Expression of B7-homolog 1 in Polymyositis

The First Affiliated Hospital of Soochow University, Suzhou 215006, China

Abstract. Objectives Costimulatory molecules are increasingly recognized as crucial for stimulation and/or inhibition of immune responses. The present study was undertaken to examine the expression and functional relevance of B7-homolog 1 (B7-H1) attributed significant immunoregulatory functions in polymyositis in vivo. Methods: 43 muscle biopsy specimens obtained from patients with polymyositis, 26 cases with limb girdle muscular dystrophies (LGMD) 2B and 21 normal muscle samples were analyzed by immunohistochemistry for B7-H1 expression. The clinical and histopathologic data were analyzed.

Results B7-H1 was not detectable on normal muscle fibers and rarely detectable from patients with LGMD-2B. In contrast, its expression was markedly increased on muscle fibers from patients with polymyositis, even after short-term immunosuppressive treatment. Positive staining mainly localized at the surface of the muscle tissue, rarely in the cytoplasm, focused in areas where inflammatory cells lay in close apposition to damaged, necrotic or degenerative muscle fibers. The expression of B7-H1 was correlated to the degrees of muscular necrosis and clinical muscular strength.

Conclusion Our results demonstrate that human muscle cells express B7-H1 in polymyositis. The muscle-related expression of B7-H1 may be helpful in the diagnosis of polymyositis and might be an indicator of prognosis of polymyositis.

Keywords: polymyositis, B7-H1, immunohistochemistry

Introduction

Polymyositis is a chronic autoimmune-mediated inflammatory muscle disease that is clinically characterized by proximal muscle weakness, myalgia and elevated serum creatine kinase (CK). The histological cornerstone of diagnosis is the identification of mononuclear cellular infiltrates in skeletal muscle tissue[1,2]. But sometimes these infiltrates can be absent in the presence of the disease, especially after immunosuppressive treatment has been initiated[3]. In the other hand, inflammatory findings in muscle biopsy have previously been described in some muscular dystrophies, such as limb girdle muscle dystrophy (LGMD)[4,5]. Bohan and Peter’s criteria become inadequate to exclude many of the conditions presenting as polymyositis[6,7].

Now it is known that cell-mediated mechanism plays an important role in the etiology of polymyositis[6,8]. Muscle cells can act as antigen-presenting cells and have been shown to effectively present endogenous and exogenous antigens. Recently, advances have been made in the characterization of positive and negative muscle-derived regulators for immune interactions, extending the view that muscle has important immunoregulatory capacities under certain conditions.

Major histocompatibility complex class I (MHC-I) expression on the sarcolemma is upregulated in polymyositis, which is essential for the interaction of muscle with CD8 cytotoxic T cells and CD4 helper T cells[9]. According
to the two-signal model of T cell activation, costimulatory signals play a key role in regulating T cell activation and are decisive in inciting and perpetuating cellular effector mechanisms [10]. B7.1/2(CD80/86) is believed to be a prerequisite for antigen-presenting cells to initiate immune responses. But cultured myoblasts do not express the classical B7.1/2 and treatment of these cultures with IFN-\(\gamma\) and TNF-\(\alpha\) has no effect on the expression of these molecules. The expression of B7 costimulatory molecules distinct from B7.1/2 has been postulated [11, 12]. The present study was undertaken to examine the expression and functional relevance of B7-H1, a novel B7 homolog in polymyositis.

Materials and Methods

Patients 43 consecutive patients (16 male and 27 female) who were diagnosed with polymyositis according to Bohan and Peter’s criteria [13] at the Department of Neurology, the first affiliated Hospital of Soochow University, China between 2008/01 and 2009/12 were included in the study. The mean age at the time of biopsies was 43.51 (range 9~77) years. The duration of muscle symptoms before the biopsy lasted from 10 days to 2.5 years. The patients accompanied with malignancy and overlap syndrome are excluded; those who had received immunosuppressive treatment for more than 4 weeks are also excluded.

The clinical features of all patients were recorded, including the age of onset, the progression of the disease, the involvement of muscles, the accompanied myalgia, the physical examination such as muscle strength and tendon reflex, and the serum CK level, etc.

According to whether receiving the corticosteroids before the biopsy, the patients were divided to untreated and shortly-treated (less than 4 weeks) groups (32 cases vs. 11 cases).

