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Original article

MHC class I antigens, CD4 and CD8 expressions in polymyositis and dermatomyositis

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ABSTRACT

Objective: To analyze the frequencies of the expression of major histocompatibility complex class I (MHC-I) antigens, and CD4 and CD8 cells in skeletal muscle in polymyositis (PM) and dermatomyositis (DM).
Methods: This was a retrospective study of 34 PM cases, 8 DM cases, and 29 control patients with non-inflammatory myopathies.
Results: MHC-I antigens were expressed in the sarcolemma and/or sarcoplasm in 79.4% of PM cases, 62.5% of DM cases, and 27.6% of controls (CD4 expression was observed in 76.5%, 75%, and 13.8%, respectively). There was a high suspicion of PM/DM (mainly PM) in participants in whom MHC-I antigens and CD4 were co-expressed. In 14.3% of PM/DM cases, we observed MHC-I antigens expression alone, without inflammatory cells.
Conclusion: MHC-I antigens expression and CD4 positivity might add to strong diagnostic suspicion of PM/DM. No cellular infiltration was observed in approximately 14.3% of such cases.

Expressão de antígenos MHC classe I e de células CD4 e CD8 na polimiosite e dermatomiosite

Palavras-chave:
Patologia muscular
Imuno-histoquímica muscular
Biópsia muscular
Antígenos de complexo principal de histocompatibilidade classe I (MHC-I)
Miopatias inflamatórias

RESUMO

Objetivo: Analisar as frequências de expressão dos antígenos de complexo principal de histocompatibilidade classe I (MHC-I) e células CD4 e CD8 no músculo esquelético na polimiosite (PM) e dermatomiosite (DM).
Métodos: Estudo retrospectivo de 34 casos de PM, 8 casos de DM e 29 controles com miopatias não inflamatórias.
Resultados: Os antígenos MHC-I expressaram-se no sarcolema e/ou sarcoplasma em 79,4% dos casos de PM, 62,5% dos casos de DM e 27,6% dos controles (a expressão de CD4 foi

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observada em 76,5%, 75% e 13,8%, respectivamente). Quando os antígenos MHC-I foram coexpressados com CD4, houve elevada suspeita de PM/DM (principalmente PM). Em 14,3% dos casos de PM/DM, observou-se a expressão isolada dos antígenos MHC-I, sem células inflamatórias.

Conclusão: A expressão dos antígenos MHC-I e a positividade do CD4 podem aumentar a suspeita diagnóstica de PM/DM. Não foi observado infiltrado celular em 14,3% dos casos.

Introduction

Inflammatory myopathies (IM) constitute a heterogeneous group of autoimmune diseases that are characterized clinically by weakness and inflammation in skeletal muscle. Accurate diagnosis is critical, as IM are potentially treatable myopathies. Based on clinical and histopathological characteristics, three main subgroups of IM were defined: polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM). The diagnosis of these diseases is based on clinical examination and laboratory tests, particularly creatine kinase, electromyogram, and muscle pathology findings obtained after biopsy.[1–3] The hallmarks for correct diagnosis on muscle biopsy are muscle fiber necrosis (usually in isolated spots) and the presence of inflammatory cells in the perimysial and endomysial regions, and also often in perivascular regions. A typical finding is lymphocytic non-necrotic fiber invasion, which is soon replaced by macrophages and T-cells after it becomes necrotic.[4] Perifascicular atrophy is specific to and a hallmark feature of DM, as rimmed-vacuoles are for IBM.[4] It is well recognized that the absence of inflammatory infiltrates does not exclude an IM. In these cases, the presence of major histocompatibility complex class I (MHC-I) antigens in sarcolemma and/or sarcoplasm might contribute to diagnostic suspicion of IM, although it is not specific to IM.

In normal muscle fibers, MHC-I antigens are only detected on blood vessels and can be easily seen on endomysial capillaries. In contrast, in IM MHC-I antigens expression were observed on the sarcolemma and also internally (sarcoplasm) in several fibers.[5,6] MHC-I antigens induction and expression occurs early, frequently before the inflammatory infiltrates, and continues throughout the evolution of this chronic disease, even with the use of immunosuppression and after apparent clinical remission.[1,7–10]

Regenerating and/or immature muscle fibers exhibited consistent MHC I antigens sarcolemmal expression irrespective of disease;[11] because of that, it is important to distinguish this normal finding from the abnormal labeling on mature fibers by using a marker for immaturity, such as neonatal myosin.[4] The expression of MHC-I in the sarcolemma and/or the sarcoplasm of mature muscle fibers is abnormal and represents a useful tool for the diagnosis of IM, particularly in the absence of inflammatory infiltrates, muscle fiber necrosis, rimmed vacuoles, or perifascicular atrophy.

