Clinical and Serologic Characterization of an Argentine Pediatric Myositis Cohort: Identification of a Novel Autoantibody (anti-MJ) to a 142-kDa Protein

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ABSTRACT. ***Objective.*** Autoantibodies are frequently found in adult patients with polymyositis (PM), dermatomyositis (DM), and overlap myositis disorders. They are less common in pediatric patients with myositis. We investigated the autoantibody pattern in a pediatric Argentine Caucasian cohort to characterize novel autoantibodies.

Methods. Sera from children that satisfied published criteria for idiopathic inflammatory myopathy were analyzed for autoantibodies by RNA and protein immunoprecipitation and immunoblotting techniques. Routine myositis-specific and myositis-associated autoantibodies as well as autoantibody specificities were determined.

Results. We tested sera from 64 consecutive pediatric myositis patients, including 40 with juvenile DM, 7 with juvenile PM, and 17 with overlap myositis syndromes. Sixteen (25%) patients were found to have anti-MJ autoantibody exclusively, which appears to identify a subset of pediatric myositis patients with severe disease characterized by muscle contractures and atrophy and significant compromise of functional status. Fourteen (22%) patients were found to have an antibody targeting 2 proteins of 155 and 140 kDa. Other myositis-specific autoantibodies were uncommon in this pediatric cohort.

Conclusion. A newly recognized autoantibody, anti-MJ, was the most common antibody found in this Argentine pediatric cohort. The clinical features indicated that this antibody is distinct from other reported antibodies in pediatric patients with myositis. (J Rheumatol First Release Oct 15 2009; doi:10.3899/jrheum.090461)

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The idiopathic inflammatory myopathies (IIM) consist of a heterogeneous group of systemic rheumatic disorders characterized by the presence of chronic inflammation of skeletal muscle. Dermatomyositis (DM) is the most common inflammatory myopathy observed in childhood, but overlap myositis syndromes, polymyositis (PM), and less frequently cancer-associated myositis or inclusion body myositis have been reported. Although the frequency of the clinical IIM subsets differs between adults and children, some clinical and histopathologic features are similar. Various classification schemes exist for adult IIM, and autoantibody markers such as the myositis-specific autoantibodies (MSA) define clinically homogeneous patient groups. Such autoantibodies also identify patients with similar immunogenetic markers, prognoses, and responses to treatment. Depending on the means of detection, autoantibodies have generally been found in 50%–75% of adults with IIM, but are significantly less common in children, particularly the MSA and myositis-associated autoantibodies (MAA) (5%–15%).

In a well defined cohort of patients with childhood myositis, we evaluated the frequency of MSA and MAA, and characterized the clinical features associated with the serologic findings.

MATERIALS AND METHODS

Patients. Sixty-four consecutive and unselected patients with childhood myositis were seen from 1987 to 1997 in the outpatient clinics at the Rheumatology Sections of the Institute of Psychophysical Rehabilitation and Ricardo Gutierrez Children's Hospital, Buenos Aires. All were tested for the presence of MSA and MAA at the University of Pittsburgh and are included in this study. All 64 patients were "Argentine Caucasian," i.e., born in Argentina to families of Spanish or Italian descent. Patients were

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classified as having juvenile DM (JDM) or juvenile PM (JPM) according to the criteria of Bohan and Peter[1], or myositis in overlap with another connective tissue disease (CTD) with an age of symptom onset of < 16 years. Patients were classified as having mixed CTD (MCTD) if they met the criteria of Kasukawa, *et al*[2]. Patients with myositis in overlap with another CTD met published criteria for that rheumatic disease. None of the children had cancer.

The clinical records were retrospectively reviewed and the following information was recorded: demographic data included sex, race, age at disease onset, duration of disease and follow-up; clinical features including the presence of Raynaud phenomenon; proximal muscle weakness, muscle/joint contractures or muscle atrophy; cutaneous signs of DM (heliotrope rash, Gottron papules and/or sign, erythematous rash of neck and upper trunk: V-sign or shawl-sign rash); inflammatory arthritis; interstitial lung disease (ILD; defined by fibrosis on chest radiograph or a decrease in forced vital capacity or diffusing capacity < 80% predicted); dysphagia (corroborated by deglutition study); and cutaneous signs of vasculitis or calcinosis.

Clinical disease activity in patients with DM was assessed (at the time the serum sample was taken for autoantibody screening) according to published criteria as "definitely active" (characteristic DM rash, elevated serum muscle-derived enzymes, and proximal muscle weakness), "possibly active" (characteristic DM rash, muscle-derived enzymes within the normal range, and mild proximal muscle weakness), and "inactive" (no physical examination or laboratory evidence of disease activity). The clinical course was termed monocyclic, polycyclic, or chronic-continuous, as described[3]. Steinbrocker criteria, although originally developed for rheumatoid arthritis, were used to evaluate functional outcome[4].

