Immunopathology of chronic myocarditis in children

Bogdan Woźniewicz¹, Lidia Ziółkowska², Wanda Kawalec², Bożena Cukrowska¹, Maciej Pronicki¹, Wiesława Grajkowska¹

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

Abstract

Chronic myocarditis is a heart dysfunction with stage NYHA I or II leading usually to dilated cardiomyopathy. The following classification of chronic myocarditis was proposed according to immunohistochemistry of heart biopsy: chronic persistent active myocarditis (CPAM) with activation of T cells (CD3+, CD4+, CD8+), macrophages (CD68+) and ICAM-1 molecules; chronic persistent resolving myocarditis (CPRgM) characterized by presence of macrophages and persistent ICAM molecules activation; chronic persistent resolved myocarditis (CPRdM) with persistent ICAM activation.

Key words: cell phenotypes, children, chronic myocarditis, heart immunopathology

Introduction

Infections (viruses, bacteria, protozoa, fungi) are the major factors causing most clinical myocarditis and heart failure [3, 8, 9, 12, 15]. The infiltration of the myocardium by inflammatory cells with necrosis of the adjacent myocytes is the most important feature in myocarditis [12]. Endomycocardial biopsy may be valuable in patients with unexplained cardiac dysfunction [1]. T lymphocytes play an important role in the cell-mediated destruction of myocytes [12]. Over expression of endothelins, adhesion molecules, cytokines, T cells, macrophages, mastocytes leads to develop cardiomyopathy and so called silent myocarditis [8]. Dallas criteria are not sufficient to confirm adequate diagnosis in chronic myocarditis without application of immunocytochemical markers [15]. Three types of reactive changes in the myocardium has been described: 1) infective – predominantly viral with mobilization of T cells, ICAM, VCAM molecules, CD 45R and CD68 positive cells; 2) autoaggressive – characterized by anti-heart antibodies (AHA Abs), anti-myosin, anti-actin Abs, anti-HLA class I and II over expression; 3) ischaemic – presented by anti-heart antibodies (AHA), B cells, troponin and neutrophiles mobilization (Table1).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Infective</th>
<th>Autoaggressive</th>
<th>Ischaemic</th>
</tr>
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<tbody>
<tr>
<td>T cells</td>
<td>AHA+</td>
<td></td>
<td>AHA+</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Anti-myosin</td>
<td></td>
<td>B cells</td>
</tr>
<tr>
<td>CD45R</td>
<td>HLA DR</td>
<td></td>
<td>Neutrophiles</td>
</tr>
<tr>
<td>CD68</td>
<td></td>
<td></td>
<td>Troponin</td>
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Material and methods

Endomyocardial biopsy of right ventricle from 70 patients with clinical diagnosis of myocarditis, arrhythmia of unknown etiology and dilated cardiomyopathy (DCM) was performed. Thirty three patients had clinical symptoms of arrhythmia and were classified as NYHA I, 37 ones were classified as NYHA II with symptoms of DCM and/or myocarditis (Table 2). Clinical history of failure was observed during 3–6 months usually after upper respiratory infection. Routine histology and immunohistochemical examination was made in all cases. The following markers were used: antibodies

Address for correspondence

Bogdan Woźniewicz
Department of Pathology
The Children’s Memorial Health Institute
Al. Dzieci Polskich 20
04-736 Warsaw, Poland

Phone: +48 22 815 19 60
E-mail: b.wozniewicz@czd.pl
Table 2

Lymphocytes and ICAM-1 density* in endomyocardial biopsy in chronic myocarditis and/or DCM.

<table>
<thead>
<tr>
<th>Marker</th>
<th>CPAM (n=10)</th>
<th>CPRgM (n=18)</th>
<th>CPRdM (n=37)</th>
<th>Normal (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 3</td>
<td>20 (+/-10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD 4</td>
<td>12 (+/-5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD 8</td>
<td>15 (+/-5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD 22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD 68</td>
<td>10 (+/-3)</td>
<td>20 (+/-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD 34</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>ICAM-1**</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>VCAM-1**</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>0</td>
</tr>
<tr>
<td>HLA class I ***</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA class II DQ</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

CPAM – Chronic persistent active myocarditis
CPRgM – Chronic persistent resolving myocarditis
CPRdM – Chronic persistent resolved myocarditis

