MHC Class I Overexpression on Muscles in Early Juvenile Dermatomyositis

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ABSTRACT. Objective. To assess muscle expression of MHC Class I complexes (heavy chain and β2-microglobulin) and to analyze the composition of infiltrating mononuclear cells, specifically cells that bear receptors for class I MHC molecules, in the muscles of children with early juvenile dermatomyositis (JDM).

Methods. Light microscopic and immunohistochemical analysis of muscle biopsies from 10 patients with JDM and 3 controls. The mean duration from initial weakness was 2.8 months. At the time of biopsy, 9 patients had not received steroid treatment or immunomodulatory drugs.

Results. MHC Class I over-expression was evident on muscle fibers in all 10 JDM samples, even in a biopsy reported as normal by conventional histology. MHC class I heavy chain and β2-microglobulin were over-expressed in an identical distribution. Variable infiltration of T cells and macrophages was seen in the JDM biopsies, with minimal lymphocytic and mononuclear infiltration in 4 cases, and none in one. Only very occasional natural killer lymphocytes were identified. Neuronal cell adhesion molecule (NCAM, CD56) staining of regenerating muscle fibers was seen in all samples and these cells were confirmed as being of muscle origin by co-staining for dystrophin.

Conclusion. MHC Class I over-expression is an early event in JDM; and may occur in the absence of lymphocytic infiltration and muscle damage. Immunostaining for MHC Class I could be used routinely in the assessment of muscle histology in juvenile dermatomyositis. (J Rheumatol 2004;31:605–9)

Key Indexing Terms: JUVENILE DERMATOMYOSITIS MHC CLASS I IMMUNOHIostoCHEMISTRY

Mononuclear cell infiltration and MHC Class I over-expression are 2 well-described phenomena in adult inflammatory muscle disease5,6 and also in dystrophies such as Duchenne’s and Becker’s muscular dystrophy7,8. Existing evidence is insufficient to determine which of these is the primary event. Because of the rarity of JDM, there have been few series reporting their immunohistochemical analyses3,9,10. We examined the immunohistological features of biopsy samples from 10 patients with JDM concentrating on the expression of MHC molecules on muscle fibers and the inflammatory cell infiltrate.

MATERIALS AND METHODS

Patients. This study was approved by the research ethics committee (IRB equivalent) of Great Ormond Street Hospital, London. All subjects gave full informed parental consent and all were treated in Great Ormond Street Hospital. We examined 10 open muscle biopsy samples from patients with JDM and 3 normal subjects (1 child and 2 adults). Data and samples were collected as part of the National Registry and Repository of Juvenile Dermatomyositis of UK and Ireland. All but one patient were naïve to either systemic steroids or second-line immunomodulatory therapies at the time of biopsy. All biopsies were from the quadriceps muscle and taken under general anesthesia.

Histopathological examination. Cryostat sections (7 µm) were mounted on gelatine-coated glass slides and stained with hematoxylin and eosin (H+E)

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Juvenile idiopathic inflammatory myopathies (JIIM) affect between 1 and 4 children per million, of which the largest group are those with juvenile dermatomyositis (JDM)1,2. The clinical spectrum of morbidity associated with JDM ranges from rash with mild weakness to severe weakness and widespread vasculitis. While many clinical features in JDM parallel those seen in adults, it is clear that others differ significantly. Ulcerative skin lesions, overt vasculitis, and constitutional symptoms are all features rarely reported in adult dermatomyositis but are common in the childhood disease and may be associated with high morbidity and even mortality1,3.
RESULTS

Clinical details and brief immunohistological findings on the 10 JDM patients included in this study are summarized in Table 2. All 10 children presented with weakness and typical rash. The mean age of onset of weakness was 7 years 8 months and the mean duration of weakness before biopsy was 2.8 months. The Childhood Myositis Assessment Scale (CMAS), a validated tool for quantifying disease activity and damage in JDM12, was used to assess these children at the time of presentation to our clinic. A healthy child with no muscular weakness could achieve a maximum score of 53; the mean CMAS score from the 8 children old enough for the test to be reliable was only 13. Seven of the 10 children were biopsied within 2 months of their initial symptom of weakness, a higher ratio than previous series3,9,13.

