Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis

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Objective. Myositis-specific autoantibodies (MSAs) may define homogeneous clinical subsets of adult patients with dermatomyositis (DM). Recently, there have been descriptions of novel autoantibodies in DM. This study was conducted to establish the clinical significance of anti-p155/140 autoantibodies in juvenile DM (JDM).

Methods. The first 116 children recruited to the JDM National Registry and Repository (UK and Ireland) were studied. Comprehensive clinical features were recorded and sera screened for anti-p155/140 autoantibodies using radio-immunoprecipitation. Sera from adults with DM (n=20), PM (n=25), SSc (n=150), SLE (n=40) and healthy subjects (n=50) were used for comparison. Immunodepletion experiments were used to establish whether the p155/140 kDa targets recognized by JDM sera were the same as adult DM sera.

Results. Twenty-seven out of 116 (23%) JDM cases were positive for anti-p155/140 in comparison with 6/20 (30%) adults with DM. Immunodepletion confirmed that the 155/140 kDa proteins recognized by JDM and adult DM sera were the same targets. All other adult control sera were negative for anti-p155/140 autoantibodies. There was a higher frequency of males in the anti-p155/140-positive JDM group (P=0.02). JDM patients with anti-p155/140 autoantibodies had significantly more cutaneous involvement including Gottron’s papules (P=0.003), ulceration (P=0.005) and oedema (P=0.013). The distribution of skin lesions was more extensive particularly periorbitally (P=0.014) and over the small (P<0.001) and large joints (P=0.003).

Conclusion. Anti-p155/140 autoantibodies are clinically significant in JDM and may define a clinical subset in terms of disease severity and outcome. The same autoantigen target is detected in adult DM patients.

KEY WORDS: Inflammatory myopathy, Dermatomyositis, Juvenile dermatomyositis, Autoantibodies.

Introduction

Juvenile dermatomyositis (JDM) is the most common of the idiopathic inflammatory myopathies (IIMs) occurring in children. The reported incidence ranges from 0.8 to 4.1 per million children per year [1–3]. JDM is a chronic, potentially debilitating disease and despite improvements in multi-disciplinary treatment approaches, the condition is associated with significant morbidity and mortality [4]. Clinical outcomes and prognosis are difficult to predict due to the heterogeneity of the condition. Children with JDM share some clinical features with adult DM patients in terms of muscle disease and characteristic skin lesions. However, certain cutaneous manifestations are more characteristic in JDM including calcinosis and skin ulceration. As these features can cause permanent scarring, including contractures, they act as predictors of a more severe disease course in JDM [4–6]. In contrast to adults with DM, both interstitial lung disease (ILD) and cancer-associated myositis are very rare in JDM [7–9].

Classifying patients using a clinico-serological approach may lead to the identification of more homogeneous subsets within the JDM spectrum and therefore have prognostic implications. In adult IIM, distinct serological markers are well described and myositis-specific autoantibodies (MSAs) are associated with homogeneous clinical subsets [10, 11]. However, MSAs in juvenile myositis including JDM are less well characterized. Previous reports have described a low frequency of anti-aminoacyl-tRNA synthetase and anti-SRP autoantibodies in JDM, but anti-Mi-2 autoantibodies are described more frequently [12, 13]. In contrast, myositis-associated autoantibodies (MAAs) including anti-PmScI, anti-Ku and anti-U1RNP autoantibodies are found in children with myositis overlap syndromes.

More recently, a number of less well-characterized autoantibodies have been described in JDM and particularly adult DM. There have been preliminary reports of autoantibodies to a 140 kDa protein (anti-MJ) and a 155 kDa protein in JDM [14, 15]. Sato et al. [16] have described autoantibodies to a 140 kDa polypeptide in adult patients with clinically amyopathic DM (CADM) and ILD. Furthermore, three studies have reported novel autoantibodies to 155 and 140 kDa nuclear polypeptides in adult DM patients [17–19]. In the study by Targoff et al. [18], the autoantibody to a 155 kDa protein was also detected in their JDM cohort.

The purpose of this study was to establish the frequency and to define the clinical significance of anti-p155/140 autoantibodies in children recruited to the UK JDM Registry. A secondary aim was to confirm whether the same autoantigen is targeted in adult DM in order to understand whether this autoantibody has any future predictive value in respect of clinical features.

