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 ß$_2$-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 ß$_2$-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 monocytic 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
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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 mortality[3,4].

Mononuclear cell infiltration and MHC Class I over-expression are 2 well-described phenomena in adult inflammatory muscle disease[5,6] and also in dystrophies such as Duchenne's and Becker's muscular dystrophy[7,8]. 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 analyses[3,9,10]. 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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or primary monoclonal antibodies (Table 1) followed by standard avidin biotin complex protocol. Primary antibodies to identify T cells and T cell subsets (CD3, CD4, and CD8), B cells (CD19) and macrophages (CD68) were used. Antibodies raised against MHC Class I heavy chains and ß$_2$-microglobulin (ß$_2$m) were used to assess the expression of the Class I glycoprotein complex. To identify cytotoxic T cells, consecutive slides were stained with antibodies against CD3 and CD8. In order to identify natural killer (NK) cells, we examined CD56 and CD16 expression. CD16 (FcgRIII) is also expressed on CD68+ macrophages, while CD56 is an isoform of neuronal cell adhesion molecule (NCAM), which is also expressed on tissues of muscle or neural origin and is elevated in regenerating muscle cells[11]. We performed staining of consecutive slides with CD56 and dystrophin (Dys-1), since cells staining for both of these proteins can be confidently identified as being of muscle origin, while small cells that stain positive for CD56 and CD16 but negative for Dys-1 are likely to be NK cells. Two different reagents specific for the CD56 molecule were used: both stained NK lymphocytes on a positive control.

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 JDM[12], 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 series[3,9,13]. Expression of both MHC Class I heavy chain (Figures 1A and E) and ß$_2$m (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 l heavy chain and ß$_2$m stained identical areas, indicating normal coexpression of the 2 chains of class l MHC (Figures 1A and B). However immunohistochemical data are qualitative, so our results cannot exclude the possibility that some MHC heavy chain might be expressed within the endoplasmic reticulum alone, if it is in excess.

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.

Antibody Against	Clone Name	Subclass	Supplier
CD3	UCHT1	IgG1	Novacastra, UK
CD4	AB12	IgG1	Novacastra
CD8	1A5	IgG1	Novacastra
CD16	DJ130c	IgG1	Novacastra
CD19	4G7/2E	IgG1	Novacastra
CD56	ERIC-1 & MOC-1	IgG1	Novacastra, UK DAKO, UK
HLA Class I (A,B,C)	W6/32	IgG2a	Novacastra
HLA DR	LN-3	IgG2b	Novacastra
ß$_2$-microglobulin	B2M01	IgG2a	In–house
Dystrophin	Dy4/6D3	IgG1	Novacastra

Table 2. Clinical features at time of biopsy and histological findings of study patients with JDM.

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

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.

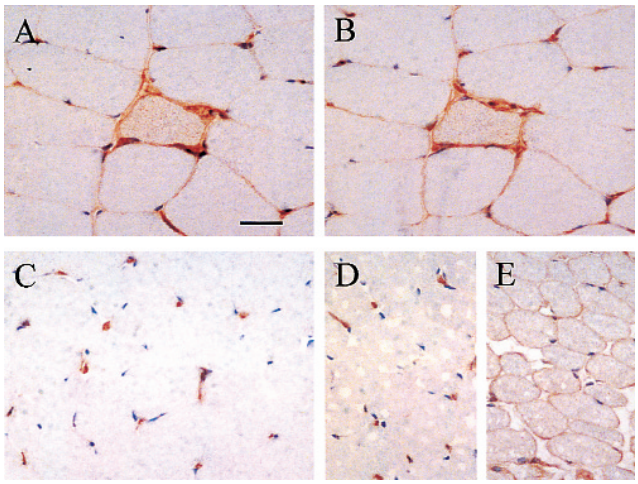

Figure 1. MHC over-expression on muscles in early juvenile dermatomyositis. (A) MHC Class I heavy chain and (B) ß$_2$-microglobulin staining of muscle biopsy from a 16-year-old girl with very early JDM. Note both sarcolemmal (fiber edge) and cytoplasmic staining. (C) MHC Class I staining on normal control: endomysial capillaries but not muscle fibers are positively stained. (D+E) Patchy MHC expression: 2 high powered fields on the same biopsy section of a 4-year-old girl with early JDM, showing a negative area on the left and a positive staining area on the right. Scalebars on all images: 30 µm.

