Abnormal cytokine profiles in patients with idiopathic dilated cardiomyopathy and their asymptomatic relatives

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Abstract

Objectives—Immunological abnormalities in idiopathic dilated cardiomyopathy (DCM) include an increase in soluble interleukin(IL)-2 receptor, disease specific cardiac autoantibodies, an HLA-DR4 association, and familial aggregation of disease; however, cytokine profiles have not been defined. Serum concentrations of IL-2, IL-4, IL-10, and IL-12 were measured in patients with DCM (WHO criteria), relatives with asymptomatic left ventricular enlargement (LVE), patients with ischaemic heart failure (IHD), and healthy controls.

Design—Serum from 20 individuals from each of the four groups was assayed for cytokine concentrations by a commercial enzyme linked immunosorbent assay.

Results—IL-2 concentrations were abnormally increased in DCM patients and relatives with LVE. Concentrations of IL-10 were increased in DCM patients. Concentrations of IL-4 and IL-12 were not increased in any of the groups.

Conclusion—These abnormalities may reflect defective/inappropriate T cell function in patients with DCM and in their relatives with LVE.

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Keywords: dilated cardiomyopathy; cytokines; pathogenesis

Idiopathic dilated cardiomyopathy is a chronic heart muscle disease of unknown aetiology characterised by a dilated and poorly contractile left ventricle.[1] It usually affects young individuals, and is the commonest indication in the United Kingdom for cardiac transplantation; this is the only curative treatment but its use has been limited by the supply of donor organs.[2]

Animal models have emphasised the role of the T cell in the induction of autoimmune myocarditis[3] and of cytokines in the maintenance of viral myocarditis,[4] but direct evidence of cellular autoimmunity in human DCM is lacking. Indirect evidence for an immune pathogenesis for this condition includes the presence of abnormal concentrations of soluble interleukin 2 receptor,[5] organ and disease specific autoantibodies by immunofluorescence,[6] abnormal cardiac endothelial major compatibility complex class II antigen expression,[7] a specific human leucocyte antigen association with the DR-4 haplotype,[7 8] and familial aggregation of disease.[9 10] Prospective family screening has identified a subset of asymptomatic first degree relatives with left ventricular enlargement consistent with early DCM[10]; immune abnormalities in these individuals include the presence of cardiac autoantibodies.[11]

Previous studies of cytokine concentrations in DCM have used methods of limited sensitivity and have not defined the Th1/Th2-type of the immune response.[12] T-helper (Th) clones can be classified according to the cytokines that they produce; definitive Th1 cells produce IL-2, IL-12, and interferon γ (IFN-γ) and are important in cellular immunity whereas definitive Th2 cells produce IL-4, IL-10, and IL-13 and predominate in humoral immunity.[13] This classification defines extremes of a spectrum of cytokine production; intermediate clones producing both Th1-type and Th2-type cytokines are termed Th0.

To characterise Th1/Th2-type responses in DCM we measured concentrations of definitive type 1 and type 2 cytokines (IL-2, IL-12, IL-4, and IL-10) in patients with DCM, in relatives with left ventricular enlargement, in patients with ischaemic heart disease, and in healthy controls.

Methods

PATIENTS WITH DILATED CARDIOMYOPATHY: DIAGNOSTIC CRITERIA AND CLINICAL CHARACTERISTICS

The clinical diagnosis of dilated cardiomyopathy was made according to strict criteria as recommended by the World Health Organisation and the National Heart, Lung and Blood Institute.[1 14] All patients had left ventricular dilatation (end diastolic diameter > 2·7 cm/m²)[14] and impaired systolic contraction (left ventricular ejection fraction < 40% or fractional shortening < 25%). Exclusion criteria included: > 50% obstruction of one or more coronary arteries, active myocarditis,[15] specific primary or secondary heart muscle disease, sustained systemic arterial hypertension, isolated right ventricular dilatation, and valve or pericardial disease. Patients with a history of chronic excess alcohol consumption, defined as > 8 units per day for men and > 6 units per day for women, were included.

The study population consisted of 20 consecutive white patients with dilated cardiomyopathy (mean (SD) age 39 (15) years, range 18–63, 15 men) who presented to St George's

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Echocardiographic and exercise characteristics of patients with dilated cardiomyopathy and relatives with left ventricular enlargement (mean (SD))

	Patients (20)	Relatives (20)
LVDD (mm)	66 (12)	58 (4)
LVDD%	144 (26)	122 (6)
Fractional shortening	14 (9)	28 (7)
VO₂ max (ml/min/kg)	18 (6)	38 (11)
VO₂%	57 (24)	101 (26)

LVDD, left ventricular diastolic diameter; LVDD%, percentage of predicted left ventricular diastolic diameter; VO_2 max, peak oxygen consumption during exercise; VO_2%, percentage of predicted peak oxygen consumption during exercise.

