Elevated serum levels of soluble interleukin-2 receptor, neopterin and β-2-microglobulin in idiopathic dilated cardiomyopathy: relation to disease severity and autoimmune pathogenesis

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Abstract

Background: It has not been assessed whether high levels of soluble interleukin 2 receptor (sIL-2R), neopterin and β-2 microglobulin in idiopathic dilated cardiomyopathy reflect heart failure severity and/or an active autoimmune process. The aim of this study was to relate serum levels of these markers to clinical and autoimmune features. Methods: We studied 60 patients with idiopathic dilated cardiomyopathy, 67 controls with ischemic heart failure and 34 normals. Results: Abnormal levels of sIL-2R, but not of neopterin and β-2 microglobulin, were more frequent in idiopathic dilated cardiomyopathy than in ischemic patients (35% vs. 16%; \( P = 0.02 \)) or in normals (35% vs. 12%, \( P = 0.01 \)); mean sIL-2R levels were, however, similar in idiopathic dilated cardiomyopathy and ischemic heart failure (842 ± 75 vs. 762 ± 93 U/ml, \( P = \text{NS} \)). In idiopathic dilated cardiomyopathy abnormal levels of sIL-2R were associated with lower peak oxygen consumption (\( P = 0.008 \)), higher neopterin and HLA class II expression in the myocardium (\( P = 0.02 \)), but were unrelated to cardiac autoantibody status or titer. In addition, abnormal levels of neopterin were associated with adverse prognosis and higher β-2 microglobulin; abnormal levels of β-2 microglobulin with lower echocardiographic percent fractional shortening, higher sIL-2R and higher neopterin. Conclusions: There is no convincing evidence that abnormal sIL-2R, neopterin and/or β-2 microglobulin are disease-specific markers of idiopathic dilated cardiomyopathy. The lack of association with cardiac autoantibodies suggests that these abnormalities are mainly related to heart failure severity rather than autoimmune pathogenesis. In keeping with this view, high levels of sIL-2R, neopterin and/or β-2 microglobulin identified a subset of idiopathic dilated cardiomyopathy patients with advanced disease and poor prognosis. © 2001 European Society of Cardiology. All rights reserved.

Keywords: Dilated cardiomyopathy; Cellular immunity; Heart failure

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1. Introduction

Recent evidence suggests that idiopathic dilated cardiomyopathy (IDC) may be an autoimmune disorder in a patient subset [1–11]. Elevated soluble interleukin-2 receptor (sIL-2R) levels represent markers of T lymphocyte activation and have been reported in IDC [8] as well as in a variety of autoimmune disorders [12–15] and in allograft rejection [16]. Neopterin is secreted by activated monocyte/macrophages; interferon-γ (IFN-γ), produced by T helper lymphocytes, is the primary inducer of neopterin synthesis [17,18]. High levels of neopterin correlate with increased tumor necrosis factor (TNF-α) secretion, which is also mainly due to activated macrophages [17–19]. β-2 microglobulin is a marker of HLA class I antigen turnover. IFN-γ upregulates cell surface expression and turnover of HLA molecules; this results in raised serum levels of β-2 microglobulin [20]. Elevated levels of neopterin and β-2 microglobulin have been reported in states of cellular immune activation, including infectious [17,20], chronic inflammatory, lymphoproliferative and autoimmune disorders [17,20–22] and in IDC [23–25]. Peripheral immune activation is also recognized in advanced heart failure, irrespective of its etiology, with the majority of studies demonstrating an increased TNF-α production [19,26–29]. Previous works in IDC have not specifically addressed the effect of heart failure per se on sIL-2R, neopterin or β-2 microglobulin levels [8,23–25].

The aim of this study was to clarify whether high sIL-2R, neopterin and β-2 microglobulin in IDC represent markers of autoimmune involvement or of non-specific inflammation in advanced heart failure. To test the disease-specific association with IDC, we measured these markers in IDC as well as in heart failure due to coronary artery disease, and in healthy subjects. In addition, to assess the clinical significance of peripheral cellular immune activation markers in IDC, their levels were related to clinical indices, to cardiac autoimmune serology [3,5,9], and to HLA and adhesion molecule expression on endomyocardial biopsy [10].