According to the level of serum CK, the patients were divided into three groups: CK1 group (200~2500 IU/L), CK2 group (2500~5000 IU/L), CK3 group (>5000 IU/L).

According to the severity and distribution of muscular fiber necrosis and inflammatory infiltration, the patients were divided into three groups: mild, moderate and severe necrotic groups. The standard of division was:

1. Severe necrotic group: extensive necrosis (>50% fibers in a x200 field) accompanied by phagocytosis and regeneration, focal necrosis was observed, mild to moderate interstitial regeneration, different degree of inflammatory infiltrates.

2. Mild necrotic group: sporadic necrotic fibers (<10% in a x200 field) accompanied by phagocytosis and regeneration, no focal necrosis was observed, different degree of interstitial regeneration, accompanied by or no inflammatory infiltrates.

3. Moderate necrotic group: between mild and severe necrotic group.

Controls Two groups of patients from the same hospital served as controls, one group included 26 cases (15 male and 11 female) with LGMD-2B based on the clinical features, biopsy and dysferlin staining. The average age was 20.31 (range 13~40) years. The course lasted from 2 to 9 years. 21 normal muscle samples (8 male and 13 female) from patients with suspected myopathy obtained by biopsy for diagnostic purposes were also included as controls. The average age was 36.66 (range 13~67) years. All the controls had been excluded accompanied neoplasma and autoimmune diseases.

Biopsy The study was approved by the local ethics committee, and written consent was obtained from all participants. Tissue was obtained under local anesthesia using the semi-open biopsy technique; biopsies were performed on those muscles clinically suspected to be affected, either in the quadriceps femoris muscle or the biceps brachii muscle. All the samples were snap-frozen in isopentane iced in liquid nitrogen within 5 minutes after surgical intervention and stored at -80°C until analyzed.

Immunostaining of B7-H1 Immunohistochemical staining was performed on 5μm serial cryo-
stat sections from human muscle tissue. Briefly, these sections were air dried for 10 minutes and dipped in acetone for 10 min at room temperature. Sections were blocked with 10% goat serum albumin (Zhongshan Goldenbridge, China) in phosphate buffered saline (PBS) for 30 minutes at room temperature and incubated with the primary rabbit anti human B7-H1 mAb (1:100 diluted in PBS, Shanghai Hushang, China) for 1 h at 37℃C. After incubation, the samples were washed in PBS and the Two-step Histostaining kit (Zhongshan Goldenbridge, China) was used. Binding of anti B7-H1 antibody was visualized using IgG-HRP polymer conjugated complex with 3,3’- diaminobenzidine tetrahydrochloride (DAB) as a chromogenic substrate solution (DAB kit, Zhongshan Goldenbridge, China). Sections were counterstained with hematoxylin, and mounted in gelatine. Negative controls with PBS buffer as primary antibody were performed.

Assessment of immunoreactivity All immunohistochemical stains for patients and controls were analyzed on coded slides on whole-tissue sections by conventional microscopic assessment by one observer who was blinded to the patient group studied. The number of positive cells related to the total area of each section on five random ×200 fields were determined, the density of B7-H1 antigens expression was assessed as follows: –, <10% positively stained fibers; +, 10–30% positively stained fibers; ++, 31–70% positively stained fibers; ++++, >70% positively stained fibers.

Statistical analysis Data were analyzed using the SAS 8.1 software program. Spearman correlation analysis, t-test, ANOVA, χ2 test and Fisher’s exact method were used. P values less than 0.05 were considered significant.

Results

Expression of B7-H1 in polymyositis, LGMD 2B and normal control Expression of B7-H1 was found in 30/43 (69.77%) of muscle biopsies from patients with polymyositis, and in 7/26 (26.92%) with LGMD 2B, only 1/21 (4.76%) healthy control detected B7-H1 positively. Besides, the density of B7-H1 expression in LGMD-2B and healthy control groups were all (+) grade, while in polymyositis group, the density were 15/30 for (+), 7/30 for (++), and 8/30 for (+++), respectively. χ2 test revealed that the expression of B7-H1 in polymyositis was different statistically from that in LGMD 2B group (p<0.001) or the healthy control (p<0.001). The density of B7-H1 expression of each group was analyzed by Fisher’s exact method, there were also statistical differences between PM group, and LGMD 2B group (p<0.05) or the healthy control (p<0.001). Positive staining mainly localized at the surface of the muscle tissue, rarely in the cytoplasm, focused in areas where inflammatory cells lay in close apposition to damaged, necrotic or degenerative muscle fibers. (tab1, fig1)