Hospital, a referral centre for heart failure and arrhythmia management, between 1989 and 1993. At presentation, seven patients were in New York Heart Association functional class I/II and the remaining 13 were in class III/IV. Patients had been symptomatic for 25 (56) months (range 0–204) before diagnosis, and their echocardiographic and exercise variables are shown in the table. Cardiac catheterisation with selective coronary angiography was performed in all patients; left ventriculography showed a mean (SD) ejection fraction of 25 (11)%. Right ventricular endomyocardial biopsy was performed in 16 patients and showed that six (37%) had established endomyocardial fibrosis and the remainder were normal with no evidence of active myocarditis.

CLINICAL CHARACTERISTICS OF RELATIVES OF PATIENTS WITH DILATED CARDIOMYOPATHY
During prospective screening of families of patients with dilated cardiomyopathy, we identified 20 unrelated and asymptomatic first degree relatives (aged 38 (16) (range 16–68), 14 men) who had left ventricular enlargement (defined as a left ventricular diastolic diameter > 117% of that predicted for age and body surface area[16]). All echocardiograms were performed by one experienced operator. The echocardiographic and exercise variables are shown in the table; the peak oxygen consumption during exercise (VO_2 max) is also presented as a proportion of that predicted for age, sex, and body surface area (VO_2%).[17]

CLINICAL CHARACTERISTICS OF PATIENTS WITH ISCHAEMIC HEART DISEASE
The disease control population consisted of 20 patients with heart failure secondary to ischaemic heart disease (age 66 (8), range

51–78, 15 men). Seven patients had suffered a Q wave myocardial infarction 12 (11) months previously (range 2–31), 15 smoked, and eight had a history of systemic hypertension. All patients were New York Heart Association functional class III/IV and had confirmed coronary artery disease.

The healthy control population comprised 20 consecutive blood donors with no evidence of ischaemic heart disease (age 45 (16), 12 men).

MATERIALS AND IMMUNOLOGICAL METHODS
Serum was stored and frozen at −20°C from all patients and controls before analysis. Serum cytokine concentrations were evaluated by enzyme-linked immunosorbent assay (R&D systems (Minneapolis) for IL-2, IL-4 and IL-12; Serotec (Oxford) for IL-10) performed according to the manufacturer's instructions. Briefly, for IL-2, IL-4, and IL-12 undiluted serum samples were incubated on microtitre plate wells precoated with murine monoclonal antibody against each cytokine for two hours at room temperature. After washing, polyclonal anti-cytokine antibody conjugated to horseradish peroxidase was added to the wells with further incubation for two hours at room temperature. After washing and development with substrate solution containing the chromogen tetramethylbenzidine the absorbance was read at 450 nm and 570 nm. For assessment of IL-10, biotinylated anti-IL-10 antibody conjugate and then streptavidin-horseradish peroxidase were used before the addition of chromogen. In each case duplicate readings were converted into pg/ml using the standard curves generated with each plate. Concentrations were defined as abnormal if they were above the lowest standard concentration used in the assay (IL-2 and IL-4, 31·2 pg/ml; IL-12, 7·8 pg/ml; IL-10, 15·6 pg/ml). Intra-assay variability of three (IL-10 and IL-12) or four (IL-2 and IL-4) known concentrations assayed in replicates of 20 (10 for IL-10) were 6·1%–9·5% for IL-2; 3·9%–7·3% for IL-4; 4·6%–7·7% for IL-10, and 1·1%–1·5% for IL-12.

DEFINITIONS AND STATISTICAL ANALYSIS
Results are provided as mean (SD). Statistical methods used Statview SE software and included analysis of discrete variables by the Chi squared test and continuous variables by analysis of variance. A probability value of less than 0·05 was regarded as statistically significant.

Results
CYTOKINE CONCENTRATIONS IN PATIENTS WITH DILATED CARDIOMYOPATHY
DCM patients had higher concentrations of IL-2 than patients with IHD (46 (37) pg/ml *v* 16 (17) pg/ml, P = 0·002) or healthy controls 17 (22) pg/ml, P < 0·001). Similarly IL-10 concentrations were greater in patients with DCM than in those with IHD (41 (17) pg/ml *v* 4 (3) pg/ml; P < 0·001) or healthy controls 2 (1) pg/ml; P < 0·001) (figs 1 and 2).