2. Methods

2.1. Study patients

Sixty consecutive IDC patients (mean age 43 ± 13 years, range 12–74, 47 male), were studied. The diagnosis of IDC was made in the presence of left ventricular dilatation and impaired systolic contraction [1]. Exclusion criteria included: ≥ 50% obstruction of one or more coronary arteries, myocarditis according to the Dallas criteria [30], specific cardiomyopathies, sustained systemic arterial hypertension, isolated right ventricular dilatation and valvular or pericardial disease. None of the 60 patients had inflammatory disease involving other organs, or cancer. All patients underwent selective coronary arteriography, left ventriculography and right ventricular endomyocardial biopsy. Patients with a history of chronic excess alcohol consumption in whom left ventricular dilatation persisted despite abstinence from alcohol were included in the study. Patients had been symptomatic for 37 ± 47 months (range 0–204); 31 patients were in New York Heart Association (NYHA) functional Class I/II and 29 in Class III/IV. Echocardiographic left ventricular end-diastolic dimension was 69 ± 9 mm, fractional shortening 13 ± 7% and left ventricular ejection fraction 25 ± 9%. Peak oxygen consumption (\( V_{O_2} \)) during metabolic exercise testing was 22 ± 11 ml/kg per min. Patients were followed for 25 ± 21 months (range 1–101) during which time 25 patients developed progressive heart failure or died; the others remained clinically stable or had symptomatic improvement.

Serum used for assessment of sIL-2R levels was obtained from all 60 IDC patients at the time of diagnosis; neopterin and β-2 microglobulin were assessed at a later stage, based upon serum availability, in 39 and 38 of them, respectively. Fourteen IDC patients who had evaluation of neopterin and β-2 microglobulin had poor prognosis (death or development of progressive heart failure) and 25 remained stable.

Control sera were obtained from 34 healthy subjects (mean age 42 ± 12 years, range 14–70, 28 male) and from 67 patients with chronic heart failure secondary to documented ischemic heart disease (mean age 57 ± 10 years, 17–76 range, 56 male). In 27 of the 34 normal subjects enough serum was available for neopterin and β-2 microglobulin testing.

2.2. Assay for sIL-2R

sIL-2R was measured by enzyme linked immunosorbent assay (ELISA) (T-cell Diagnostics Cellfree system, UK). The normal range of sIL-2R using this technique has been established by the manufacturer as < 913 U/ml; this cut-off was the mean + 2 S.D. the levels observed in 42 healthy individuals. The detection limit of the assay was 24 U/ml. In brief, serum was added to an ELISA plate pre-coated with monoclonal antibody against interleukin-2 receptor followed by an enzyme-labeled anti-sIL-2R monoclonal antibody. The resulting mixture was then incubated for 3 h at room temperature after which unreacted products were removed by washing in phosphate buffered saline/surfactant solution. Anti-sIL-
2R antibody bound to sIL-2R was then detected by adding 0-phenylenediamine which resulted in the generation of a coloured product which was quantified by measuring the spectrophotometric absorbance at 490 nm after addition of a stop solution. For each assay standard curves were constructed by assessing sIL-2R calibration of six known concentrations. In our laboratory, intra assay variability (assessed as the mean coefficient of variation between duplicate wells) was 3.4%, and the inter assay variability was 6.4%.

2.3. Assays for neopterin and β-2 microglobulin

Neopterin levels were assessed by radioimmunoassay (Hennig, Berlin, Germany). The manufacturer has established the normal range for this assay as being ≤ 10 nmol/l based on the mean + 2 S.D. of the levels documented in 76,000 healthy blood donors. The detection limit of the assay was 0.3 nmol/l. Briefly, 50 μl of serum sample was incubated with 100 μl of 125I radio labeled anti-neopterin antiserum. The tubes were mixed and incubated at room temperature for 1 h in the dark. Tubes were centrifuged, decanted and the resulting pellet was counted in a gamma counter for 1 min. For each assay standard curves were constructed by assessing neopterin calibration of six known concentrations. All standards and samples were analyzed in duplicate. In our laboratory, intra assay variability was 2.5% and inter assay variability was 0.7%.