Autoantibody studies. Serologic testing included assessment of MSA and MAA at the University of Pittsburgh by protein and RNA immunoprecipitation (IP) and resolved on an 8% polyacrylamide gel (sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SDS-PAGE) as described[5,6].

IP Western blots. Because the MJ protein[7] is similar in size to the 140-kDa protein of p155/140[8], all patients' immunoprecipitating proteins of approximately 140 kDa were rerun on a 5% gel (Figure 1), where these proteins are easily resolved. As shown in Figure 1, the MJ protein migrates slightly above the 140 kDa protein of p155/140. We therefore chose to identify it as a 142-kDa protein to distinguish it from the other similar-size autoantigens. Because many patients immunoprecipitating the 142-kDa protein did not immunoblot the immunoprecipitates of our index patient MJ on IP Western blot (data not shown), 61 of 64 patients were evaluated by a technique similar to that described by Targoff, *et al* as "reverse" IP Western blot[8]. For this technique a 60-µl serum sample was bound overnight at 4°C to 6 mg Protein A Sepharose CL-4b beads (GE Life Sciences, Piscataway, NJ, USA), washed 3 times with IP buffer (10 mM Tris/HCl, pH 8.0, 500 mM NaCl, 0.1% Igepal CA630), and incubated 2 h at 4°C with unlabeled extract from 2×10^7 rapidly dividing K562 cells. The beads were washed 3 times with IP buffer, suspended in 2× Laemmli sample buffer, and electrophoresed at 200 V on a 5% SDS-PAGE standard-size gel with a 5% stack. The proteins were electrophoretically transferred to nitrocellulose membrane. The membrane was blocked overnight in 10% milk/0.01 M phosphate buffered saline (PBS)/0.05% Tween and probed for 1 h with a 1/100 dilution of a patient serum previously found to immunoblot the immunoprecipitates of our index patient MJ (data not shown). A 1/10,000 dilution of the secondary antibody, anti-human IgG Fc-horseradish peroxidase conjugate, was added and incubated an additional 1 h at room temperature. The membrane was washed 4 times with PBS and developed with Renaissance Plus® (NEN Life Science Products, Boston, MA, USA) chemiluminescence and visualized by autoradiography.

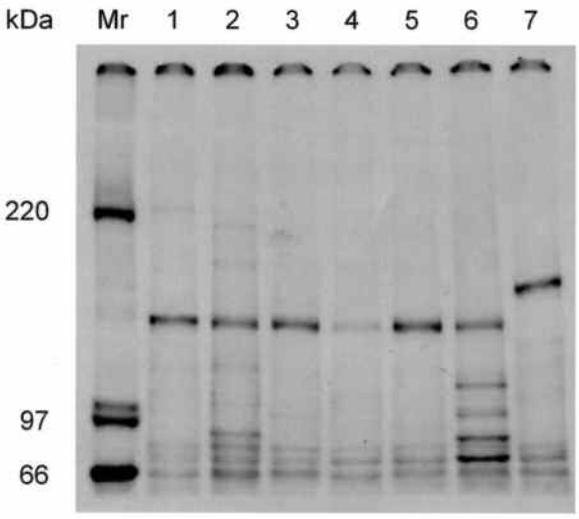

Figure 1. Seven patients immunoprecipitated proteins of apparent molecular weight 142 kDa from ^{35}S methionine-labeled K562 whole-cell extract, separated on an 8% SDS polyacrylamide 20-cm gel (data not shown). Immunoprecipitation was repeated for these 7 patients and the proteins electrophoretically resolved on a 5% SDS polyacrylamide minigel, enhanced with sodium salicylate and visualized by autoradiography (shown here). Lanes 1–6 represent patients included as "MJ" in Table 2; Lane 7, a patient included as "p155/140" in Table 2. Also shown is a ^{14}C molecular weight marker (Mr).

RESULTS

The demographic features of the 64 children are outlined in Table 1. Forty (63%) had JDM, 17 (27%) had myositis overlap syndromes (including 11 in overlap with different CTD, 3 with MCTD, and 3 with SLE), and 7 (10%) had JPM. Most patients were female (48/64, 75%) with a mean age at myositis onset of 8.3 years. At the time of study entry, the mean disease duration was 4.5 years (range 1.2–17) with a mean followup time of 3 years (range 5–17).