The present study was figured out to emphasize the importance of routinely making the MHC I antigens together with subtypes of T cells expression on muscle biopsy techniques if there is IM suspicion. In spite of the IM pathological hallmark finding being an inflammatory infiltrate (as stressed above), sometimes this could be missed.

Material and methods

Patients

Seventy-one patients assisted at the Hospital de Base, Faculdade de Medicina de São José do Rio Preto (FAMERP) were studied, from June 2005 to June 2013. They were referred for muscle biopsy from several medical specialties, particularly neurology and rheumatology.

Two patient groups were constituted for evaluation. Group 1 consisted of 42 patients with a consistent clinical picture and muscle pathology characteristic of PM or DM, as follows: muscle necrosis, endomysial and/or perivascular inflammatory infiltrate (Fig. 1A and B), invasion of non-necrotic muscle fibers, and/or perifascicular atrophy. Group 2 consisted of 29 patients referred for muscle biopsy with suspicion of a myopathy other than IM, but with normal or non-specific and non-inflammatory muscle pathology abnormalities.

Muscle biopsy

All muscle biopsies from *Deltoideus* were performed by a physician specialized in neuromuscular disorders, using an open technique under local anesthesia. Each muscle sample was forwarded to the Laboratory of Neuromuscular Investigation in a fresh state with no fixatives or additives, and was immediately frozen in liquid nitrogen and stored at −176 °C until processing. Frozen muscle specimens were cut into sections of 5 μm thickness using a cryostat at a temperature of −30 °C, and slices were mounted on glass slides coated in poly-lysine. The distribution of MHC-I antigens was investigated using a monoclonal antibody by the immunoperoxidase technique. Antibodies labeling to be seen under optical microscopy were performed using a polymer detection system (NovoLink Max Polymer Detection System, Novocastra Laboratories Ltd., Newcastle upon-Tyne, England) according to the manufacturer's instructions. The antibodies used were: anti-MHC-I (Rhea Biotech, Campinas, Brazil), anti-CD8 (monoclonal mouse, 1A5 clone, IgG1 isotype), and anti-CD4 (monoclonal mouse, 4B12 clone, IgG1 isotype). CD4 and CD8 antibodies were from DakoCytomation Denmark A/S, Denmark.

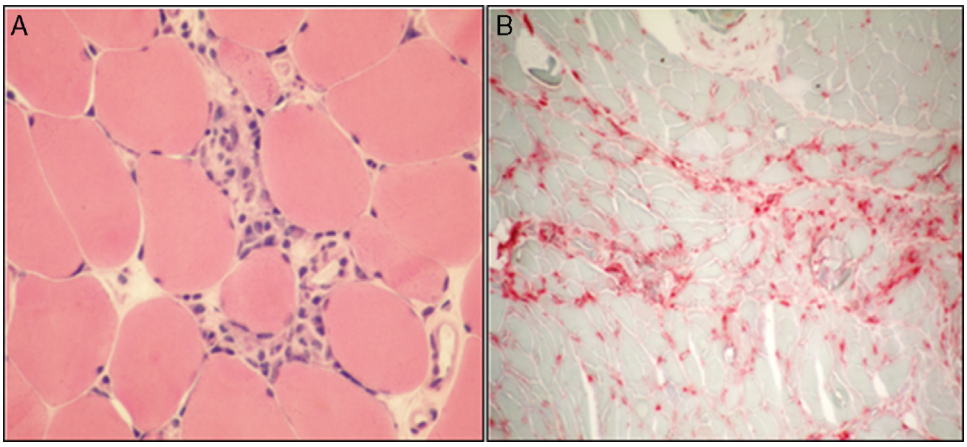

Fig. 1 – Deltoideus muscle biopsy from a patient with inflammatory myopathy. (A) Muscle fiber necrosis and endomysial inflammatory infiltrate (hematoxylin and eosin). (B) Increased lysosomal activity (acid phosphatase).

Statistics

A Chi-squared test was used for the comparison of two proportions, expressed as a percentage. *p*-values <0.05 were defined as statistically significant.

