Suppressor T lymphocyte function in patients with idiopathic congestive cardiomyopathy

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SUMMARY Suppressor T lymphocyte function was examined in 11 patients with idiopathic congestive cardiomyopathy and in 11 age and sex matched patients with a similar degree of heart failure resulting from ischaemic heart disease. Suppressor T lymphocyte function was also assessed in a control population of 11 normal subjects. Suppressor T lymphocyte function was reduced in both groups of patients with heart failure but not significantly, and a wide range of suppression was demonstrated in all groups.

These data do not support the hypothesis that there is a defect in T lymphocyte function in patients with congestive cardiomyopathy, but they do suggest that there may be a non-specific reduction in T lymphocyte suppressor function associated with heart failure in general.

The aetiology of idiopathic congestive cardiomyopathy is, by definition, unknown and is likely to represent the end stage of several different pathogenetic mechanisms. The possibility that immunological abnormalities may affect the pathogenesis of some cases has received much attention in recent years. There have been reports of impaired suppressor T lymphocyte function in patients with congestive cardiomyopathy and this could be the result of a quantitative or a qualitative abnormality. Such a defect might be determined genetically by predisposing an individual to viral infection, myocarditis, and subsequent development of cardiomyopathy. This hypothesis has not, however, been accepted by all workers and some studies have shown no specific abnormality of T lymphocyte function in congestive cardiomyopathy.

These doubts regarding the role of immunological abnormalities in the pathogenesis of congestive cardiomyopathy prompted a study to determine whether patients with congestive cardiomyopathy have a defect of suppressor T lymphocyte function that is specific to their disease.

Patients and methods

Patients

Eleven patients with idiopathic congestive cardiomyopathy (according to the classification of Goodwin and Oakley) that was confirmed by cardiac catheterisation, coronary angiography, and left ventricular endomyocardial biopsy were studied and compared with eleven individually age and sex matched patients who had congestive cardiac failure of ischaemic origin, also confirmed at cardiac catheterisation. We also studied eleven healthy controls recruited from hospital staff with no personal or family history of ischaemic heart disease.

The severity of congestive cardiac failure and the treatment regimens were similar in the patient groups (table). All patients in the heart failure groups were being treated with digoxin and warfarin. Ejection fractions were determined by volumes derived from end systolic and end diastolic frames acquired at left ventricular cineangiography.

Methods

Lymphocytes were separated from heparinised blood by density gradient centrifugation. Suppressor activity was induced by culturing cells with concanavalin A (con A) at two concentrations—30
Suppressor T lymphocyte function in patients with idiopathic congestive cardiomyopathy

Table Data on the groups of patients and controls that were studied

<table>
<thead>
<tr>
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<th>Congestive cardiomyopathy</th>
<th>Ischaemic heart disease</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr) (mean (SD))</td>
<td>47 (13)</td>
<td>52 (10)</td>
<td>44 (16)</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.32 (0.12)</td>
<td>0.29 (0.12)</td>
<td>—</td>
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<tr>
<td>Average daily dose</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>of frusemide (mg)</td>
<td>69</td>
<td>73</td>
<td>—</td>
</tr>
<tr>
<td>(range 40–120)</td>
<td>(range 40–120)</td>
<td>(range 40–120)</td>
<td>—</td>
</tr>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>taking vasodilators</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

and 10 μg/ml—and incubating them for 48 hours at 37°C in a humidified atmosphere containing 5% carbon dioxide. Parallel cultures in medium alone provided the control cells. The cell division of suppressor cells was inhibited by treatment with mitomycin C (2.5 μg/10⁶ lymphocytes). Three normal healthy adults (responders 1, 2, and 3) were used as the source of responder cells. Lymphocytes from responders were incubated with suppressor cells in medium alone or with phytohaemagglutinin (PHA) 25 μg/ml in ratios of suppressor to responders of 2:1 and 1:1. Cultures were incubated in triplicate for three days at 37°C in a humidified atmosphere containing 5% carbon dioxide. Sixteen hours before harvesting 0.6 μCi of [3H]thymidine was added to each culture and uptake of label was measured as disintegrations/minute (dpm) in a scintillation counter. Percentage suppression was calculated by the formula:

$$100 \times \left(1 - \frac{\text{dpm} \text{ responser cells + PHA Con A treated cells}}{\text{dpm} \text{ responser cells + PHA + control cells}}\right)$$

where dpm = mean uptake by stimulated cultures minus mean uptake by unstimulated cultures.