β-2 Microglobulin was measured by ELISA (Pharmacia, Milton Keynes, UK). The normal range for this assay has been established by the manufacturer as being ≤ 2.5 mg/l for individuals > 60 years and ≤ 2.0 mg/l for < 60 years, defined as the 95th centile of values identified in 60 healthy blood donors. Twenty-five microliters of sample (standard or control) was added to an ELISA plate pre-coated with monoclonal antibody against β-2 microglobulin followed by 100 μl of an enzyme-labeled anti-β-2 microglobulin monoclonal antibody. The resulting mixture was then incubated for 1 h at room temperature with agitation. Unreacted products were removed by washing in phosphate-buffered saline-Tween solution. Anti-β-2 microglobulin antibody bound to β-2 microglobulin was then detected by adding 200 μl of the development solution containing the enzyme substrate which resulted in the generation of a coloured product which was quantified by measuring the spectrophotometric absorbance at 405 nm after addition of a stop solution. For each assay standard curves were constructed by assessing β-2 microglobulin calibration of six known concentrations. In our laboratory, intra assay variability was 9% and inter assay variability was 20%.

2.4. Cardiac antibody testing by indirect immunofluorescence (IFL) and ELISA

All 60 samples from IDC patients were tested at 1:10 dilution on 4-μm-thick unfixed fresh frozen cryostat sections of blood group O normal human atrium and skeletal muscle [3,5]. Cardiac antibody titers were measured by doubling dilutions of sera in phosphate-buffered saline. Antibody patterns were classified as reported [3,5]. Two sera were used as standard positive (antibody titer 1:40) and negative controls and titrated in every assay. The intensity of immunofluorescence (IFL) of the positive standard at 1:40 dilution was used as the threshold for positivity. All sera tested at 1:10 dilution were read blindly against these standards. An additional positive control serum was titrated to assess reproducibility [5]. Endpoint titers for this serum were reproducible within one double dilution in all assays.

The ELISA method for detection of anti-α myosin antibodies has been previously detailed [9]. The α-(atrial-specific) myosin samples were prepared as described [4]. Briefly, ELISA plates (Immulon 1; Dynatech, W. Sussex, UK) were coated with sequential duplicates of 100 μl purified human α-myosin at a concentration of 5 μg/ml. In 54 of the 60 patients with IDC enough serum was available for ELISA testing; sera were diluted at 1:320 in phosphate-buffered saline (Sigma, UK) containing 0.1% Tween 20 and 1% bovine serum albumin. Absorbance was assessed using a Pasteur Diagnostics ELISA reader at 450 nm. All antibody levels are expressed as mean absorbance at 450 nm (S.E.M.). The upper limit of normal for the assay was defined as 2 S.D. above the mean value obtained from normals.

2.5. HLA and ICAM-1 expression by double IFL

Serial 4-μm frozen cryostat sections of endomyocardial biopsies from the IDC patients were fixed in acetone for 3 min and then incubated for 30 min with mouse monoclonal antibodies to HLA Class I, Class II (DR, DP, DQ), ICAM-1, pan T lymphocyte and macrophage markers [10]. Mouse anti-thyroglobulin monoclonal antibody was used as a control. The sections were subsequently washed for 15 min in phosphate-buffered saline and incubated for 25 min with affinity purified fluorescein isothiocyanate conjugated goat antibodies to mouse IgG (gamma-chain specific) (Southern biotechnology, Birmingham, USA). As a third layer rabbit anti-human immunoglobulin to Factor VIII (marker for endothelial cells) (Dakopatts, Denmark) was applied for 25 min; sections were again washed, and the reaction was revealed after 20 min incubation with tetraethylrhodamine isothiocyanate.
conjugated goat anti-rabbit immunoglobulin (Nordic Laboratories, UK).

A Zeiss III photomicroscope (Zeiss, New York, NY) with ultraviolet epillumination and equipped with filters for two-color fluorescence was employed. For positive HLA and ICAM-1 molecule expression on endothelial cells, vessels were examined under alternate filters for green and red IFL and the visual observation was completed by double exposure photography [10]. The IFL intensity was graded as negative, weak, positive and strong positive.