Table 2 outlines the serologic findings in our pediatric cohort. Only 4/64 (6%) patients had an antibody currently defined as an MSA, all with JDM and the anti-Mi-2 autoantibody. MAA were found alone in 10 (16%) patients; 7 with anti-U1RNP, 2 with anti-PM-Scl (although 3 additional patients had PM-Scl with another antibody), and 1 with anti-Ku. Sixteen patients (25%) precipitated anti-MJ alone (while 2 patients had an additional autoantibody). Consistent with prior reports, none of the 18 MJ-positive

Table 1. Demographic features of 64 patients with childhood idiopathic inflammatory myopathies. Of the 17 patients with myositis overlap syndromes, 3 had mixed connective tissue disease, 3 had systemic lupus erythematosus, and the remainder had myositis in overlap with other connective tissue diseases.

Feature	Total, n = 64	JDM, n = 40	JPM, n = 7	Myositis Overlap Syndromes, n = 17
Sex F/M	48/16	30/10	5/2	13/4
Mean age at onset, yrs	8.3	8.7	9.8	10.8
Mean disease duration, yrs	4.5	4	3.5	4.8
Mean followup, yrs	3	3	3	3.8

JDM: Juvenile dermatomyositis; JPM: Juvenile polymyositis.

Table 2. Clinical features in patients with single myositis-specific autoantibody (MSA) or myositis-associated autoantibody (MAA). Four patients not included in the table had 2 autoantibodies: 2 with PM-Scl/Ku, 1 with PM-Scl/MJ, 1 with MJ/Mi-2. Four patients also possessed the anti-Sm autoantibody, which is not an MSA or MAA.

Clinical Manifestation	Mi-2 (n = 4)	PM-Scl (n = 2)	U1RNP (n = 7)	Ku (n = 1)	MJ (n = 16)	p155/140 (n = 14)
Sex F/M	3/1	1/1	5/2	1/0	12/4	11/3
Diagnosis						
JPM	—	—	—	—	2	—
JDM	4	1	3	—	13	11
JOS	—	1	4	1	1	3
Dysphagia	2	1	—	—	5	3
Muscular						
Proximal weakness	4	2	7	1	14	14
Contractures	—	1	1	—	7	7
Atrophy	4	1	—	—	7	2
DM cutaneous signs	4	—	1	—	14	11
Cutaneous vasculitis	—	1	1	—	6	8
Calcinosis	1	—	—	—	5	5
Interstitial lung disease	—	1	2	—	4	1
Arthritis	1	1	3	—	6	9
Raynaud phenomenon	—	2	3	—	1	4

JOS: juvenile overlap syndrome; JPM: juvenile polymyositis; JDM: juvenile dermatomyositis.

patients precipitated an MJ-associated RNA[7]. No patient precipitated a 140-kDa protein in the absence of the 155-kDa protein. All patients precipitating the 142-kDa protein were positive for anti-MJ by either standard or "reverse" IP Western blot. All other patients were negative by "reverse" IP Western blot. Four patients had anti-Sm, 2 with SLE and myositis and 2 having polyarthritis in overlap with JDM. Fourteen patients (13%) precipitated 2 proteins of 155 and 140 kDa — consistent in size with the newly described anti-155/140[8] and no associated RNA, but further confirmatory investigations were not completed. No defined autoantibodies were detected in 12 patients.

The clinical features of this cohort in conjunction with the autoantibody profile are described in Table 2. All patients had proximal muscle weakness and, as expected, anti-Mi-2 antibody-positive patients had typical cutaneous features including a heliotrope rash and Gottron sign. Dysphagia and calcinosis were uncommon, but were noted in 3 anti-Mi-2-positive patients. Both anti-PM-Scl-positive patients had typical Raynaud phenomenon, and one had severe ILD in overlap with systemic sclerosis (SSc). Raynaud phenomenon, symmetric non-erosive polyarthritis, and mild ILD were most common in the subset of U1RNP-positive patients. One patient with the anti-Ku autoantibody alone had SLE in overlap with myositis and severe muscle weakness. Seven of 16 patients that precipitated the MJ 142-kDa protein demonstrated severe muscle atrophy with contractures. Six of these 16 children also had large-joint arthritis (elbows, knees, and ankles) and 5 had dysphagia. Six developed cutaneous vasculitis with persistent ulcerations, and calcinosis universalis occurred in 5 children. The children with anti-155/140 also had muscle contractures but less atrophy and suffered from more cutaneous vasculitis and arthritis than the MJ subset.

Table 3 correlates the disease course and functional status with the autoantibody findings, but only patients with 2 or more years of followup (36/64) were included. Anti-Mi-2 patients had a more benign course compared to the remaining serologic subsets, as the latter children tended to have a polycyclic or chronic-continuous disease course. Anti-MJ-positive patients had more severe disease as evidenced by their disease course consisting of functional status and more persistent disease activity, while p155/140 children generally had a more benign disease course.