Expression of both MHC Class I heavy chain (Figures 1A and E) and ß2m (Figure 1B) on muscle fibers was clearly increased in all 10 of the JDM samples, compared to controls (Figure 1C). Both sarcolemmal and cytoplasmic pattern staining were seen in the over-expression. The pattern of MHC staining on muscle fibers was frequently patchy, often with negatively staining fibers in proximity to heavily stained ones on the same section (Figure 1D). Antibodies against class I heavy chain and ß2m stained identical areas, stained ones on the same section (Figure 1D). Antibodies often with negatively staining fibers in proximity to heavily stained ones on the same section (Figure 1D). Antibodies stained ones on the same section (Figure 1D). Antibodies.

Although 50% of the biopsies in our series showed moderate or abundant inflammatory infiltrate (Figure 2A), 4 samples had minimal inflammatory cell infiltration, and 1 sample did not have any infiltrate at all (Figure 2B). At the time of muscle biopsy this patient was exhibiting typical manifestations of JDM, including myometric demonstration of proximal muscle weakness, CMAS score of 7, and laboratory findings of elevated muscle enzymes. By conventional histological examination, all aspects of her muscle biopsy were reported as normal. However this and the other 4 samples with minimal inflammatory infiltration did show increased MHC expression on muscle fibers, as did the 5 other samples with clear inflammatory changes.

Muscle fiber necrosis, fiber size variation, perifascicular atrophy, and increased perimysial connective tissue space

Table 1. Primary murine antibodies used for immunostaining.