Figure 1 Concentrations of IL-2 in patients with dilated cardiomyopathy, relatives with left ventricular enlargement, controls with ischaemic heart failure, and healthy individuals. Serum IL-2 was increased in patients with dilated cardiomyopathy and in many relatives with left ventricular enlargement but only in a few controls. The dotted line is the upper limit of normal for IL-2 (31·2 pg/ml).
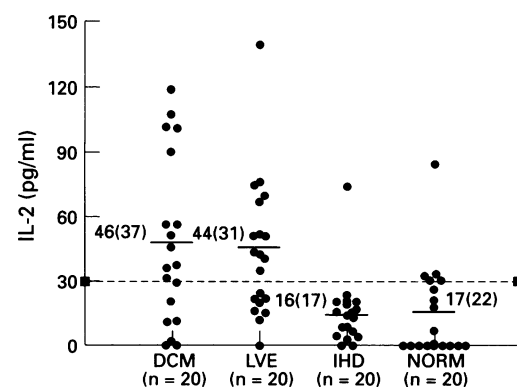
Figure 2 Concentrations of IL-10 in patients with dilated cardiomyopathy, relatives with left ventricular enlargement, controls with ischaemic heart failure, and healthy individuals. IL-10 was increased in most patients with dilated cardiomyopathy, but not in relatives with left ventricular enlargement or in controls. The dotted line is the upper limit of normal for IL-10 (15·6 pg/ml).
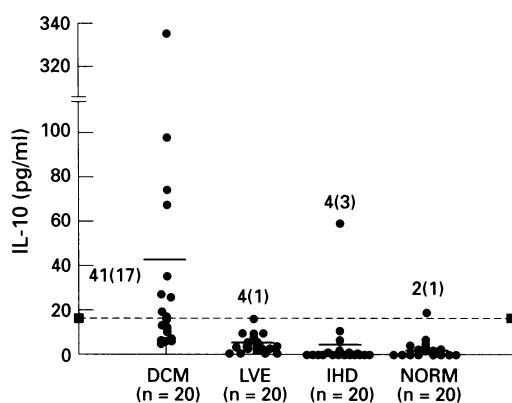
Abnormal concentrations of IL-2 were more common in DCM patients than patients with IHD (13 out of 20 (65%) v 1 out of 20 (5%); P = 0·0001) or healthy controls (3 out of 20 (15%); P = 0·001). Raised IL-10 concentrations were also detected more frequently in DCM patients than IHD patients (10 out of 20 (50%) v 1 out of 20 (5%); P = 0·001) or healthy controls (none; P = 0·0003). Only one of the IHD patients showed raised concentrations of IL-10. Serum concentrations of IL-4 and IL-12 were undetectable in all patients and controls except for one DCM patient, who had raised concentrations of IL-12.

CYTOKINE CONCENTRATIONS IN RELATIVES OF PATIENTS WITH DILATED CARDIOMYOPATHY.
Concentrations of IL-2 were higher in relatives with LVE than in patients with IHD (44 (31) pg/ml v 16 (17) pg/ml; P = 0·001) or healthy controls (17 (22) pg/ml; P = 0·001). IL-2 concentrations were similar in patients with IHD and healthy controls (NS). Raised concentrations of IL-2 (but not any other cytokine) were more frequent in relatives with LVE than in IHD patients (12 out of 20 patients (60%) v 1 out of 20 (5%); P = 0·0002) or healthy controls (3 out of 20 (15%); P = 0·003).

CLINICAL CORRELATIONS
There were no significant associations between concentrations of cytokines and any clinical variable in patients or relatives—including duration or severity of symptoms, ventricular dimensions or contractility, endomyocardial fibrosis, exercise performance, or clinical outcome.

Discussion
In this study abnormal concentrations of IL-2 were found in 65% of patients with dilated cardiomyopathy and in 60% of their asymptomatic relatives with left ventricular enlargement compared with 5% of patients with ischaemic heart disease and 15% of healthy controls. Raised IL-10 concentrations were present in 50% of patients with dilated cardiomyopathy but in only 5% of those with ischaemic heart disease and in none of the relatives or healthy controls. These observations suggest that an increase in these cytokines is

specific to dilated cardiomyopathy and not an epiphenomenon related to heart failure per se. This supports the concept that left ventricular enlargement may represent early DCM.

