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Identification of GRASP-1 as a novel 97 kDa autoantigen localized to endosomes

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Abstract

We have identified an autoantigen that is recognized by antibodies from an 18-year-old female with a history of recurrent infections who later in her clinical course developed Raynaud's phenomenon and telangiectasias. By indirect immunofluorescence (IIF), the index serum produced a unique cytoplasmic discrete speckled (CDS) staining pattern that partially colocalized with early endosome antigen 1 (EEA1) but not Golgi complex or other cytoplasmic organelles in HEp-2 cells. When HEp-2 cells were treated with 0.1 N HCl, the cytoplasmic speckled staining of the index serum was markedly decreased, suggesting that the reactive antigen was soluble. Western blot analysis showed a reactive ~97 kDa protein in a saline soluble protein preparation from HeLa cells. Mass spectrometric analysis of the excised 97 kDa band that was immunoprecipitated from HeLa cell extracts identified GRASP-1 as a possible target. The index serum and anti-GRASP-1 antibodies colocalized to structures in the cytoplasm of HEp-2 cells. Synthetic peptides representing the full-length GRASP-1 protein were used to identify reactive epitopes. Like many other cytoplasmic autoantigens, GRASP-1 has numerous coiled-coil domains throughout the protein with the exception of short segments at the amino and carboxyl terminus.
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Keywords: Autoantibodies; Autoimmunity; Endosomes; GRASP-1; Coiled-coils

Introduction

Autoantibodies are important serological markers of autoimmune diseases and have previously been used as reagents to successfully isolate and characterize a number of cellular antigens. For example, novel nuclear [1,2], Golgi complex [3,4], mitochondrial [5,6], ribosomal [7], and endosomal [8,9] antigens have been identified using human autoantibodies.

Several autoantigens have been identified in the endosomal trafficking pathway. They include early endosome antigen 1 (EEA1) [9,10], lysobisphosphatidic acid (LBPA) [11], and cytoplasmic linker protein-170 (CLIP 170) [12]. EEA1 is a hydrophilic peripheral membrane protein associated with the cytoplasmic side of early endosomes [13,14]. Autoantibodies to EEA1 have been found in patients with neurological diseases, subacute cutaneous lupus [9,10], Raynaud's phenomenon, Wegener's granulomatosis, and proteinuria [15]. LBPA is an anionic phospholipid enriching the internal membranes of late endosomes [16]. Anti-LBPA antibodies are present in the plasma of some patients with anti-phospholipid antibodies (APLAs), which are associated with thrombosis and/or recurrent pregnancy loss [17,18]. CLIP-170 is a member of the cytoplasmic linker proteins that facilitate the interaction of cellular organelles, such as endosomes, to microtubules. CLIP-170 was localized to the plus ends of microtubules,

Abbreviations: cANCA, cytoplasmic anti-neutrophil cytoplasmic antibodies; CLIP, cytoplasmic linker protein; EEA1, early endosome antigen 1; ELISA, enzyme linked immunosorbent assay; GRASP-1, glutamate receptor interacting protein-associated protein-1; GW/GWB, glycine (G) and tryptophan (W) rich proteins localized in distinct cytoplasmic foci referred to as GW bodies; Hrs, hepatocyte growth factor receptor substrate: LBPA, lysobisphosphatidic acid; MS, mass spectroscopy.

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binds to newly polymerized tubulin, and is an activator of the microtubule-based motor, dynactin [19–22]. Thus far, autoantibodies to CLIP-170 have only been found in patients with systemic sclerosis, glioblastoma, and idiopathic pleural effusion [12].

Autoantibodies to lysosomes are characterized as a large irregular speckled staining pattern distributed throughout the cytoplasm [23,24]. Except for the well-characterized antigens in neutrophils characterized as cANCA [25,26], lysosome autoantigens have not been extensively characterized or understood. One such autoantigen was identified as the glycoprotein h-lamp-2 in the cytoplasm of neutrophils [27]. H-lamp-2 is a transmembrane protein with extensive glycosylation. Anti-h-lamp-2 antibodies were identified in the sera of patients with necrotizing and crescentric glomerulonephritis (NCGN). It was found that 14 out of 16 patients with NCGN and anti-neutrophil cytoplasmic antibodies contained antibodies to h-lamp-2 [27].