2.6. Statistical analysis

Baseline measurements in the patient section are presented as mean ± standard deviation. All data in the result section and tables are expressed as mean ± standard error (S.E.) for continuous variables and as frequency for categorical variables. Continuous variables were compared among the study groups by analysis of variance ANOVA. Quantitative data were correlated by linear regression analysis. Categorical variables were compared through \( \chi^2 \) analysis or Fisher’s exact test as appropriate. A probability value of less than 0.05 was taken as being of statistical significance.

3. Results

3.1. Levels of sIL-2R, neopterin and \( \beta \)-2 microglobulin in the study patients

Fig. 1 shows the scattergram distribution of the individual data points of each patient for sIL-2R, neopterin and \( \beta \)-2 microglobulin. Mean sIL-2R levels were higher (842 ± 75 vs. 544 ± 53 U/ml, \( P = 0.007 \)) and abnormally elevated levels were more frequent in IDC compared to healthy subjects (21/60, 35% vs. 4/34, 12%, \( P = 0.01 \)). IDC patients had similar mean sIL-2R levels than those with ischemic heart disease (842 ± 75 vs. 762 ± 93, \( P = \text{NS} \)), although abnormally elevated levels were more frequent in IDC compared to ischemic heart disease (21/60, 35% vs. 11/67, 16%, \( P = 0.02 \)). There was no difference in sIL-2R levels in patients with ischemic heart failure compared to healthy subjects and the proportion of individuals with elevated levels was similar in both groups. There was no difference between mean neopterin levels in IDC, disease or normal controls (9.5 ± 1.5; 8.1 ± 0.6 and 6.0 ± 0.4, respectively, \( P = \text{NS} \)); the frequency of abnormal results was also similar (9/39, 23%; 10/67,15% and 2/27,7% respectively, \( P = \text{NS} \)). Conversely both the mean level and the proportion of abnormal results for \( \beta \)-2 microglobulin were higher in ischemic heart disease compared to IDC or normals (2.5 ± 0.1; 2.0 ± 0.2 and 1.9 ± 0.1 respectively, \( P = 0.006 \); and 28/67, 42%, 11/38, 29% and 3/27, 11%, \( P = 0.005 \), respectively).

3.2. sIL-2R, neopterin and \( \beta \)-2 microglobulin in IDC: correlation with clinical and immunological features

Fig. 2 shows significant correlation of the exercise data in IDC with some of the immunological markers by linear regression analysis. The other clinical and immunological features in IDC patients with and without abnormal sIL-2R, neopterin and \( \beta \)-2 microglobulin are shown in Tables 1–3, respectively. In IDC abnormal sIL-2R levels correlated with lower peak \( V_O_2 \) during exercise (\( P = 0.008 \)) (Fig. 2, top graph) and were associated with higher neopterin levels (\( P = 0.04 \)) (Table 1); sIL-2R levels tended to be higher in patients with lower %fractional shortening and serum sodium (\( P = 0.06 \)) (Table 1). In addition sIL-2R levels correlated with both \( \beta \)-2 microglobulin (\( r = 0.4, P = 0.01 \)) and neopterin (\( r = 0.4, P = 0.02 \)) (not shown). Abnormal neopterin results were associated with higher \( \beta \)-2 microglobulin levels (\( P = 0.0001 \)) (Table 2). The proportion of IDC patients who later died or developed progressive heart failure was higher among those with abnormal neopterin (77%) compared to those in whom neopterin was normal (27%, \( P = 0.02 \)) (Table 2). Levels of neopterin correlated with those of \( \beta \)-2 microglobulin (\( r = 0.7, P = 0.0001 \)) (not shown). Abnormal \( \beta \)-2 microglobulin results were associated with higher sIL-2R (\( P = 0.03 \)), higher neopterin (\( P = 0.0001 \)) and lower fractional shortening (\( P = 0.01 \)) (Table 3). Eight (53%) patients with NYHA III/IV symptoms had abnormal \( \beta \)-2 microglobulin compared to 3 (14%) of those with milder (NYHA I/II) symptoms (\( P = 0.02 \)).