<table>
<thead>
<tr>
<th>Antibody Against</th>
<th>Clone Name</th>
<th>Subclass</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>UCHT1</td>
<td>IgG1</td>
<td>Novacastra, UK</td>
</tr>
<tr>
<td>CD4</td>
<td>AB12</td>
<td>IgG1</td>
<td>Novacastra</td>
</tr>
<tr>
<td>CD8</td>
<td>IA5</td>
<td>IgG1</td>
<td>Novacastra</td>
</tr>
<tr>
<td>CD16</td>
<td>D1130c</td>
<td>IgG1</td>
<td>Novacastra</td>
</tr>
<tr>
<td>CD19</td>
<td>4G7/2E</td>
<td>IgG1</td>
<td>Novacastra</td>
</tr>
<tr>
<td>CD56</td>
<td>ERIC-1 &amp; MOC-1</td>
<td>IgG1</td>
<td>Novacastra, UK</td>
</tr>
<tr>
<td>HLA Class I (A,B,C)</td>
<td>W6/32</td>
<td>IgG2a</td>
<td>Novacastra</td>
</tr>
<tr>
<td>HLA DR</td>
<td>LN-3</td>
<td>IgG2b</td>
<td>Novacastra</td>
</tr>
<tr>
<td>ß2-microglobulin</td>
<td>B2M01</td>
<td>IgG2a</td>
<td>In–house</td>
</tr>
<tr>
<td>Dystrophin</td>
<td>Dy4/6D3</td>
<td>IgG1</td>
<td>Novacastra</td>
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Table 2. Clinical features at time of biopsy and histological findings of study patients with JDM.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Onset of Weakness</th>
<th>CMAS Score</th>
<th>CK at Biopsy (U/l)</th>
<th>LDH at Biopsy (U/l)</th>
<th>Time from Weakness to Biopsy (months)</th>
<th>Therapy Before Biopsy</th>
<th>Inflammatory Cell Infiltration</th>
<th>MHC I Over-expression</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>4y 0m</td>
<td>3</td>
<td>6193</td>
<td>6318</td>
<td>2</td>
<td>Nil</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>43</td>
<td>2y 7m</td>
<td>NA</td>
<td>378</td>
<td>1364</td>
<td>2</td>
<td>Nil</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>48</td>
<td>8y 10m</td>
<td>0</td>
<td>2103</td>
<td>1830</td>
<td>8</td>
<td>Steroids</td>
<td>Abundant</td>
<td>Yes</td>
</tr>
<tr>
<td>49</td>
<td>11y 9m</td>
<td>43</td>
<td>74</td>
<td>842</td>
<td>3</td>
<td>Nil</td>
<td>Minimal</td>
<td>Yes</td>
</tr>
<tr>
<td>56</td>
<td>4y 7m</td>
<td>19</td>
<td>1132</td>
<td>1735</td>
<td>2</td>
<td>Nil</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>62</td>
<td>16y 6m</td>
<td>7</td>
<td>564</td>
<td>1321</td>
<td>1</td>
<td>Nil</td>
<td>Nil</td>
<td>Yes</td>
</tr>
<tr>
<td>69</td>
<td>13y 4m</td>
<td>4</td>
<td>2771</td>
<td>1704</td>
<td>1</td>
<td>Nil</td>
<td>Minimal</td>
<td>Yes</td>
</tr>
<tr>
<td>71</td>
<td>9y 1m</td>
<td>21</td>
<td>1420</td>
<td>2311</td>
<td>2</td>
<td>Nil</td>
<td>Minimal</td>
<td>Yes</td>
</tr>
<tr>
<td>73</td>
<td>2y 4m</td>
<td>NA</td>
<td>3381</td>
<td>3136</td>
<td>5</td>
<td>Nil</td>
<td>Abundant</td>
<td>Yes</td>
</tr>
<tr>
<td>75</td>
<td>4y 4m</td>
<td>7</td>
<td>&gt; 4500</td>
<td>2436</td>
<td>2</td>
<td>Nil</td>
<td>Minimal</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Y: Years; M: months; CK: creatine kinase, upper limit of normal range 250 U/l; LDH: lactate dehydrogenase, upper limit of normal range 640 U/l. * First presented with typical rash including Gottron’s papules age 15 years 11 months.
were apparent in many of our 10 biopsies, but particularly marked in those where the time lag from the onset of weakness to biopsy was longer (Figure 2A), and those with inflammatory cell infiltration. In the 5 biopsies with significant inflammatory changes, the infiltrating cells were found in both perivascular and endomysial areas.

We analyzed the infiltrates for the presence of cells that carry specific receptors for MHC class I molecules, i.e., CD8+ T cells and NK cells. The latter generally express CD56 and may also express CD8, though typically at lower levels than conventional CD8 T cells. The predominant cell types seen in the JDM infiltrates were CD3+ T cells in a predominantly perivascular manner (Figures 2C and D), CD68+ (Figure 2G), HLA-DR+ (Figure 2H), and CD16+ (data not shown) macrophages throughout the tissue. CD19+ cells (B cells) were rarely observed. Only very occasional mononuclear cells stained for CD56, including some that were positively stained for CD8+ on adjacent sections. However the monoclonal reagents for CD56 did identify cells positive for the NCAM isofrom of CD56 (Figure 2E). NCAM is expressed on cells of neuronal and muscle origin. NCAM staining of regenerating muscle cells was seen on all but one sample. To distinguish small muscle fibers staining for NCAM from mononuclear cells, consecutive sections were stained for the muscle protein dystrophin-1 (Dys-1). Nearly all the NCAM stained cells were also positive for Dys-1. No CD56 positive Dys-1 negative cells were clearly seen on any of the 10 samples (Figures 2E and F). Infiltrating inflammatory cells were not seen on any of the control samples. Control staining confirmed that both monoclonal antibodies for CD56 could indeed identify NK cells (data not shown).