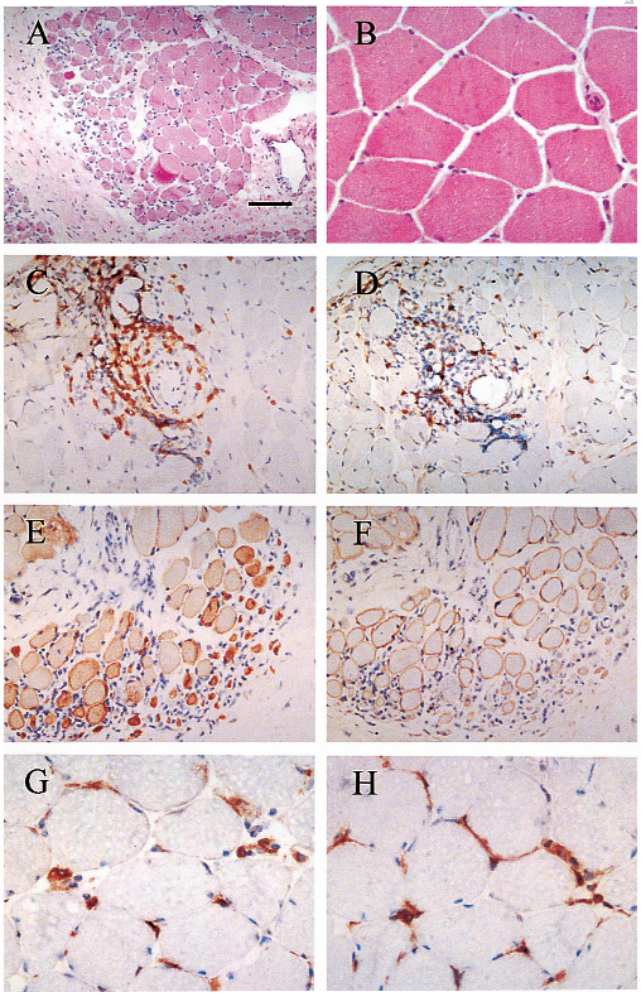

Figure 2. (A) H+E staining of muscle biopsy of an 8-year-old girl with severe JDM showing perifascicular atrophy, muscle fiber degeneration and inflammatory infiltrates. (B) H+E staining of biopsy from a child with early JDM (same patient as Figure 1A and B) showing no inflammatory infiltrate, no variation in muscle fiber size, and no fiber necrosis. (C-H) Inflammatory mononuclear cell infiltration in severe JDM. (C) CD3 and (D) CD8 staining in a perivascular pattern. (E) Muscle fibers, including atrophic perifascicular fibers stained positive for CD56/NCAM. (F) Consecutive section immunostained with anti-dystrophin showing that CD56+ cells are of muscle origin. (G) CD68 and (H) MHC Class II staining on consecutive sections showing endomysially distributed macrophages as the predominant inflammatory cells. Scalebars on (A): 120 µm; (B,G,H): 30 µm; (C-F): 60 µm.

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 isoform of CD56 (Figure 2E). NCAM is expressed on cells of neuronal and muscle origin[10,13]. 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[15]. McDouall, *et al* have shown the same phenomenon in both JDM and muscular dystrophy[7,9]: 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*[16] were able to show, using a murine model, that transgenic over-expression of self MHC class l 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[17]. MHC Class I expression on muscles has been reported in 3 cases of JDM with minimal histological changes[10]. 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[18].

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 is done soon after the onset of symptoms. In a recent series of 35 patients with JDM from Brazil[13] 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[19,20] first reported more T cells in inflammatory infiltrates in adult polymyositis. NK cells were rarely seen in adult myositis[21,22]. In JDM, one report[23] 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[15], 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[6,21]. McDouall, *et al*[9] 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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