Our findings are consistent with previous reports of increased concentrations of soluble interleukin-2 receptor in about a third of patients with DCM[5] as well as in patients with myocarditis.[18] T-cell activation is accompanied by the release into the circulation of interleukin-2 which acts via a specific receptor on the cell surface, but concentrations of cytokine receptor in the serum are only an indirect assessment of T cell function. A recent study found that abnormal concentrations of IL-2 were present in only 4% of a cohort of Asian patients with DCM, but not in the ischaemic heart disease patients or healthy controls[12]; the assay used in that study was of low sensitivity and patients with a comparable degree of heart failure secondary to ischaemic heart disease were not evaluated. Experimental models of autoimmune myocarditis have emphasised the role of T cells in induction of disease[3]; the observation in the present study of an increase in both IL-2 and IL-10 but not IL-4 or IL-12 in a high proportion of patients with DCM in comparison with controls is compatible with a Th0-type profile, in which there is an increase in cytokine concentrations associated with both Th1-type (gamma-IFN, IL-2, and IL-12) and Th2–type (IL-4, IL-5, IL-6, and IL-10) immune function.

Left ventricular dysfunction was common among the asymptomatic relatives of patients with DCM in whom it may represent early disease.[9][10] An immune component to disease pathogenesis has been recently suggested by the detection of an organ and disease specific anti-heart antibody by immunofluorescence in 30% of these relatives, particularly those with left ventricular enlargement.[11] Circulating cytokines have not been previously evaluated in asymptomatic relatives of patients with DCM, but the description of raised IL-2 in 50% of individuals with left ventricular enlargement who are otherwise asymptomatic supports the idea of a generalised activation of lymphocytes.

The presence of both IL-2 and IL-10 in the symptomatic DCM patients has two possible interpretations. One explanation might be that a dominant Th1-type (cell mediated) response is protective against the induction of disease or that a switch to a mixed Th1/Th2 (Th0)-type response might contribute to the development of clinically overt disease; we do not, however, show data to prove that this phenomenon occurs longitudinally. Cytokine switching, suggesting a change from Th1 to Th2 immunodominant states with disease progression, has been reported in several infections such as tuberculosis, leprosy, and leishmaniasis as well as autoreactive states including systemic lupus erythematosus and graft versus host disease.[13][19] Conversely, organ specific autoimmune disorders such as rheumatoid arthritis[20] and multiple sclerosis[21] have been associated with a Th1-type profile, although other data suggest an increase in Th0-type ·

responses in multiple sclerosis and myasthenia gravis.[22]

It is possible that a Th1-type immune response as suggested by the occurrence of high serum IL-2 concentrations shown in DCM patients in this study may be either protective or pathogenic. The subsequent development of raised IL-10, which is consistent with a Th2 type response, could be pathogenic owing to down-regulation of antigen presentation by monocyte/macrophages and/or to reduced production of IFN-g, which is a powerful immunomodulator, and anti-viral Th1 type cytokine. Coxsackie B3 virus is an important cause of myocarditis and has been associated with monocyte/macrophage induced inflammatory responses which may in themselves be responsible for the raised IL-10 concentrations in this study.[23] Nevertheless, abnormal cytokine mediated immune responses to this virus may be clinically relevant in the pathogenesis of DCM. Failure of an appropriate immune switch to T helper (Th2-type) cells producing IL-4 at the onset of clinically significant disease in DCM could also lead to IL-10 production; this explanation would be consistent with the finding in the present study of an absence of detectable IL-4 in any of the patients.

Raised concentrations of IL-10 may have been produced by monocyte/macrophage cells[24]—if these cells were activated by a different pathogenic mechanism in DCM patients. In the absence of direct measurements of Th-1 or Th-2 cytokine profiles in isolated lymphocyte populations (such as by fluorexene activated cell sorter (FACS) analysis of intracellular cytokines), this remains a strong possibility. We are addressing these issues by longitudinally studying serum and isolated lymphocyte analysis of DCM patients and their asymptomatic relatives with left ventricular enlargement. Quantitative analysis of mRNA of different cytokines from different cell types as well as cardiac tissue, in addition to in situ hybridisation analysis of cardiac biopsies may clarify these issues. Further studies may suggest a therapeutic strategy for stimulating or inhibiting immunoregulatory cytokines and their antagonists in DCM.

In conclusion, DCM is associated with an activated immune response involving both IL-2 and IL-10, whereas IL-2 was raised only in the serum of asymptomatic relatives with LVE. The data do not prove a causal or protective role for IL-2 or IL-10 in DCM, but they do strengthen support for an immune pathogenesis in this disease. Longitudinal study of DCM patients and their relatives may permit exploration of specific immunomodulatory treatments.

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