In this study, our attention focused on a serum that produced a unique cytoplasmic staining pattern in HEp2 cells and reacted with an unidentified protein in immunoblots of tissue culture cell extracts. The index serum was from an 18-year-old female with a history of recurrent infections and a presumed immune deficiency who later developed Raynaud's phenomenon and telangiectasias. Based on mass spectrometry, colocalization, and epitope mapping studies, we have identified the target autoantigen as GRASP-1.

Materials and methods

Human sera and patients

The index serum was from an 18-year-old female with a history of recurrent infections and a presumed immune deficiency who later developed Raynaud's phenomenon and telangiectasias. Other human sera used in this study were obtained from the Advanced Diagnostics Laboratory, University of Calgary. Control sera were collected from healthy volunteers or randomly selected from a bank of 2000 blood donors [28]. The serum samples were stored at −20°C or −70°C.

Indirect immunofluorescence (IIF) and ELISA

IIF was performed on commercially prepared HEp-2 cells (Immuno Concepts Inc., Sacramento, CA) using a fluorescein (FITC)-conjugated affinity-purified goat anti-human IgA + IgG + IgM (H + L) (Jackson Immuno Research, West Grove, PA) as previously described [29–31].

Colocalization

Colocalization studies used affinity-purified Cy3™-conjugated donkey anti-mouse IgG (H + L) (Jackson Immuno Research) or affinity-purified Cy3™-conjugated donkey anti-rabbit IgG (H + L) (Jackson Immuno Research) and the appropriate primary antibodies. Primary polyclonal antibodies raised in rabbits included: EEA1 [9], Hrs (hepatocyte growth factor receptor substrate) (a gift from Dr. E.K.L. Chan, University of Florida, Gainesville, USA), and GRASP-1 (a gift from Dr. R Huganir, The John Hopkins University School of Medicine, Baltimore, Maryland). Murine monoclonal antibodies were: EEA1 (Transduction Laboratories, Mississauga, ON), golgin-97 (CytoStore, Calgary, AB), LAMP2 (lysosome associated membrane protein-2; a gift from J. August and J. Hildreth, The John Hopkins University).

Acid extraction

Saline-soluble proteins were extracted from HEp-2 cells (ImmunoConcepts Inc.) by immersion of slides in 0.1 N HCl for 30 min at room temperature and then washing them in phosphate-buffered saline (PBS) for 10 min followed by IIF as previously described [32]. Human sera with anti-histone [32] and anti-GW antibodies [33] were used as controls. The slides were overlayed with a cover slip using Vectashield® mounting media (Vector Laboratories Inc., Burlingame, CA) that contained 4′,6-diamidino-2-phenylindole (DAPI) to visualize cell nuclei. The slides were then viewed with a Leica DMRXA2 confocal microscope with Chroma HiQ filter sets. Images were taken with a Princeton Instrument Inc. digital camera, and Adobe Photoshop (version 6.0) was used to process the images.

ELISA

A commercially available EIA Kit (anti-phospholipid-8Pro-G, Alpco Diagnostics, Windham, NH) was used to test for the presence of antibodies directed against β2-glycoprotein I, cardiolipin, cardiolipin combined with β2-glycoprotein I in the same well, phosphatidyl–choline, phosphatidyl–ethanolamine, phosphatidyl–inositol, phosphatidyl–serine, and sphingomyelin according to the manufacturer's instructions. The reactivities were read at an absorbance of 450 nm on a Biomek 1000 (Beckman Coulter Canada Inc., Mississauga, ON).

Cell lines and extracts

HeLa cells (ATTC CCl 2.2; American Type Culture Collection, Rockville, MD) were grown in Dulbecco's Modified Eagle Media (DMEM, Gibco) supplemented with 10% FCS, 2 mmol/l L-glutamine, and 1% penicillin–streptomycin. Cells were grown to confluence, lysed by adding Buffer A (150 mM NaCl, 10 mM Tris pH 7.5, 1.5 mM $MgCl_2$, 0.5% NP-40) for 5 min, scraped from the flask and centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was collected and stored at −70°C until required. The protein concentrations of the homogenates

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