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Review

Identification of a novel myositis-associated antibody directed against cortactin

Moisés Labrador-Horrillo [a,*], Maria Angeles Martínez [b], Albert Selva-O'Callaghan [a], Ernesto Trallero-Araguás [a], Josep M. Grau-Junyent [c], Miquel Vilardell-Tarrés [a], Candido Juarez [b]

[a] Internal Medicine Department, Vall d'Hebron General Hospital, Universitat Autonoma de Barcelona, Barcelona, Spain
[b] Immunology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autónoma de Barcelona, Barcelona, Spain
[c] Muscle Research Unit, Internal Medicine Service, Hospital Clínic de Barcelona, Universitat de Barcelona, Fundación Cellex, Barcelona, Spain

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ABSTRACT

Objective: The aim of this study is to describe a novel myositis-associated autoantibody (anti-cortactin antibody) and assess related clinical and immunological manifestations and its clinical significance.
Methods: Adult patients with myositis (dermatomyositis, polymyositis, immune-mediated necrotizing myopathy, and inclusion body myositis), as well as patients with other autoimmune diseases and non-inflammatory myopathies were analyzed for the presence of anti-cortactin antibody using in-house developed ELISA and immunoblotting techniques with a commercial source of purified cortactin. The cut-off for positive status was determined in a group of healthy volunteers.
Results: Antibody against cortactin was positive in 7/34 (20%) polymyositis patients, 9/117 (7.6%) dermatomyositis, 2/7 (26%) immune-mediated necrotizing myopathy, and none of the 4 patients with inclusion body myositis. The antibody also tested positive in 3/101 patients with other autoimmune diseases (2 systemic sclerosis and 1 systemic lupus erythematosus), and in 1/29 patients with non-inflammatory myopathy. No relevant association with specific clinical features was found in patients with these antibodies. Anti-cortactin antibody was more frequently positive in patients with polymyositis and immune-mediated necrotizing myopathy than in the remaining myositis patients, and was the only myositis autoantibody found in sera of 3 patients from these groups.
Conclusions: Our data indicate that cortactin is a novel target antigen in patients with autoimmune diseases, especially patients with polymyositis or immune-mediated necrotizing myopathy. Anti-cortactin can be considered a new myositis-associated antibody.

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* Corresponding author at: Internal Medicine Department, Vall d' Hebron General Hospital, Passeig Vall d' Hebron, 119-129, 08035 Barcelona, Spain. Tel./fax: +34 93 2746169.
E-mail address: mlabrador@vhebron.net (M. Labrador-Horrillo).

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1. Introduction

Autoimmune myopathies comprise a spectrum of acquired diseases characterized by skeletal muscle inflammation leading to chronic dysfunction and disability. Dermatomyositis (DM) and polymyositis (PM) are the main clinical forms, in which immune-mediated pathways of muscle injury are prominent; immune-mediated necrotizing myopathies (IMNM) and inclusion body myositis (IBM) are also considered members of this group [1–5]. One of the hallmark characteristics of autoimmune myopathies is the presence of serum autoantibodies. These autoantibodies are detected in around 60% to 70% of myositis patients and are traditionally classified into two groups based on their diagnostic accuracy: myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs), the latter mainly occurring in myositis-overlap syndrome patients, but also in connective tissue diseases with no evidence of myositis [6].

During the process of identifying autoantibodies in our myositis patients, we came across a new autoantibody reactive with a human protein. We noticed that some patients who were anti-MDA5 or anti-HMGCR-positive using our in-house developed ELISA were negative for these antigens on confirmatory immunoblotting, but unexpectedly, we observed a 68-kD band. The protein was identified as a contaminant in two commercial recombinant proteins supplied by different companies, regularly used for diagnosing myositis. By in-gel digestion and mass spectrometry analysis of the band, we were able to identify the protein as cortactin (CTTN). In humans, cortactin is encoded by the CTTN gene (previously EMS1) on the long arm of chromosome 11. Cortactin was first identified as a prominent substrate of the oncogene Src tyrosine kinase and has a recognized association with progression of cancer [7,8].

Our objective was to determine the prevalence of this new autoantibody in a large series of patients with myositis from a single center and to investigate the existence of a characteristic associated clinical profile.