DISCUSSION

Childhood IIM comprises a rare group of disorders characterized by chronic inflammation of skeletal muscle. JDM is the most common IIM subset in children, but overlap disorders that include myositis are increasingly recognized. Although childhood myositis shares similar clinical manifestations with adult IIM, patients with JDM have more overt vasculitis with the potential for life-threatening gastrointestinal involvement. The frequency of autoantibodies (MSA or MAA) in pediatric myositis is lower than that in adults as at least one-third of adult patients with myositis have MSA with distinct clinical syndromes[9]. MAA are commonly observed in patients with adult or childhood myositis and overlap syndromes are seen in patients who share similar clinical and immunogenetic features. Anti-U1RNP is found in MCTD, and anti-PM-Scl defines a syndrome that includes myositis and/or scleroderma as observed in our

Table 3. Correlation of disease course with autoantibody in a subset of juvenile myositis patients with 2+ years of followup.

Feature	Mi-2, n = 4	PM-Scl, n = 2	U1RNP, n = 6	Ku, n = 1	MJ, n = 10	p155/140, n = 13
Course						
Monocyclic	3	1	4	—	5	10
Polycyclic	1	1	2	1	4	2
Chronic-continuous	—	—	—	—	1	1
Functional status						
I–II	4	2	6	1	7	13
III–IV	—	—	—	—	3	—
Disease activity						
Active	1	1	1	1	4	8
Inactive	3	1	5	—	6	5

pediatric cohort. The anti-Ku autoantibody has been described in children with DM, MCTD, and SLE.

There are few reports of autoantibodies in children with myositis, and MSA in childhood myositis are infrequent. Feldman, *et al*[10] reported MSA (Mi-2) in only 2 of 49 patients, while Rider, *et al*[9] identified a higher frequency (12/77; 16%) in a pediatric cohort in which 11 of the 12 patients had a Hispanic background. A newly recognized autoantibody, anti-transcriptional intermediary factor 1-γ (anti-TIF1-γ)[11], targets proteins of apparent molecular weights 155 and 140 kDa. Targoff, *et al* first reported this antibody as strongly associated with DM in both children and adults[8], and Kaji, *et al* confirmed this finding, also noting its association with malignancy in adult patients with DM[12]. This antibody was found in a cohort of patients with JDM in the UK where 27/116 (23%) cases were positive for anti-p155/140, again confirming this antibody in pediatric and adult patients with myositis[13]. In our series, 14 (22%) of 64 JDM patients precipitated proteins consistent in size with this new autoantigen, but rigorous studies to demonstrate the identity of these proteins were not undertaken.

Wedderburn, *et al* recently demonstrated clear serological differences between JDM (n = 87) and JDM-SSc (n = 27) subgroups in a UK cohort of 114 patients. Seven had an MSA, 5 (7%) anti-Mi-2-positive patients in the JDM cohort and 2 (8%) Jo-1-positive patients in the JDM-SSc subgroup[14].

The main findings in our pediatric series of Argentine Caucasian patients with myositis with IIM include a low frequency of traditional MSA and the clinical description of anti-MJ-positive pediatric patients. Anti-Mi-2 was observed in only 6% of our patients, all of whom had JDM with typical cutaneous features of heliotrope, Gottron sign, and a fairly benign course. Anti-MJ was the most prevalent autoantibody in this cohort of patients, being detected alone or in combination with another autoantibody in 18 of 64 (28%) of our pediatric myositis patients. This serological group featured no major demographic differences compared to other patients, and the antibody marker was most commonly found in JDM patients but also seen in JPM patients and 1 child with overlap myositis. There was a greater frequency of muscle atrophy and contractures and greater functional compromise in children with anti-MJ autoantibody. In this Argentine Caucasian population this antibody defines a subset of pediatric patients with severe disease.

The MJ autoantibody was preliminarily described in patients with JDM who presented with severe muscle weakness, some of whom also had joint contractures, intestinal vasculitis, and polyarthritis[7]. Recently, it was reported that autoantibodies to the MJ antigen react with NXP-2, which has RNA-binding and nuclear matrix-binding domains[15]. Also, NXP-2 has been shown to be a small ubiquitin-like modifier activating enzyme (SUMO) target and to have a possible role in SUMO-mediated transcriptional repression. This is important since antibodies to SUMO-1 activating enzyme have been found in DM[16]. Although our study is limited by the small number of patients in each pediatric myositis subgroup subject to investigation, the identification of an autoantibody targeting the MJ autoantigen makes it the most prevalent autoantibody (28%) in this Argentine Caucasian pediatric cohort. Given the recent descriptions of antibodies targeting the 155/140 doublet and our description of anti-MJ, consideration should be given to including these 2 antibodies under the rubric of MSA. Future studies of larger, more ethnically heterogeneous populations of pediatric patients with IIM are necessary to further characterize the clinical features, immunogenetic associations, and specificity of the newly described anti-MJ autoantibody.

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