Prognostic value of ICAM-1 and IL-10 in children with biopsy proven myocarditis

Elżbieta Czarnowska, Bożena Cukrowska, Lidia Ziółkowska, Ilona Rosiak, Magdalena Brudek, Wiesława Grajkowska, Wanda Kawalec

1 Department of Pathology
2 Department of Cardiology
The Children’s Memorial Health Institute
Warsaw, Poland

Abstract

Prognostic factors of myocarditis outcome are still not clear, however abnormalities of both humoral and cellular immunity have been demonstrated. We investigated whether biopsy and serum cytokine levels at early stage of myocarditis correlate with outcome in dilated cardiomyopathy (DCM). Myocardial biopsy and blood serum cytokine level at the time of initial presentation of myocarditis and six months after the first examination were performed in 12 patients (10 boys, 2 girls, aged 6.6 – 17.6 yrs). The patients were divided into the group, in which DCM developed (Group I, n = 5) and into the group recovered from myocarditis with no abnormalities in left ventricle diameter and function (Group II, n = 7). The number of inflammatory cells CD3-, CD4-, CD8-, CD68-positive in biopsies and level of sICAM-1, IL-6, IL-10, TNF-α, and INF-γ in sera were analyzed. Data showed a markedly higher level of sICAM-1, and lower IL-10, and lower number of macrophages in the right ventricle endomyocardial samples at initial presentation of disease in patients with DCM outcome (Group I) when compared to those recovered from myocarditis (Group II). In conclusion, these parameters appear to predict the DCM outcome of myocarditis in paediatric patients, however further study is needed on higher number of patients.

Key words: cytokines, dilated cardiomyopathy, ICAM-1, inflammatory cells, myocarditis

Introduction

Accumulating data on myocarditis biology has revealed that this disease and dilated cardiomyopathy (DCM) are closely related. It is believed the viral infection is caused of DCM in great majority of cases, however in some cases the presence of virus has not been confirmed [10]. Patients may exhibit various clinical symptoms and different immune response. It is accepted that myocarditis is a triphasic process [16]. During first phase related to active viral infection cell lysis occurs by normally replicating viruses. In the second phase the cytokine activation contributes to cellular and humoral autoimmunity related to recovery from infections [12] or worsening cellular injuries [17]. It is known from animal models that viral infection may activate various mechanisms, including myocytolysis in the absence of specific immune responses [5], and this phenomenon may play a role in human fulminating myocarditis, while, an increased level of cytokines is characteristic in myocarditis and DCM [6, 11, 17]. The significance of an increased cytokine level in the pathomechanism of human myocarditis remains uncertain. Data from experimental myocarditis revealed that interleukins: IL-6, IL-10, IL-12, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) play pro-inflammatory role [20, 7], while IL-10 possesses a variety of immunoregulatory properties, including the inhibition of activation of nuclear factor-kappaB (NF-kB), i.e. transcriptional factor of inflammatory processes. [2]. Cytokines are produced by macrophages and Th1 and Th2 lym-
phocytes, and Th-1-associated response is responsible for proinflammatory reactions, whereas Th2 enhances humoral immunity. These findings imply that cytokines profile might be a prognostic factor for myocarditis outcome.

In the present study we have examined whether serum cytokine level in relation to immunophenotype infiltrating cells present in endomyocardial tissue samples obtained from right ventricle enables to determine the disease outcome.

Material and methods

Patients
The study includes 12 children (10 boys, 2 girls; aged 6.6–17.6 yrs; mean 13 ±3.8 yrs) presenting symptoms of myocarditis who were consecutively hospitalized in Pediatric Cardiology Department from 2005 to 2007. They were clinically and histopathologically diagnosed according to Dallas criteria [3] and Lieberman et al [14] as having myocarditis. The mean time from onset of symptoms of myocarditis to diagnosis was 2.52 ± 3 weeks. All patients presented fatigue, ventricular arrhythmias (n = 5), chest pain (n = 5), heart failure (n = 4). In all patients positive titer of viral antibodies against Coxackievirus B or Parainfluenza type 3 or Influenza A, B were observed. In the history all children presented flu-like disease one to two weeks before onset of clinical symptoms of myocarditis. Endomyocardial biopsy (EMB) was performed at the time of initial presentation of myocarditis and 6 months after the first examination. Patients were treated with immunoglobulins (n = 6), beta-blockers (n = 3), ACE-inhibitors (n = 3), diuretics (n = 4), carvedilol (n = 2).

Patients were divided into the group, in which DCM developed (Group I, n = 5) and into the group recovered from myocarditis in follow up (Group II) at the time when the first biopsy was taken (Tabela 1). During the first biopsy amounts of infiltrating leukocytes were similar in both groups, while the number of macrophages was markedly decreased in Group I (Fig. 1). Expression of ICAM-1 on endothelial cells did not differ in both groups of patients, being expressed by various numbers of vessels with different reaction intensity.

In second biopsy performed after 6 months only the number of cytotoxic CD8-positive lymphocytes differs in both groups of patients being elevated in Group I (Fig. 1).

Systemic pro-inflammatory cytokines IL-6, TNF-α and INF-γ did not differ between the groups at the time of the first biopsy, and during the second biopsy differences were found only at the level of IL-6 (Fig. 2). This cytokine markedly increased in Group I, whereas decreased in Group II after 6 months.