Patients and methods

Patients and sera (JDM)

The first 116 patients recruited to the JDM National Registry and Repository (UK and Ireland) were studied [20]. The diagnosis of probable or definite myositis was based on the Bohan and Peter criteria [21, 22]. Children were recruited consecutively on visits to paediatric rheumatology departments if they had a diagnosis of JDM or DM with overlap features of another connective tissue disease but where myositis was the predominant manifestation. Serial clinical data were collected prospectively using standardized proformas and stored using anonymous codes in a central database. The median age at symptom onset was 6 yrs.
Patients and sera (adults)

For adults, a diagnosis of probable or definite DM or PM was based on the Bohan and Peter criteria [21, 22]. Clinical information on patients with IIM attending the Royal National Hospital for Rheumatic Diseases, Bath, UK has been recorded prospectively and for the purpose of this study, patient notes were re-reviewed to confirm clinical details. Sera was taken at the time of diagnosis and stored at −80°C until required. Adult serum samples were analysed from 20 DM, 25 PM, 150 SSc and 40 SLE patients. All patients with SSc and SLE fulfilled the published criteria for those conditions [24, 25]. Sera from 50 age-matched healthy individuals (blood donors) were taken from our Bath biomarkers repository. The adult IIM patients reported here are separate to the UK-wide AOMIC study coordinated by two of the co-authors (H.C., R.G.C.) [19].

The study had both multi-centre and local regional ethics committee approval. All subjects gave written parental consent or full informed written consent before recruitment to the study.

Indirect immunofluorescence

Indirect immunofluorescence (IF) was performed using Hep-2 cells as substrate and FITC-conjugated anti-human IgG (Sigma, UK).

Immunoprecipitation

Immunoprecipitation (IPP) was used to detect anti-p155/140 autoantibodies and all other known MSAs/MAAs (including the anti-synthetases, anti-Mi-2, anti-SRP, anti-CADM-140, anti-Pm-Scl, anti-Ku, anti-U1RNP, anti-U3RNP and anti-Ro autoantibodies). IPP from K562 cell extracts was performed as previously described [26]. Briefly, 10 μl of sera was mixed with 2 mg protein-A-Sepharose beads (Sigma) in IPP buffer (10 mM Tris–Cl pH 7.4, 150 mM NaCl, 0.1% v/v Igepal) at room temperature for 30 min. Beads were washed in IPP buffer prior to the addition of 120 μl [35S]-methionine-labelled K562 cell extract. Samples were mixed with end-over-end rotation at 4°C for 2 h. Beads were washed in IPP buffer followed by TBS buffer (10 mM Tris–Cl pH 7.4, 150 mM NaCl) before being resuspended in 50 μl SDS sample buffer (Sigma). After heating, proteins were fractionated by 10% SDS–PAGE, enhanced, fixed and dried at 70°C for 80 min. Labelled proteins were analysed by autoradiography.

Immunodepletion experiments

The immunodepletion studies were undertaken in order to ascertain whether the IPP pattern seen in JDM and adult DM sera was due to precipitation of the same autoantigens. Cell extracts were depleted of autoantibody targets using anti-p155/140 JDM-positive serum or adult DM anti-p155/140-positive serum and normal serum (NS) as a negative control. These extracts were then used in further immunoprecipitations using both juvenile and adult anti-p155/140-positive serum. In brief, duplicate samples each containing 10 μg protein A sepharose beads in 1 ml IPP buffer and 50 μl patient serum were mixed with end-over-end rotation at room temperature for 30 min. The beads were washed four times in 1 ml IPP buffer and 1 tube (A) was placed on ice whilst 150 μl [35S]-methionine-labelled K562 cell extract and 350 μl IPP buffer was added to the remaining tube (B). Tube B was mixed with end-over-end rotation at 4°C for 2 h after which the supernatant was transferred to tube A, which was mixed with end-over-end rotation at 4°C for a further 2 h. The supernatant from tube A was then transferred to a fresh tube (C) and stored at −80°C. IPPs using JDM or adult DM serum and either 150 μl control [35S]-methionine-labelled cell extract or the immunodepleted supernatants (C) were completed as described in the paragraph above.

Statistical analysis

Statistics were conducted using SPSS for Windows (version 12) software. The frequencies of clinical features were compared using the chi-squared test with Yates’ continuity correction or the Fisher’s exact test for groups with small numbers. Where data was not normally distributed the Mann–Whitney U-test was used to compare continuous data. Median values (IQR) were expressed where appropriate and P-values <0.05 were considered statistically significant.

Results

Following IPP, sera from a number of JDM patients recognized two distinct proteins forming a doublet with molecular weights of 155 and 140 kDa. The same pattern was observed in a subset of adult DM patients (Fig. 1). Non-specific weak nuclear patterns were observed on IIF between anti-p155/140 patients (data not shown).