Table 1. Expression of B7-H1 in PM, LGMD 2B and healthy control

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>n</th>
<th>Number of positive staining (%)</th>
<th>Density of B7-H1 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>43</td>
<td>30 (69.77)</td>
<td>13</td>
</tr>
<tr>
<td>LGMD2B</td>
<td>26</td>
<td>7 (26.92)</td>
<td>19</td>
</tr>
<tr>
<td>Healthy control</td>
<td>21</td>
<td>1 (4.76)</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig.1 Immunohistostaining of B7-H1. (A) Negative control with PBS buffer as primary antibody. (B, C, D) Positive expression in polymyositis with rabbit anti human B7-H1 mAb as primary antibody. Positive buffy to brownish staining mainly localized at the surface of the muscle cell (B), rarely in the cytoplasm (C), focused in areas where inflammatory cells lay in close apposition to damaged, necrotic or degenerative muscle fibers (D). (×400)
Expression of B7-H1 in untreated, shortly-treated polymyositis and normal control
Proportions of positive B7-H1 expression in the polymyositis patients without treatment, with preceding short-term corticosteroids treatment, and normal control were 23/32 (71.88%), 7/11 (63.64%), and 1/21 (4.76%), respectively. Both patients without or with treatment were statistically higher than the normal control (p<0.001) using Fisher’s exact method. The group without treatment was higher than that with corticosteroids, but there was no statistical difference (p>0.05). (Fig.2)

Expression of B7-H1 in different CK groups of polymyositis
According to the serum CK level, the proportion of positive B7-H1 expression of the polymyositis patients in CK1, CK2, and CK3 groups were 4/10 (40.00%), 10/14 (71.43%), and 16/19 (84.21%), respectively. Fisher’s exact method revealed there were no statistical difference (p>0.05) between CK1 and CK2 group, or between CK2 and CK3 group, but CK3 group showed the statistically higher positive percentage than CK1 group (p<0.05). (Fig.3)

Correlation of clinical data and expression of B7-H1
Some clinical data of the PM patients and the proportion of positive expression of B7-H1 protein were correlation analyzed, such as age, sex, the onset of disease, muscular strength, and accompanied myalgia, difficulty in raising head or swallow, and tendon reflex. Only muscular strength showed positive correlation with the percentage of B7-H1-positive-expression samples (r=0.389, p=0.011), more severe the muscle weakness, higher percentage of B7-H1-positive samples, but other factors did not correlate with the protein expression. (tab.2)

Table 2. Correlation analysis of B7-H1 expression and some clinical data

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.121</td>
<td>0.439</td>
</tr>
<tr>
<td>Sex</td>
<td>0.089</td>
<td>0.570</td>
</tr>
<tr>
<td>Onset of disease</td>
<td>0.156</td>
<td>0.317</td>
</tr>
<tr>
<td>Muscle strength</td>
<td>0.389</td>
<td>0.011</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0.237</td>
<td>0.125</td>
</tr>
<tr>
<td>Difficulty in raising head</td>
<td>0.177</td>
<td>0.256</td>
</tr>
<tr>
<td>Difficulty in swallow</td>
<td>0.228</td>
<td>0.141</td>
</tr>
<tr>
<td>Tendon reflex</td>
<td>0.176</td>
<td>0.258</td>
</tr>
</tbody>
</table>

Discussion

Muscle is an immunologic microenviroment[9], under pathologic conditions muscle cells can
express a variety of immunologically important molecules, though there is no MHC expression can be detected normally. Under inflammatory conditions such as polymyositis, endogenous antigens can be presented via MHC-I on the surface of a muscle cell to CD8+ T cells, which after activation induce necrosis through the release of lytic granzymes[12,14-16]. Costimulatory molecules are prerequisite in the process. The expression of B7 costimulatory molecules distinct from B7.1/2 has been postulated, B7-H1 protein is expressed in TE671 rhabdomyosarcoma cells and cultured human myoblasts in the presence of IFN-γ[17].