The level of regulatory cytokine IL-10 at time of the first biopsy was higher in Group II when compared with pa-

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Sex</th>
<th>NYHA</th>
<th>% SF</th>
<th>EF</th>
<th>LV diameter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>8, 62±3,17</td>
<td>5M</td>
<td>II (n=4)</td>
<td>21,5±3,6</td>
<td>0,44±0,03</td>
<td>160±22,8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>III (n=1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>12,52±5,02</td>
<td>2F, 5M</td>
<td>II (n=7)</td>
<td>38,4±4,5</td>
<td>0,66±0,03</td>
<td>100</td>
</tr>
</tbody>
</table>

LV (left ventricle) diameter = % of mean normal range relative to body surface area
SF = shortening fraction  EF = ejection fraction  M = male  F = female
tients, who developed DCM from Group I, while during the second biopsy its level increased in Group I (Fig. 2).

Soluble adhesion molecule ICAM-1 showed tendency to be increased in Group I when compared with Group II at the early stage of the disease, whereas 6 months later its levels were comparable in both groups (Fig. 2).

**Discussion**

In the present study we have focused to find possible prognostic markers that could predict the myocarditis outcome and its progression to DCM. In the study two major observations were made:

---

**Fig. 1.** Inflammatory cell infiltration in endomyocardial biopsy samples analyzed in area of 1 mm$^2$.

**Fig. 2.** The level of sICAM-1, IL-10 and proinflammatory cytokines in sera of patients with myocarditis at different stages of disease.
(i) a high level of sICAM-1 and low of IL-10 in blood serum, and low number of macrophages in endomyocardial biopsy sample are characteristic feature in patients with myocarditis and its progression to DCM;

(ii) neither the serum level of TNF-α, IL-6 nor INF-γ correlates with clinical outcome.

In the literature there is a disagreement associated with the presence of virus genome and myocardial inflammation and deterioration of left ventricle function. Some data demonstrate positive correlation between these factors [11] while the others are in contrast [10]. However, it is fully accepted that virus persistence leads to DCM in triphasic course of myocarditis [15]. Therefore the increased duration of the disease is significant for DCM outcome. In our study the presence of antiviruses antibodies characterized both groups of patients, but since the presence of virus genome was not investigated it cannot be determined a role of viruses in the disease outcome.

In spite of the lack of straight indication for virus persistence in our patients we expected the presence of other predictive factors of unfavorable disease outcome. Unexpectedly among these factors there was the lack of pro-inflammatory cytokines (IL-6, TNF-α, INF-γ), but the presence of IL-10, a regulatory cytokine was observed. Generally, IL-10 is thought to exhibit immuno-suppressive effects on Th1 lymphocytes [18], but its role in myocarditis is still not clear. In experimental myocarditis it was demonstrated the protective effect of IL-10 [24]. It also has been suggested that high level of IL-10 could be a predictive factor of fulminating myocarditis [8, 19].

The second characteristic factor found in our study was the low number of macrophages infiltrating endomyocardium in the first biopsy of patients, who developed DCM. It is known that these cells eliminate viruses and are responsible for induction local cell damage through the release of reactive oxygen species [1]. It can not be excluded that the low number of macrophages does not only impair the elimination of viruses and infected cells but also is related to the low level of TNF-α. The low level of TNF-α can be not only the effect of the limited number of macrophages, but also the lack or inadequate production by cardiomyocytes [1].

In our study the proinflammatory cytokines IL-6, TNF-α and IFN-γ were in both groups on similar levels at early stage of disease, but in late stage the amount of IL-6 increased in Group I. Overexpression of the IL-6 has been demonstrated in experimental myocarditis as an effect of poor elimination of viruses and impaired release of TNF-α in early phase [23]. Therefore, it might be that the high level of IL-6 after 6 months of disease onset is a feature of chronic process related to DCM.

At the stage of disease when the first biopsies were obtained we observed doubled level of sICAM-1 in sera of patients who later developed DCM. The soluble ICAM-1 is a circulating form of the adhesion molecule which is membrane-bound to endothelial cells and macrophages. It is believed that the level of sICAM-1 reflects the expression of this molecule on those cells [13]. However, we did not observed correlation between the expression of ICAM-1 in sera and endomyocardial tissue. The expression of ICAMS on endothelial cells is induced by several inflammatory mediators, e.g. IL-1 [4, 9], TNF-α [9], angiotensin [21] and others. In our study the level of TNF-α did not differ in both groups of patients and this could explain relatively low ICAM-1 expression on endothelial cells at the disease stage when biopsy was obtained. It can not be excluded that the strong release of this molecule was induced in earlier phase of the disease.

It becomes increasingly clear that the activation of the immune system occurs in patients with DCM, but the prognostic markers that could predict the myocarditis progression to DCM are still looking for. The establishment of these markers will provide new targets for specific early therapeutic intervention. Our observations suggest that sICAM-1 and the low number of macrophages in biopsy specimen and low level of IL-10 in blood are the markers of unfavorable myocarditis outcome.

Acknowledgements

This study was supported by grants S95/06 and 83/2005 financed by CMHI.

References