2. Patients and methods

2.1. Patient population

The study included 162 white, adult myositis patients (121 women), 117 diagnosed with DM, 34 with PM, 7 with IMNM, and 4 with IBM. In addition, we included 101 patients with other systemic autoimmune diseases (41 systemic sclerosis [SSc)], 25 systemic lupus erythematosus [SLE], 25 rheumatoid arthritis [RA] and 10 primary Sjögren syndrome [pSS]), and 29 with non-inflammatory myopathies (18 limb girdle muscle dystrophies [LGMD]: 14 dysferlinopathy [LGMD 2B], and 4 calpainopathy [LGMD 2A]); 10 with other types of dystrophies: 3 Becker muscular dystrophy, 5 facioscapulohumeral dystrophy, 2 myotonic dystrophy; and 1 Pompe disease). Twenty-five healthy controls were studied to determine the cut-off value for establishing positive status for anti-cortactin (anti-CTTN) antibody by ELISA. All the myositis patients included belong to a historical cohort diagnosed with idiopathic inflammatory myopathy at Vall d'Hebron General Hospital in Barcelona (Spain) between 1983 and 2014. Our center is a single teaching hospital with approximately 700 acute care beds, attending a population of nearly 450,000 inhabitants. Serum samples from these patients are routinely collected at diagnosis and during follow-up in our outpatient clinic, and stored at −80 °C. Patients and controls included in the study gave informed consent for the use of their serum for research purposes. The institutional review board of our hospital approved the study. The diagnosis of DM and PM was based on the criteria of Bohan and Peter [1,2]. Only patients with definite or probable disease were included. The Sontheimer criteria were used to diagnose amyopathic DM [9]. Distinctive pathological features enable the diagnosis of IMNM [3], and IBM was diagnosed according to established clinical and histological criteria [10]. Clinical data were obtained retrospectively by review of the patients' medical records.

2.2. Laboratory tests and serological assay

Serum samples from each patient were screened by indirect immunofluorescence for antinuclear antibodies (ANA) using HEp-2 cells, and by a commercial ELISA used in our routine laboratory setting for antibodies against extractable nuclear antigens (Ro, La, RNP, Sm) and anti-histidyl-tRNA synthetase (anti-Jo-1). Anti-TIF1γ and anti-MDA5 antibodies were detected by an in-house ELISA and confirmed by immunoblotting [11,12].

In addition, all samples were tested by protein and RNA immunoprecipitation [13], which confirmed the ELISA results and enabled detection of other synthetases and myositis-specific and myositis-associated antibodies (anti-Mi-2, anti-SRP, anti-Ro52, anti-Ro60, anti-La, anti-PM/Scl, anti-p155, and anti-U1RNP) that may have been overlooked by ELISA.

2.3. Anti-CTTN antibody detected by ELISA

Briefly, 96-well ELISA plates (Costar, Corning, New York) were coated with 2 μg/mL of purified recombinant CTTN (OriGene, Rockville, MD) diluted in phosphate buffered saline (PBS) and left to stand overnight at 4 °C. Wells were incubated for 1 h at room temperature (RT) with blocking buffer (10% nonfat dry milk in PBS). Plates were then washed in PBS, and human serum samples diluted 1:100 in HRP Sample Diluent, (INOVA Diagnostic Inc., San Diego, CA) were added in triplicate: two to CTTN-coated wells and one to a PBS-coated well (without antigen) to determine the background absorbance of each sample. Plates were incubated at RT for 1 h. After washing with HRP Wash (INOVA Diagnostic Inc., San Diego, CA), HRP-labeled goat anti-human IgG antibody (INOVA Diagnostic Inc., San Diego, CA) was added to each well, and plates were incubated for 1 h at RT and washed again. Color development was performed with peroxidase reagent TMB Chromogen (INOVA Diagnostic Inc., San Diego, CA) and absorbances at 450 nm were determined. For each sample, the background absorbance from the PBS-coated well was subtracted from the average absorbance of the two CTTN-coated wells. Sample absorbance was expressed as optical density units. The same positive serum was used as the reference in each assay.

2.4. Anti-CTTN antibody detected by immunoblotting

Briefly, 5 μg of purified recombinant CTTN (OriGene, Rockville, MD) or 5 μg of purified recombinant MDA5 (OriGene, Rockville, MD) or 5 μg of HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase, catalytic domain human, recombinant GST fusion protein expressed in *E. coli*) (Sigma-Aldrich, St Louis, MO) was run on 4% to 12% polyacrylamide-SDS minigels with MOPS running buffer, and western blot was performed on a nitrocellulose membrane using the Invitrogen NuPAGE (Carlsbad, CA) electrophoresis system, as previously described [11,12]. CTTN-transferred nitrocellulose was vertically cut into several strips and incubated for 1 h at RT in PBS containing 3% nonfat dry milk (blocking buffer). Each strip was then incubated with the corresponding human serum sample diluted 1:100 in blocking buffer for 1 hour at RT. After washing, phosphatase alkaline-labeled goat anti-human immunoglobulin antibody (Invitrogen Frederick, MD) was added to each strip