Ethics

The study was approved by the ethics committee of the FAMERP.

Results

Group 1 encompassed of 42 patients (PM or DM): 28 female (66.7%) and 14 male (33.3%), mean age 44.7 ± 19.9 years (range 6–80 years). Thirty-four of these 42 cases (80.1%) were PM patients [22 female (64.7%) and 12 male (35.3%); mean age 49.3 ± 17.7 years (range 8–80 years)]. The remaining 8 cases (19.9%) were DM patients [6 female (75%) and 2 male (25%); mean age 25.3 ± 17.4 years (range 6–51 years)]. Group 2 consisted of 29 patients: 17 female (58.6%) and 12 male (41.4%), mean age 34.2 ± 21.9 years (range 1–71 years).

The frequency of MHC-I antigens expression in muscle fibers are shown in detail in table. Fig. 2A–C depicts its expression in the sarcolemma or sarcoplasm, as well as lack of expression. MHC-I antigens expression in either sarcoplasm or sarcolemma were observed in 79.4% of PM patients, 62.5% of DM patients, and 27.6% of controls. No MHC-I antigens expression were observed in either the sarcolemma or the sarcoplasm in 20.6% of PM patients, 37.5% of DM patients, and 72.4% of controls.

The frequencies of antibody positivity for CD4 and CD8 are detailed in Table 1. Fig. 3A–C depicts CD4/CD8 expression mainly in the endomysium. The majority of PM cases (76.5%) tested positive for CD4 expression, while 23.5% tested negative for both CD4 and CD8. Similarly, 75% of DM cases tested positive for CD4, and 25% tested negative for both CD4 and CD8. Either CD4 or CD8 expression was observed in 24.1% of control samples; however, expression of both CD4 and CD8 was observed in 0% of control samples, and 75.9% tested negative for both CD4 and CD8.

MHC-I antigens expression in either the sarcoplasm or the sarcolemma were observed together with CD4 positivity in 88.2% of PM, 50% of DM, and 3.5% of control samples. In comparison, MHC-I antigens expression in either the sarcoplasm or the sarcolemma were observed together with CD8 positivity in 35.3% of PM, 12.5% of DM, and 0% of control samples.

In 5 PM cases (14.7%) and in 1 DM case (12.5%), MHC-I antigens were expressed either in sarcolemma or sarcoplasm in the absence of both CD4 and CD8.

Discussion

The present study demonstrated that the expression of MHC-I antigens in either the sarcolemma or the sarcoplasm occurred more often in patients with PM/DM than in controls, although only the difference between controls and PM patients was statistically significant. Overall, MHC-I antigens were expressed in 79.4% of PM/DM patients. Overall, the sensitivity of the test for diagnosing IM was 78%, similar to ours (79.4%).[12] When we considered only sarcolemmal MHC-I antigens expression, there was no significant difference between controls and either PM or DM patients. When we considered only sarcoplasmic MHC-I antigens expression, the difference was significant only between controls versus PM patients. MHC-I antigens were not expressed in both sarcolemma and sarcoplasm in most controls, which differed significantly only from PM cases. According Karpati et al.,[11] in PM the majority of muscle fibers exhibited strong sarcolemmal MHC-I antigens expression and in DM, muscle fibers situated perifascicularly or distributed in random clusters revealed strong expression.

Overall, 76.2% of PM/DM patients tested positive for CD4 and/or CD8. The positivity of inflammatory cells (CD4) was higher in cases with PM/DM versus controls, with a significant difference for both. CD8 positivity was less pronounced but still significant for PM cases, although not for DM cases. The rate at which controls tested negative for CD4 and CD8 was significant when compared with both PM and DM. None on the controls were positive for both CD4 and CD8 and represented a striking finding.