IDC patients with and without abnormal sIL-2R, neopterin and \( \beta \)-2 microglobulin levels had similar frequencies of cardiac antibodies by IFL (\( P = \text{NS} \)); mean anti-myosin antibody titers by ELISA were also similar (Tables 1–3). In addition, mean sIL-2R, neopterin and \( \beta \)-2 microglobulin levels did not differ (\( P = \text{NS} \)) in patients with and without cardiac antibodies by IFL (820 ± 130 vs. 861 ± 85 U/ml; 8.3 ± 0.9 vs. 7.2 ± 0.7 nmol/l; 1.9 ± 0.2 vs. 1.7 ± 0.2 mg/l, respectively) or by ELISA (765 ± 97 vs. 886 ± 111 U/ml; 8.0 ± 0.7 vs. 7.4 ± 1.0 nmol/l; 2.0 ± 0.2 vs. 1.8 ± 0.2 mg/l, respectively). Increased HLA class II (-DR and -DP) on coronary endothelium and on myocardial interstitial cells correlated with high sIL-2R serum levels (\( P = 0.04 \)) (not shown) and lower peak \( V_O_2 \) (\( P = 0.02 \)) (Fig. 2, middle graph). High ICAM-1 ex-
pression on coronary endothelium and on myocardial interstitial cells tended to be correlated with high sIL-2R levels \((P = \text{NS}, \text{not shown})\) and was related with lower peak \(V_{O_2}\) \((P = 0.03)\) (Fig. 2, bottom graph). No significant associations were found between HLA or ICAM-1 expression and serum neopterin or \(\beta-2\) microglobulin levels.

4. Discussion

The pattern of sIL-2R, neopterin and \(\beta-2\) microglobulin levels that we observed in IDC does not provide convincing evidence for disease-specificity. In fact, we failed to find an association between neopterin...
and β-2 microglobulin and IDC; although a greater proportion of IDC patients had abnormally high sIL-2R compared to disease controls, mean levels were similar in IDC and ischemic heart failure. Our sIL-2R results were entirely in keeping with previous studies [8,31,32]. However, the interpretation of such findings was varied [8,31,32]. Those workers who compared mean values but did not assess frequencies of abnormal sIL-2R results, concluded that there was no disease-specific association with IDC [31,32]. Conversely, Limas et al, who found, similar to us, a higher frequency of abnormal sIL-2R levels but lack of difference in mean values, concluded in favor of the disease-specificity for IDC [8]. The latter interpretation would be in agreement with the lack of IL-2/sIL-2R abnormalities in severe heart failure due to coronary artery disease or hypertension described in one previous study [33] and with the disease-specific cytokine profiles observed in IDC by Marriott et al. [34] but in contrast with recent work where peripheral cytokine activation was independent of the heart failure etiology [35].

In keeping with this report [35], in the present study we provide further evidence suggesting that high sIL-2R, neopterin and β-2 microglobulin are likely to represent markers of immune activation in advanced heart failure, irrespective of its etiology, and, possibly, of poor prognosis. First of all, in our study abnormal sIL-2R levels in IDC were associated with lower peak \( \bar{V}O_2 \), an objective assessment of heart failure severity [27] as well as ejection fraction. [8] Secondly, we found that abnormal neopterin levels were associated with the development of progressive heart failure and/or death, and abnormal β-2 microglobulin with lower %fractional shortening. Thirdly,