**DISCUSSION**

Over-expression of MHC Class I was first reported in adult inflammatory myopathies over 20 years ago. Constitutive MHC Class I expression on normal muscles is very low. McDouall, et al have shown the same phenomenon in both JDM and muscular dystrophy; however, interestingly we did not parallel their finding that fibers showing internal...
cytoplasmic staining were predominantly located in a perifascicular manner. This may be related to differences in the duration of disease before the biopsies were carried out.

It is remarkable that Nagaraju, et al. were able to show, using a murine model, that transgenic over-expression of self MHC class I protein in skeletal muscles led to an inflammatory myopathy with features that closely parallel human idiopathic myositis. This raises the question whether MHC Class I over-expression may be a primary trigger or central event in the pathogenic process in myositis. A recent study in adult idiopathic inflammatory myopathies found that MHC over-expression on muscle cells may be seen in the absence of inflammatory infiltrates, both in early disease and in late inactive disease. MHC Class I expression on muscles has been reported in 3 cases of JDM with minimal histological changes. We have extended these observations and shown that when patients are biopsied early, MHC Class I over-expression can occur before significant inflammatory changes, and in one case, before any inflammatory cell migration. While this feature is not specific to JDM or to the inflammatory myopathies as a whole, it does appear to be an early and sensitive feature of conditions that involve muscle damage. Whether MHC itself could be directly toxic to muscle cells remains to be answered.

Apart from collections of inflammatory lymphocytes and macrophages, features of fiber size variability in particular perifascicular atrophy and overt muscle necrosis are commonly quoted histological features (Figure 2A). In our series these are not common features, and are absent in the muscle samples where the biopsy was done soon after the onset of symptoms. In a recent series of 35 patients with JDM from Brazil where the mean time to biopsy was 11 months (± 16 months) the authors reported that myofibrillar loss, fiber necrosis, and fiber regeneration were commonly observed. This would concur with our impression that these are relatively late features.

The relative proportion of T cells, macrophages, and NK cells within muscle samples from patients with dermatomyositis in published series has been contradictory. Arahata and Engel first reported more T cells in inflammatory infiltrates in adult polymyositis. NK cells were rarely seen in adult myositis. In JDM, one report has suggested an increased proportion of NK cells in muscle biopsies of untreated cases. We have been unable to identify NK cells positively in our series of 10 JDM biopsies taken in early disease, but have confirmed that the predominant cell within the perivascular infiltrates at this stage is the T cell, typically with more CD4 than CD8 cells. The reasons for the discrepancy between our data and the previous reports are unclear but may represent differences in time of biopsy, chronicity of disease, or different patient populations. We are confident that in our series of biopsy samples, the majority of cells staining for NCAM/CD56 are muscle tissue as evidenced by their positive Dys-1 staining.

The 5 patients where we found minimal or no inflammatory cell infiltration were those in whom the biopsy was performed very early after the onset of symptoms (all within 2 months of onset of weakness). In all these samples MHC Class I upregulation on muscles was clearly evident. This would be consistent with our hypothesis that the latter is an early event rather than secondary to cytokine stimulation from infiltrating cells. Taken together with the novel murine model described above, further evaluation of this phenomenon is clearly indicated.

On only one sample in our 10 cases did we find evidence of significant numbers of CD19+ B cells within the mononuclear infiltrates. This is in contrast to some reports that inflammatory infiltrates in adult dermatomyositis are predominantly perimysial and composed of CD4+ and B lymphocytes. McDouall, et al. found evidence of B cells in JDM samples but commented that they were never the predominant cell type.

We conclude that MHC Class I molecules are over-expressed on muscles in early JDM. Our results suggest that this phenomenon may occur independent of inflammatory cell infiltration and that it may be a highly sensitive, although not specific, indicator of muscle damage. In terms of cells with receptors that recognize the MHC (and any peptides presented therein), CD8+ T cells are predominantly located in perivascular areas, while NK cells were not found in excess of normal controls in any of our biopsies.

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