Our study showed the expression of B7-H1 in areas characterized by the presence of inflammatory cells and muscle fiber damage in vivo in polymyositis. B7-H1 (PD-L1) is a type I transmembrane glycoprotein with 20% amino acid identity to B7.1 and 15% amino acid identity to B7.2[18], originally defined as a molecule expressed by antigen presenting cells. Recent data revealed that B7-H1 expression is not restricted to macrophages and dendritic cells. Its messenger RNA was also detected in the heart, placenta, skeletal muscle and lung[19]. B7-H1 was shown to inhibit proliferation and cytokine production by activated T cells through interaction with the PD-1 receptor[20-22]. Some evidences showed that B7-H1 exerted strong immune-inhibitory properties for CD4 and CD8 T cells in co-culture assays in that it reduced cytokine production and up-regulation of T-cell activation markers [20]. Thus, B7-H1 could serve as a negative immune-regulatory principle exerted by muscle cells and induced upon inflammatory stimuli. As hypothesized, B7-H1 could protect muscle fibers from cell-mediated injury in autoimmune muscle disorders.

Besides its broad implications for the immunobiology of muscles, the muscle-related expression of B7-H1 may be helpful in the diagnosis of polymyositis. When the criteria of Bohan and Peter are used, sometimes it is very difficult to differentiate from secondary inflammation seen in dystrophies or when the infiltrates are absent after immunosuppressive treatment. New diagnostic criteria were recently introduced [7, 23, 24], in which the MHC/CD8 complex is considered a specific immunopathological marker because it distinguishes the antigen-driven inflammatory cells that characterize polymyositis from the non-specific, secondary inflammation seen in other disorders[25-27]. CD8 cells and MHC-I expression can be seen in certain dystrophies, but in polymyositis do the CD8 cells consistently invade MHC-I-expressing muscle fibers[28]. Double immunostaining is needed to detect the MHC/CD8 complex as a diagnostic method. Furthermore, the sensitivity for the detection of MHC-I expression on the sarcolemma for a diagnosis of IIM decreases from 89% to 78% after the chronic immunosuppressive treatment before biopsy[24]. As a costimulatory molecule in the activation of MHC/CD8, B7-H1 was rarely expressed under physiologic conditions and much less expressed in LGMD-2B. Another potential benefit shown in this study was that B7-H1 was still detectable after short-term immunosuppressive treatment, unlike the inflammatory infiltrates. Thus the immunohistological detection of B7-H1 on the sarcolemma might serve as a diagnostic test for the diagnosis of polymyositis. Its value in the diagnosis of polymyositis still needs to be verified in larger samples.

Furthermore, the expression of B7-H1 was related to the degrees of muscular necrosis and clinical muscular strength in this study, reflecting that the expression of B7-H1 was relevant to the severity of disease both in clinic and pathology, which suggested the expression of B7-H1, might be used to assess the severity of polymyositis. Nowadays, there is no satisfied index that is in accordance with the severity of the clinical symptoms [2]. The elevated serum CK is an important marker reflecting the existence of muscular damage [1], but its value is not always coincident with the severity of necrosis [7]. CK levels may fluctuate from day to day (increasing significantly after major exercise), even in the absence of any intervention. This may be also the reason why there was no relevance of expression of B7-H1 and serum
CK level in this study. The relationship of B7-H1 expression and the severity of the disease still need extensive follow-up observation. We suppose the possible explanation is that more severe the muscular damage, more intense immune reactions, increased expression of B7-H1 inhibitory regulator is the physical reaction to counterbalance the immune disturbance.

The immunological role of B7-H1 is far from being completely understood. The mechanism of B7-H1 involved in the pathogenesis of polymyositis needs to be further explored. Insight into the immune regulatory properties of muscle is important for a better understanding of the desirable and undesirable immune reactions occurring in this tissue and necessary in order to control novel therapeutic strategies[29].

Acknowledgement

This work was supported by the nature science fund of Jiangsu Province (BK2006542) and preresearch fund of SooChow University (Q3128289). We thank Professor Yan Chuangzhu and his colleagues of the laboratory of neuromuscular pathology (Shandong University, China) for kindly assistance of laboratory technique, and for Professor Guo Lingchuan and Deng Haizhen (Department of pathology, First affiliated hospital of Soochow University, China) for kindly support.

Reference


27. Sundaram C, Uppin MS, Meena AK. Major histocompatibility complex class I expression can be used as a diagnostic tool to differentiate idiopathic inflammatory myopathies from dystrophies. Neurol India 2008; 56(3): 363-367.