The expression of MHC-I antigens in either the sarcolemma or the sarcoplasm in combination with CD4 positivity were

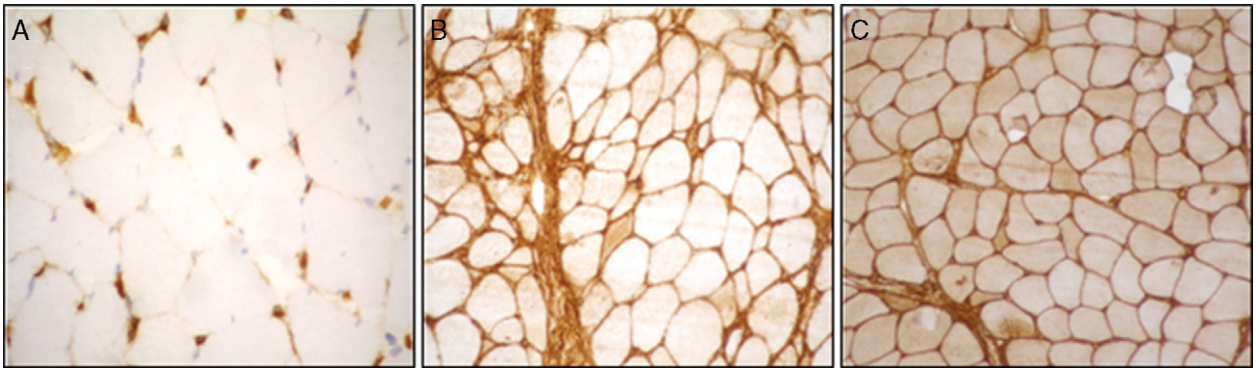

Fig. 2 – MHC-I antigens expression in muscle fibers. (A) negative; (B) sarcolemma; (C) sarcolemma and sarcoplasm.

the most common finding in PM versus controls, and this was the most useful finding for PM diagnosis. This finding was less useful for DM diagnosis, although it was still a useful finding in that context. It is also worthwhile to emphasize that we did not observe any association between MHC-I antigens expression (either in sarcolemma or sarcoplasm) and CD8 expression in control samples. This finding likely represents a very useful tool to rule out PM/DM.

We observed no inflammatory infiltrate in 5 PM and 1 DM cases, although MHC-I antigens were expressed either in the sarcolemma or in the sarcoplasm. This finding showed that approximately 15% of PM/DM cases could not be diagnosed based only on inflammatory infiltrate, corroborating the statement from Dalakas[1] that the expression of MHC-I antigens are a useful marker to confirm the diagnosis of IM even when there is no evidence of inflammatory cells in the muscle biopsy.

According to muscle biopsy results from van der Pas et al.,[13] the expression of MHC-I antigens were observed in 67% patients with DM and in 61% of patients with PM. In DM cases, the immunohistochemical analysis revealed significantly higher MHC-I antigens expression in the juvenile form (96.4%) than in the adult form (50%).[12] MHC-I antigens expression were observed in 11% of biopsies from patients with muscular dystrophy and in 4% of biopsies from patients with a miscellaneous neuromuscular disorder, totaling 15% (less than our observed expression of 27.6%).

Most muscle fibers invaded by CD4 and/or CD8 express MHC-I antigens on the surface.[7] However, as noted above, sometimes the MHC-I antigens expression were observed without invasion by mononuclear cells. Nyberg et al.[14] has also emphasized the importance of MHC-I antigens expression in inactive chronic PM or DM with persistent muscle weakness in the absence of both inflammatory infiltrates and signs of inflammation on magnetic resonance imaging. In addition, the expression of MHC-I antigens are not modified by prior treatment with immunosuppressive drugs, although van der Pas et al.[13] reported a decrease of sensitivity for the MHC-I antigens test after 4 weeks of immunosuppressive therapy.

It should be emphasized that MHC-I antigens can also be expressed in muscular dystrophies (mainly dysferlin deficiency, which clinically presents as limb-girdle dystrophy and distal myopathy).[15] In these cases, an increased inflammatory response was observed together with an active dystrophic pattern. The cellular infiltrates suggest that the

Table 1 – Expression of MHC-I antigens (sarcolemma and sarcoplasm), CD4 and CD8 in muscle biopsies from patients with polymyositis (PM), dermatomyositis (DM) and controls (C).