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normal sIL-2R (n = 39)</th>
<th>Elevated sIL-2R (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41 ± 2</td>
<td>45 ± 3</td>
<td>0.30</td>
</tr>
<tr>
<td>Duration of symptoms (months)</td>
<td>40 ± 7</td>
<td>32 ± 12</td>
<td>0.70</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>70 ± 2</td>
<td>70 ± 2</td>
<td>1.00</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>15 ± 1</td>
<td>11 ± 1</td>
<td>0.06</td>
</tr>
<tr>
<td>Angiographic LV ejection fraction</td>
<td>24 ± 2</td>
<td>26 ± 3</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum Sodium (mmol/l)</td>
<td>138 ± 2</td>
<td>131 ± 3</td>
<td>0.06</td>
</tr>
<tr>
<td>D/PHF (%)</td>
<td>13 (33%)</td>
<td>12 (57%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cardiac antibody positive by IFL (%)</td>
<td>46%</td>
<td>48%</td>
<td>0.91</td>
</tr>
<tr>
<td>Anti-myosin antibody ELISA titers</td>
<td>310 ± 44</td>
<td>287 ± 43</td>
<td>0.73</td>
</tr>
<tr>
<td>Neopterin (nmol/l)</td>
<td>6.7 ± 0.6</td>
<td>9.1 ± 0.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values expressed as mean ± standard error (S.E).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Normal neopterin (n = 30)</th>
<th>Elevated neopterin (n = 9)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41 ± 2</td>
<td>40 ± 5</td>
<td>0.90</td>
</tr>
<tr>
<td>Duration of symptoms (months)</td>
<td>40 ± 9</td>
<td>19 ± 15</td>
<td>0.30</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>69 ± 2</td>
<td>69 ± 4</td>
<td>0.95</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>15 ± 1</td>
<td>10 ± 2</td>
<td>0.08</td>
</tr>
<tr>
<td>Angiographic LV ejection fraction</td>
<td>27 ± 2</td>
<td>22 ± 6</td>
<td>0.40</td>
</tr>
<tr>
<td>Peak oxygen consumption (ml/Kg/min)</td>
<td>24 ± 2</td>
<td>17 ± 4</td>
<td>0.20</td>
</tr>
<tr>
<td>Serum Sodium (mmol/l)</td>
<td>138 ± 2</td>
<td>135 ± 6</td>
<td>0.50</td>
</tr>
<tr>
<td>D/PHF (%)</td>
<td>8 (27%)</td>
<td>7 (77%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cardiac antibody positive by IFL (%)</td>
<td>42%</td>
<td>40%</td>
<td>0.94</td>
</tr>
<tr>
<td>Anti-myosin antibody ELISA titers</td>
<td>371 ± 53</td>
<td>385 ± 88</td>
<td>0.91</td>
</tr>
<tr>
<td>β-2 microglobulin (mg/l)</td>
<td>1.7 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\( ^{a} \) Values expressed as mean ± S.E.
Table 3
Levels of β-2 microglobulin in patients with IDC: relationship to clinical features and autoantibody status

<table>
<thead>
<tr>
<th></th>
<th>Normal β-2 microglobulin (n = 27)</th>
<th>Elevated β-2 microglobulin (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40 ± 3</td>
<td>41 ± 4</td>
<td>0.90</td>
</tr>
<tr>
<td>Duration of symptoms (months)</td>
<td>38 ± 10</td>
<td>35 ± 18</td>
<td>0.90</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>69 ± 2</td>
<td>69 ± 4</td>
<td>0.90</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>16 ± 1</td>
<td>10 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td>Angiographic LV ejection fraction</td>
<td>29 ± 2</td>
<td>19 ± 5</td>
<td>0.08</td>
</tr>
<tr>
<td>Peak oxygen consumption (ml/kg/min)</td>
<td>24 ± 2</td>
<td>19 ± 5</td>
<td>0.40</td>
</tr>
<tr>
<td>Serum Sodium (mmol/l)</td>
<td>139 ± 2</td>
<td>135 ± 4</td>
<td>0.30</td>
</tr>
<tr>
<td>D/PHF (%)</td>
<td>8 (30%)</td>
<td>7 (64%)</td>
<td>0.051</td>
</tr>
<tr>
<td>Cardiac antibody positive (%)</td>
<td>42%</td>
<td>33%</td>
<td>0.65</td>
</tr>
<tr>
<td>Anti-myosin antibody ELISA titers (± SE)</td>
<td>356 ± 65</td>
<td>386 ± 52</td>
<td>0.77</td>
</tr>
<tr>
<td>sIL-2R (U/ml)</td>
<td>733 ± 90</td>
<td>1117 ± 163</td>
<td>0.03</td>
</tr>
<tr>
<td>Neopterin (mmol/l)</td>
<td>6.4 ± 0.5</td>
<td>10.7 ± 1.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± S.E.