STATISTICAL ANALYSIS

Analysis of variance was used to assess the statistical significance of differences between the groups; p < 0.05 was taken to indicate statistical significance.

Results

There was a wide variation in the percentage inhibition produced by suppressor cells tested with cells from different responders. This variation was most pronounced in both patient groups (fig 1).

Figure 2 shows the wide range of suppression in all groups; this was more pronounced in the patient groups. Initial experiments indicated that for optimum reproducibility suppressor cells should be induced with concanavalin A; however, use of different concentrations of concanavalin A (30 and 10 μg/ml) made no difference to the range of suppression.
Lowry, Gammage, Gentle, Baynham, Thompson, Littler

Discussion

Abnormalities of cellular immunity are known to occur in heart diseases such as rheumatic fever,12 Chagas's disease,13 and even in association with ischaemic heart disease.14 Whether these abnormalities are pathogenic, however, remains unclear. It has been suggested that the normal cell mediated immunological process is in some way deficient in congestive cardiomyopathy3,15; attention has been directed towards the immunoregulatory function of T lymphocytes and a quantitative or qualitative abnormality of the ratio of helper to suppressor T lymphocytes has been suggested.5–7 Suppressor T lymphocytes regulate helper T lymphocytes and thereby influence antibody production and cell mediated immunity.16 Failure of suppression by suppressor T lymphocytes has been demonstrated6 in patients with congestive cardiomyopathy but not in patients with ischaemic heart disease.5 This finding has led to the hypothesis that B lymphocyte activity, autoantibody formation, and cell mediated immunity may be abnormal in patients with congestive cardiomyopathy.7 A report that lymphoma developed in six of 37 patients who had cardiac transplants because of congestive cardiomyopathy in contrast with none of 54 patients who had cardiac transplants because of ischaemic heart disease was believed to add weight to the suggestion that impaired suppressor T lymphocyte function was of aetiological importance in congestive cardiomyopathy.17 A deficiency of suppressor activity was demonstrated in only one of the six patients with lymphoma, however.

We found a statistically non-significant trend for suppression in patients with congestive cardiomyopathy to be lower than in normal controls; there was a similar trend in patients with ischaemic heart disease when compared with controls, which reached statistical significance on two occasions (at concanavalin A concentrations of 10 and 30 μg/ml with cell ratio of suppressor to responder of 2:1) but with only one of the three responders. No statistically significant difference in percentage suppression was demonstrated between congestive cardiomyopathy and ischaemic heart disease at either concentration or ratio. No significant abnormality of suppressor T lymphocyte function was found in congestive cardiomyopathy and the trend towards lower suppression in patient groups suggests a non-specific abnormality related to illness. Other studies have failed to confirm an abnormality of suppressor T lymphocyte function specific to congestive
cardiomyopathy\(^9\) \(^1\) or ischaemic heart disease.\(^5\)
Low suppressor T lymphocyte activity has been demonstrated, however, in myocarditis.\(^6\) Does this represent a primary immunological defect responsible wholly or in part for pathogenesis or does it merely reflect a secondary phenomenon of heart disease? The possibility that medication or chronic illness per se may affect T lymphocyte function must be considered; this may account for the similarity in results between the two patient groups in this study with a reduction in suppression in both groups.

Immunological response, including suppressor T lymphocyte function, changes with age.\(^19\) The mean ages of study groups are therefore important and sometimes are not given in published reports.\(^6\) Even in a study in which low suppressor activity has been demonstrated in a group of patients with congestive cardiomyopathy, normal individual controls may show variation in suppression from 0% to 100%.\(^6\) Shou et al demonstrated important variation between individuals and in some cases they were unable to demonstrate suppressor activity at all.\(^20\) Use of different responder cells in this study has highlighted this variability even in normal subjects. A study of change in suppressor T lymphocyte activity with time in patients with congestive cardiomyopathy, other heart diseases, and normal controls may clarify the role of suppressor T lymphocyte function and the variability in results of reported studies.

The ratio of suppressor to responder cells is also important—the greater the ratio the better—as is the length of incubation period during generation of suppressor cells.\(^19\) Initial experiments in our laboratory indicated that optimum reproducibility occurred with induction of suppressor cells with concanavalin A and stimulation of responder cells by phytohaemagglutinin.

Using this assay we have not been able to demonstrate an innate suppressor T lymphocyte defect which is specific to congestive cardiomyopathy. Poor cardiac function (resulting from either cardiomyopathy or myocardial ischaemia), however, may be associated with abnormalities of suppressor T cell function.

References