	PM	DM	Controls	*p*	*p*
N	34	8	29	PM/C	DM/C
Age	49.3 ± 19.9	25.3 ± 17.4	34.2 ± 21.9		
Male	35.3% (12)	25% (2)	41.4% (12)		
Female	64.7% (22)	75% (6)	58.6% (17)		
MHC-I (+) sarcolemma	47.1% (25)	62.5% (5)	24.1% (7)	0.1037	0.1035
MHC-I (+) sarcoplasma	73.5% (16)	50.0% (4)	13.8% (4)	<0.0001	0.0860
MHC-I (+) both	41.2% (14)	50.0% (4)	10.3% (3)	0.0135	0.0424
MHC-I (+) (either)	79.4% (27)	62.5% (5)	27.6% (8)	0.0001	0.1579
MHC-I (–) both	20.6% (7)	37.5% (3)	72.4% (21)	0.0001	0.1579
CD8 (+)	38.2% (13)	12.5% (1)	10.3% (3)	0.0247	0.6410
CD4 (+)	76.5% (26)	75.0% (6)	13.8% (4)	<0.0001	0.0027
CD4 and CD8 (+)	38.2% (13)	12.5% (1)	None (0)	0.0006	0.4846
CD4 and/or CD8 (+)	76.5% (26)	75.0% (6)	24.1% (7)	0.0001	0.0243
CD4 and CD8 (–)	23.5% (8)	25.0% (2)	75.9% (22)	0.0001	0.0243
MHC-I (either) and CD4 (+)	88.2% (30)	50.0% (4)	3.5% (1)	<0.0001	0.0048
MHC-I (either) and CD8 (+)	35.3% (12)	12.5% (1)	None (0)	0.0012	0.4846

$p < 0.05$ was considered statistically significant.

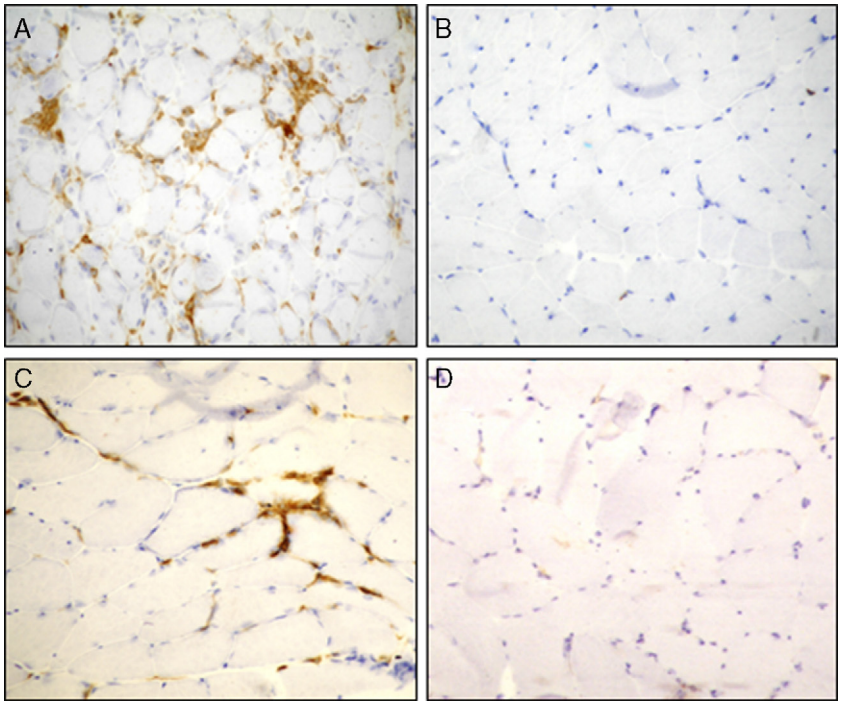

Fig. 3 – CD4 and CD8 expressions: (A) CD4-positive; (B) CD4-negative; (C) CD8-positive; (D) CD8-negative.

inflammatory reaction is secondary to necrosis. MHC-I antigens were overexpressed mainly in association with fiber phagocytosis and regeneration.[15,16] In dysferlinopathies, CD8 lymphocytes are rare and T lymphocytes invade muscle fibers only occasionally, while both are common findings in PM.[17] Dysferlinopathy should be considered in the differential diagnosis of IM that are unresponsive to steroids.

Conclusion

The presence of MHC-I antigens and subtypes of T cells expression could be useful to help the clinician differentiate in those cases where differential between inflammatory versus other non-inflammatory myopathies cases, sometimes difficult to distinguish on clinical grounds. MHC-I antigens was more often expressed in PM; more cells were positive for CD4 in PM and DM; MHC-I antigens were expressed without inflammatory cells in 14.6% of PM/DM cases; there is a high suspicion of PM/DM (mainly PM) when MHC-I antigens were expressed in combination with CD4 positivity; and there is a high probability to rule out both PM and DM in the absence of both MHC-I antigens and CD4 expression.

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Conflicts of interest

The authors declare no conflicts of interest.

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