high sIL-2R in our IDC patients correlated with increased neopterin and β-2 microglobulin, suggesting that all of these were abnormal in a patient subset. This has been previously suggested [24,25,32], although the present work is, to our knowledge, the first where all three markers were measured and compared in IDC, ischemic heart failure and in normals. Finally, we observed that high HLA class II and ICAM-1 expression on coronary endothelium and on cardiac interstitial dendritic cells in IDC was associated with high sIL-2R, and with lower peak VO₂. The elevation of sIL-2R and neopterin is a marker of increased secretion of IL-2 and TNF-α respectively, that are both potent inducers of HLA and adhesion molecules on vascular endothelium [15]. Thus the correlation between expression of these molecules in the myocardium and high sIL-2R in the periphery is not surprising. The association between immune activation and poor prognosis is unclear, but may relate to cytokine-induced nitric oxide (NO) independent and NO-dependent negative inotropic effects and, possibly, myocyte death by apoptosis [26–28]. In keeping with this, recent evidence has been produced for increased TNF-α [27] and inducible NO synthase [37,38] expression and for apoptosis [39,40] in the myocardium of patients with advanced heart failure independent of the heart failure etiology.

In the present study there was no association between sIL-2R, neopterin or β-2 microglobulin and autoantibody status or titer. Conversely, Limas et al. [8] found that high autoantibody titers were more common among IDC patients with abnormal sIL-2R. We measured cardiac-specific autoantibodies, whereas Limas et al. did not distinguish between cardiac-specific and cross-reactive autoantibodies; the anti-β1-receptor autoantibodies were not measured in our study, because these are not detected by IFL. sIL-2R may be related with distinct autoantibody specificity, e.g. in Graves’ disease high sIL-2R was associated with anti-TSH receptor autoantibodies, but was unrelated to the autoantibodies to intracellular antigens (anti-microsomal and anti-thyroglobulin) [12]. The same may apply to IDC, high sIL-2R being present in association with antibodies to extracellular, e.g. the β1 receptor, rather than intracellular antigens, e.g. α-myosin and the other cardiac-specific antigens involved in the IFL reaction [3–5]. This may explain at least in part the discrepancy between their results [8] and our data. Nonetheless, the fact that we did not find high autoantibody titers in association with high sIL-2R and more severe disease is in keeping with the reduction in antibody titers with disease progression, seen in organ-specific autoimmune diseases [41]. In agreement with this, in IDC cardiac-specific autoantibodies are detected in patients who are less symptomatic [3], as well as in a proportion of symptom-free relatives [5]. In addition, it has been shown by prospective follow-up that antibody titers in IDC are reduced with disease progression and inversely related to peak and maximal VO₂ [11]. Altogether, these data indicate that cardiac-specific autoantibodies in IDC are markers of early disease and do not reflect secondary immunopathogenesis or tissue necrosis/ degeneration in advanced heart failure.

In conclusion, there is no convincing evidence that abnormal sIL-2R, neopterin and/or β-2 microglobulin are disease-specific markers of IDC. The lack of association with cardiac autoantibodies suggests that these abnormalities are mainly related to heart failure severity rather than autoimmune pathogenesis. In keeping with this view, high levels of sIL-2R, neopterin and/or β-2 microglobulin identified a subset of IDC patients with advanced disease and poor prognosis.
Fig. 2. The figure shows significant correlation of the exercise data in IDC with some of the immunological markers by linear regression analysis. Overlapping data points are shown as coincident. Top graph: negative correlation between peak $V_O_2$ and sIL2-r levels ($r^2 = 0.154$, $P = 0.008$). Middle graph: negative correlation between peak $V_O_2$ and HLA-class II score on endomyocardial biopsy tissue ($r^2 = 0.097$, $P = 0.02$). neg = negative, weak pos = weak positive; pos = positive; strong pos = strong positive. Bottom graph: negative correlation between peak $V_O_2$, and ICAM-1 score on endomyocardial biopsy tissue ($r^2 = 0.045$, $P = 0.03$). neg = negative, weak pos = weak positive; pos = positive; strong pos = strong positive.

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References

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