Anti-PM1-Alpha antibodies are frequently accompanied by antibodies to GW bodies in patients with PM/Scl overlap syndrome

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INTRODUCTION

Autoantibodies to intracellular structures represent important biomarkers for the diagnosis of autoimmune disorders. PM/Scl autoantibodies target components of the exosome and are a serological feature of patients with scleroderma (Scl), polymyositis (PM) and most frequently PM/Scl overlap syndrome. In a recent study, it was shown that a synthetic peptide comprising amino acids 231-245 of PM/Scl-100 represents a sensitive and specific marker for a subset of patients with Scl and/or PM. Antibodies targeting a novel cytoplasmic structure named GW bodies (GWBs) contain unique proteins that are involved in mRNA processing and the majority of patients have neurological disease and/or Sjögren's' syndrome. A monoclonal antibody specific for GW182 (4B6) was generated and characterized.

MATERIALS AND METHODS

We tested 28 sera from patients with PM/Scl overlap syndrome for anti-PM1-Alpha peptide antibodies by ELISA (PM1-Alpha ELISA, Dr. Fooke Laboratories, Neuss, Germany) and by indirect immunofluorescence on HEp-2 cells (HEp-2000, Immuno Concepts, Sacramento, CA). Samples that showed a staining pattern resembling GWB staining were tested for colocalization with GWBs using a monoclonal antibody to recombinant GW182 (4B6). These sera were also assayed using an addressable laser bead immunoassay (ALBIA) that detected antibodies to recombinant GW182, GW2, and GW3.

RESULTS

13/28 (46%) PM/Scl patients were positive for anti-PM1-Alpha antibodies and 7/28 (25%) patients showed a staining pattern indicative of antibodies to GWBs. 4/28 (14%) had both antibodies. Colocalization experiments with 4B6 revealed that 5/7 sera showed cytoplasmic dots that partly colocalized with GWBs, but analysis of those sera by ALBIA showed no binding to GW182, GW2, GW3.

Figure 1

Colocolization of anti-PM1-Alpha and anti-GWB antibodies. Indirect immuno-fluorescence of HEp-2 cells using a human serum from a patient with PM/Scl overlap syndrom (A) show cytoplasmic dots that partly colocolize with a monoclonal antibody to GW182A (4B6) (B). The nuclei are stained with DAPI (C) and the images were merged (D). As secondary antibodies fluorescein labelled anti-mouse immunoglogulin G + M (green) and Cy3 labelled anti-human (red) IgG, IgM, IgA were used. Colocolizing spots are indicated by white arrows.

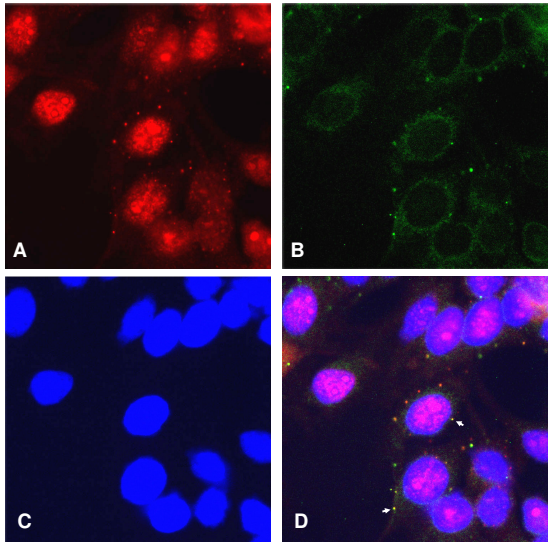

Table 1

Overview of reactivity of 28 serum samples from patients with PM/Scl overlap syndrome.

	ELISA	IIF		
ID	PM1-Alpha	Nuclear	Cytoplasmic	Colocolization with 4B6
1	0.7	Nsp/NLR	-	-
2	0.7	Nsp	-	-
3	0.5	Nsp	-	-
4	1.8	-	-	-
5	1.3	-	CDS/GWB	+
6	0.5	CENP	-	-
7	1.9	Nsp/NLR	CDS/GWB	+
8	1.8	Nsp/NLR	-	-
9	1.5	Nsp/NLR	-	-
10	11.2	Nsp/NLR	CDS/GWB	+
11	7.0	Nsp	-	-
12	4.0	Nsp	-	-
13	6.4	Nsp/NLR	-	-
14	0.7	Nsp	-	-
15	4.0	Nsp/NLR	-	-
16	0.9	Nsp/NLR	-	-
17	0.7	Nsp	-	-
18	1.9	-	CDS/GWB	+
19	1.4	Nsp	-	-
20	1.1	Nsp/NLR	-	-
21	0.8	Nsp	Fs/D	-
22	0.5	-	CDS/GWB	+
23	7.3	Nsp/NLR	-	-
24	0.9	Nsp	-	-
25	10.1	Nsp/NLR	CD *	-
26	6.0	Nsp/NLR	-	-
27	1.7	Nsp/NLR	-	-
28	1.9	-	CDS/GWB	+

Nsp = nuclear speckled pattern; NLR = nucleolar; CDS = cytoplasmic discrete speckles; Fs = fine speckled; D = diffuse; CD = cytoplasmic dots; * = most likely artefacts

CONCLUSION

We conclude that PM/Scl patients frequently have antibodies to PM1-Alpha and to yet unidentified components of GWBs. Since a number of other components of GWBs in addition to those tested in this study (i.e. exonuclease Xrn, decapping enzyme hDcp, slicer, dicer, phosphatidylethanolamine) have been identified, further studies are required to identify the target proteins that accompany PM/Scl antibodies. The potential relationship of GWBs to exosomes and RNA processing is of importance to cell biology and immunology.

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