The immunodepletion results support the co-identity of the p155/140 kDa doublet precipitated by sera from both JDM and

![Image](http://rheumatology.oxfordjournals.org/Downloaded from University College London on June 12, 2015)
Frequency of anti-p155/140 autoantibodies

From 116 juvenile myositis sera, 27 (23%) had anti-p155/140 autoantibodies and of this group 26 had JDM and one had anti-p155/140-positive sera, the autoantigens were no longer detectable in juvenile or adult anti-p155/140-positive sera, respectively. This provides good evidence that the sera from JDM and adult DM contained the same autoantibody specificity.

Clinical features of the JDM patients with anti-p155/140 autoantibodies

Information on the degree of skin involvement and other selected clinical features are outlined in Table 1. There was a higher frequency of males in the anti-p155/140-positive children compared with anti-p155/140-negative children (P = 0.02). Anti-p155/140-positive JDM patients had an increased frequency of skin lesions (Gottron’s papules P = 0.003, ulceration P = 0.005, oedema P = 0.013) with a wider distribution of cutaneous involvement, particularly periorbitally (P = 0.014) and over the small joints (P < 0.001) and large joints (P = 0.003). Overall, there was no significant difference in those with elevated muscle enzymes at diagnosis/during disease course or those with an abnormal MRI/muscle biopsy between anti-p155/140-positive and -negative groups (data not shown). However, not all children had data on this, in particular, some did not have an MRI or biopsy performed. There was a trend towards lower CMAS (lower values indicate more severe weakness) and higher PGA in anti-p155/140-positive children at baseline and during follow-up, although this did not reach statistical significance. The frequency of other clinical signs including lipatrophy, arthritis, Raynaud’s phenomenon, sclerodermatous skin changes, dysphagia, mouth ulcers and alopecia was not significantly different between children with or without anti-p155/140 (data not shown). There was no history of malignancy in the entire JDM cohort during the follow-up period.

Clinical features of the adult DM patients with anti-p155/140

Similar to the anti-p155/140 JDM cohort, adult patients with anti-p155/140 had more skin involvement. In comparison with anti-p155/140-negative adult DM patients, there was a significantly higher frequency of the V-sign (P < 0.05) and Shawl-sign rash (P < 0.05). Other cutaneous features including Gottron’s papules, heliotrope rash and periungual changes tended to be more frequent in anti-p155/140 patients, although none of these findings reached statistical significance. Three adults with anti-p155/140 had a history of malignancy that was diagnosed either at onset or within 3 months of their presentation of classic DM. The remaining three adult patients with anti-p155/140 had CADM with no history of malignancy. There was no history of malignancy in the remainder of the adult IIM patients.

Discussion

Knowledge of an autoantibody profile is an important cornerstone in the diagnosis of patients with a wide variety of autoimmune connective tissue disorders, to the extent that certain autoantibodies form part of the diagnostic criteria. MSAs are...
Significance of anti-p155/140 autoantibodies in JDM

Autoantibodies directed against a p155/140 kDa protein are a major autoimmune target in JDM.

The clinical specificity of anti-p155/140 autoantibodies is distinct and identifies children with more severe cutaneous disease.

Acknowledgements

The authors would like to thank Miss Charlotte Carmichael for her advice on statistical analysis and for her assistance in collecting informed consent from the adult patients in the study. The Juvenile Dermatomyositis Research Group would like to thank all local research coordinators and principal investigators. The members who contributed were: Mr Ian Roberts, The Royal Liverpool Children’s Hospital, Alder Hey, Liverpool and Booth Hall Children’s Hospital, Manchester; Dr Eileen Baildam, Booth Hall Children’s Hospital, Manchester; Mrs Janis Scott and Dr Clive Ryder, Birmingham Children’s Hospital, Birmingham; Mrs Gillian Jackson and Dr Sue Wyatt, Leeds General Infirmary, Leeds; Ms Elizabeth Camp and Dr Janet Gardner-Medwin, The Royal Hospital for Sick Children, Yorkhill, Glasgow; Mrs Alison Swift, Dr Helen Foster and Dr Mark Friswell, The Royal Victoria Infirmary, Newcastle; Mrs Elizabeth Hutchinson and Dr Helen Venning, Queens Medical Centre, Nottingham; and Dr Clarissa Pilkington and Ms Sue Maillard, Great Ormond Street Hospital, London, UK.

Funding: The UK Raynaud’s and Scleroderma Association, the Cathal Hayes Research Foundation and Myositis Support Group, UK supported this work.

Disclosure statement: The authors have declared no conflicts of interest.

References