing,²⁰ for example, in Mantoux positive and negative individuals.²¹ In this study, no skin test was available, whereas in studies with other antigens in which skin testing was

possible a migration index of 0.80 has separated the positive and negative responders^{20 22} and has been applied in similar studies.²³

In this study, neither leucocyte migration inhibition nor lymphocyte transformation to heart antigens showed significant differences between the groups. There did, however, appear to be a group of patients in the cardiomyopathic group who did show leucocyte migration inhibition (migration index <0.80) when compared with the other groups using a normal heart and congestive cardiomyopathic antigens; the numbers did not reach statistical significance but the finding did raise some interesting points. One of the difficulties of this study was that by the very nature of the illnesses, patients in the ischaemic and cardiomyopathic groups died before all testing was complete. The group of 10 congestive cardiomyopathy patients showing inhibition to normal heart antigen and congestive cardiomyopathic (A) antigen had a 50% mortality compared with 11% for the rest of the group. The haemodynamic data suggested that the cardiomyopathy patients showing inhibition had worse ventricular function than the rest of the group. The congestive cardiomyopathy patients inhibited with congestive cardiomyopathic (A) antigen had worse haemodynamic data, a shorter duration of illness, and higher mortality (71%) compared with the remainder of the group. These trends may indicate that patients with congestive cardiomyopathy showing leucocyte migration inhibition to cardiac antigens have a more malignant illness and poorer prognosis. It is not clear whether this is cardiac tissue specific since other tissue preparations were not evaluated in this study, which concentrated on showing an effect of cardiac antigen preparation in the first instance. The few patients with ischaemic heart disease who showed

Fig 3 Lymphocyte transformation results to normal heart antigen and congestive cardiomyopathic (A) (CCM (A)) antigen at two different antigen concentrations (mg/ml) expressed as the mean (SEM) ratio of test to control counts. CCM, congestive cardiomyopathy; IHD, ischaemic heart disease.

inhibition with normal heart or congestive cardiomyopathic (A) antigens were not necessarily those with poor left ventricular function (Table 3) suggesting that such sensitisation is not merely the result of end stage cardiac damage whatever the cause.

These results are similar to those of other groups who have also found small numbers of patients within the cardiomyopathy group showing hypersensitivity to heart antigens.^{10,24} There are several possible explanations for these findings. Differing aetiologies may lead to different antigens being exposed; for example, a virus might predispose to the exposure of a particular antigen so that only those patients sensitised to that particular antigen would show hypersensitivity. It is interesting that the congestive cardiomyopathic (A) heart antigen was from a boy who had a rapidly progressive illness, as had those showing inhibition with his heart. Different congestive cardiomyopathy antigens did not, however, identify separate and distinct groups of patients, but overlap was seen. The nature of the antigens in such preparations of heart muscle was very important. They undoubtedly consist of a mixture of soluble cytoplasmic and membrane proteins. Sterility was achieved by ultrafiltration, and this excluded the bulk of possible antigenic material especially membrane bound antigens. The use of a soluble antigen preparation in any case is less likely to cause inhibition than a particulate antigen.¹⁷ It is possible that the major part of the antigen of prime importance is lost altogether in the preparation. It may be that hypersensitivity occurs terminally, and this would explain why inhibition was often present within three months of death. Also the response to the heart preparation did not seem to be specific to the cardiomyopathy group or indeed to patients with heart disease since even the normal group had individuals showing a positive response. It was of interest that the twin brothers both showed inhibition with normal heart antigens; perhaps it is these people who, with the appropriate stimulus, will proceed to develop congestive cardiomyopathy.

In order to probe these findings further the preparation of a purer and more appropriate antigen will be necessary using both biochemical and immunological methods.

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