* number of cells per 0,5 square mm
** semiquantitatively +, ++, +++ (mild, moderate, extensive)

anti-ICAM-1 anti-CD3, -CD4, -CD8, -CD 22, -CD68, -CD34, -HLA class I and II. Detection of antibodies were made using APAAP method on frozen sections or when it was possible on paraffin serial sections. Twenty one patients underwent second endocardial biopsy for evaluation of the influence of treatment procedures. Five patients with active form of persistent myocarditis received infusions of gamma globulins 2 g/kg and 16 patients with chronic persistent resolving type had received steroid therapy. Patients with chronic persistent resolved myocarditis (ICAM+) were not treated.

Results

In the majority of cases the conventional histology with haematoxillin and eosin staining showed only interstitial inflammatory cells, not sufficient to recognize myocarditis. In contrast, immunohistochemical methods displayed lymphocyte and/or macrophage infiltration (number of cells) ranged between 7–50 per 0.5 square millimeter of tissue section. Such immunohistochemical staining was done in 65 cases. In 5 cases nor histologically nor immunohistochemically myocarditis features were seen. The lymphocyte infiltration correlated with expression of adhesion molecules ICAM-1 (Table 2). The reactions for B lymphocytes (anti-CD22) always were negative.

On the basis of immunohistochemical reactions it was possible to classify chronic myocarditis into three groups. Chronic persistent active myocarditis (CPAM) was recognized in 10 patients, chronic persistent resolving myocarditis (CPRgM) in 18 or resolved myocarditis (CPRdM) in 37 patients (Fig. 1–3).
Discussion

Immunohistochemical reaction may be helpful to qualify the cellular reaction of the heart into inflammatory, ischemic and autoimmune reactions [1, 6]. Active myocarditis is observed when T cell mobilization is over 7 cells per field and over expression of HLA class II molecules can be determined [2, 13, 15]. The inflammatory reaction of the heart is characterized by T cell populations and ICAM molecules [4, 8, 9, 12, 14, 15]. In normal control heart ICAM and T cell expression is usually negative. The ischemic reactions are characterized by neutrophiles, B cells and endothelin involvement. Cardiac troponin seems to be the gold marker for the new millennium in evaluation of cardiocyte damage. In many patients with symptoms of myocarditis anti-heart and anti myosin antibodies as well as T cell reactive antibodies can be detected. This indicates that autoimmunity can be an additional mechanism in cardiac injury [4, 11, 12]. In experimental cardiac injury two types of animal models are discussed: adjuvant induced myocarditis, in which animal are given multiple immunization with heart proteins (myosin, adenine nucleotide translocator), and virus induced myocarditis, in which animals are infected with viruses predominantly associated with human cardiotropism. In mouse autoimmune myocarditis was inhibited by administration of an antagonist of IFN [4].

Patients with so called borderline myocarditis on conventional histology should be confirmed by immunohistochemical markers. T lymphocyte density correlates with over expression of MHC class I and II, and ICAM-1 molecules. Manifestation of myocarditis depends on the interplay between initiated agents and host responses. T cells expressing alpha-beta T cell receptor seem to play a central role in the pathogenesis of tissue injury in virus-induced myocarditis. In active fulminant lymphocytic – virus induced myocarditis infiltration of the heart tissue contain predominantly gamma-delta T cells [7]. It is worthwhile to emphasize that no evidence of virus could be obtained by in situ hybridization with different specific DNA/RNA probes and by reverse transcriptase polymerase chain reaction using specific probes for enteroviruses, adenoviruses, herpes simplex, influenza A and B and CMV. This finding has been important in order to identify subgroup of patient who may benefit from immunosuppressive therapy. Myocarditis susceptibility depends upon activation of gamma-delta T cell receptor that induce

Treatment effects

Ten patients with active persistent lymphocytes (CD3, CD4, and CD8 positive) reaching density of 20–50 per 0.5 mm², received gammaglobulin infusion 2 g/kg. After such treatment 5 patients showed improvement (normalization) in second biopsy performed 6 months after first biopsy. T cells diminished and the complete recovery was noted including ICAM-1. In 5 patients with active changes second biopsy showed disappearance of T cells, but persistence of macrophages with density 20 per 0.5 mm². In those patients transition from active into resolving myocarditis was recognized during 6–8 months.

Sixteen patients with immunocytochemical diagnosis of resolving myocarditis underwent steroid therapy using Encorton (1mg/kg) within 4 weeks. Complete recovery was observed in 30% (5 patient). In those patients diminishing of T cells, macrophages and ICAM-1 expression was seen. In the remaining 11 patients resolved myocarditis was recognized in second biopsy with persistence of ICAM-1 expression (Table 3).

The ICAM-1 molecules expression was an indicator that myocardial cell damage persists immunologically for a long period and may be a risk factor. Such patient should be protected against physical effort. Using immunocytochemical markers of inflammatory reaction was concluded that Dallas criteria of myocarditis could be improved.

Table 3

Comparison of immunohistochemistry of myocarditis in primary and secondary biopsy in 21 cases treated with gammaglobulin or steroids (intervals between biopsies approximately 6 months).

<table>
<thead>
<tr>
<th>Biopsy I ( n=21)</th>
<th>Biopsy II (n–21)</th>
<th>% recovery after therapy</th>
</tr>
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<tbody>
<tr>
<td>CPAM NYHA I/II</td>
<td>10</td>
<td>5 recovery, 5 persists</td>
</tr>
<tr>
<td>CPRgM</td>
<td>16</td>
<td>6 recovery, 10 persists</td>
</tr>
</tbody>
</table>

CPAM – Chronic persistent active myocarditis
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CPRdM – Chronic persistent resolved myocarditis
Fas-dependent apoptosis in inflammatory dilated cardiomyopathy [7]. It is postulated that in myocarditis cellular immunity plays an important role in two major mechanisms: a secretory mechanism in which perforin and granzymes are key players, and no secretory mechanism involving Fas/Fas ligant activation [5, 7]. There is little information about the relationship between the expression of ICAM-1 and VCAM-1 in myocarditis in children. In our investigation ICAM-1 and VCAM-1 expression was not evident. Both intercellular adhesion molecules have been implicated in cardiac allograft rejection. Persistent ICAM-1 expression observed in series of our cases suggests that myocardial damage persists immunologically for a long period in so called silent myocarditis. Although an autoimmune mechanism has been postulated in chronic myocarditis, immunosuppressive therapy has had beneficial effect in about 30% of patients [10, 11]. In patients with significant myocardial infiltration of T cells, infusion of intravenous gamma globulin was effective as measured by an improvement of ventricular function and disappearance of T cell infiltration in control biopsy.

Reference

Introduction

Sensor neural hearing loss resulting cochlear hair cell damage is a major cause of human deafness. While hair cell regeneration in the mammal occurs naturally over a very limited time during development, stem cell transplantation could therefore be beneficial for restoring the inner ear hair cells [3]. Experimental studies allowed us to know specific markers for progenitors of hair cells [4, 5, 6, 9, 11]. It seems that transplanted neural stem cells (NSCs) have the potential to differentiate and restore inner hair cells [5, 6]. Grafts of neural stem cells transplanted into the mouse inner ear after drug induced injury expressed glial fibrillary acidic protein (GFAP) and/or microtubule-associated protein-2 (MAP-2). A few grafted cells were positive for nestin, a marker for premature neural cells, and myosin VIIA, the specific marker for inner ear hair cells.

Recently several studies have shown that using stem cells from adults as an alternative to stem cells from embryos carries important implications for the treatment of degenerative diseases such as hearing loss [1, 7, 10].

Material and methods

As a source of progenitor hair cells we used both adult human and guinea pig cochleas. Retroauricular incision for approach to the temporal bones was used in guinea pig. The cochleas were removed and separation of sensory epithelium of the cochlea from other tissues was performed. The sensory epithelium of the cochlea was immersed in the Chang Medium C (Irvine Scientific) at 4°C for 0.5 h of transportation. The tissue was dissected and cultivated in Chang Medium C with antibiotics (penicillin and ceftriaxone) and 10% fetal calf serum at 37°C in humid air containing 5% CO₂. After 28-day lasting cultivations cells were collected and used for immunohistological analysis. The fate of cultured cells was determined by using antibodies against MAP-2, nestin, vimentin.