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Clinical and Serological Features of Patients with Autoantibodies to GW/P Bodies

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Abstract

GW bodies (GWBs) are unique cytoplasmic structures involved in messenger RNA (mRNA) processing and RNA interference (RNAi). GWBs contain, mRNA, components of the RNA-induced silencing complex (RISC), microRNA (miRNA), Argonaute proteins (i.e. Ago2), the *Ge-1/Hedls* protein and other enzymes involving mRNA degradation. The objective of this study was to identify the target GWB autoantigens reactive with 55 sera from patients with anti-GWB autoantibodies and to identify clinical features associated with these antibodies. Analysis by addressable laser bead immunoassay (ALBIA) and immunoprecipitation of recombinant proteins indicated that autoantibodies in this cohort of anti-GWB sera were directed against *Ge-1/Hedls* (58%), GW182 (40%) and Ago2 (9%). GWB autoantibodies targeted epitopes that included the N-terminus of Ago2 and the nuclear localization signal (NLS) containing region of *Ge-1/Hedls*. Clinical data was available on 42 patients of which 39 were female and the mean age was 61 years. The most common clinical presentations were neurological symptoms (i.e. ataxia, motor and sensory neuropathy) (33%), Sjögren's syndrome (SjS) (31%) and the remainder had a variety of other diagnoses that included systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and primary biliary cirrhosis (PBC). Moreover, 44% of patients with anti-GWB antibodies had reactivity to Ro52. These studies indicate that *Ge-1* is a common target of anti-GWB sera and the majority of patients in a GWB cohort had SjS and neurological disease.

Keywords

autoantibodies; P bodies GW182; Ago2; *Ge-1*

Introduction

Historically, human autoantibodies have been used to identify novel cellular proteins and were linked to clinical subsets of autoimmune diseases (reviewed in [1]). Small nuclear ribonucleoprotein (snRNPs), components of the spliceosome complex involved in pre-mRNA splicing, were discovered with the aid of anti-Sm autoantibodies from systemic lupus

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erythematosus (SLE) patients [2-5]. Several other RNA binding autoantigens were also identified using patient sera including U1RNP, SS-A/Ro and SS-B/La [6-10]. More recently, use of human autoantibodies led to the discovery of a novel cellular compartment named GW bodies (GWB) which contained a glycine/tryptophan (G/W)-rich mRNA binding protein GW182 [11].

GWB, also known as mammalian processing (P) bodies and hereafter referred to as GW/PB, are sites for mRNA processing and degradation, and are important in the RNA interference (RNAi) pathway (reviewed in [12;13]). Some of the proteins identified in GWBs include the decapping proteins hDcp1, the human homolog of the yeast decapping enzyme subunit Dcp1; hDcp2 which has the enzymatic decapping activity; the exonuclease hXrn1, a 5'-3' exonuclease active in deadenylation dependent and independent cytoplasmic mRNA turnover pathways; members of the heptameric decapping activator complex LSm1-7; hCcr4, a yeast homolog of deadenylase shown to have exonuclease activity *in vitro*; the human enhancer of decapping large subunit (Hedls, also known as Ge-1 or RCD8), and Edc3; eukaryotic translation initiation factor and cap binding protein (eIF4E) and the eIF4E binding protein, eIF4E-T; cytoplasmic polyadenylation element-binding protein CPEB1; proteins that comprise the RNA induced silencing complex (RISC) that include Argonaute 2 (Ago2), and human Rck/p54, a homolog of yeast Dhh1p, that is involved in decapping and translation initiation (reviewed in [12-14]). More recently, additional components of GW/PB were identified using an approach referred to as autoantigenomics [15].

GW/PBs contain a unique family of proteins referred to as GW182 (TNRC6-A), GW2 (TNRC6-B), and GW3 (TNRC6-C), all of which have a RNA recognition motif (RRM) and multiple GW repeats throughout most of the protein (reviewed in [12;13]). GW182 associates with the Ago2 protein, a subset of mRNA, and has a role in miRNA-mediated gene regulation (reviewed in [12-14]). Recent data suggests that GW2 (TNRC6-B) may also interact with Ago2 [16], whereas GW3 remains to be characterized.

In this report we identify target autoantigens in GW/PB and the retrospective clinical diagnoses for 55 patients with autoantibodies to GW/PBs. Previous studies have independently identified GW182 [17], Ge-1 [18;19], RAP55 [20], hAgo2 [21] and diacyl-phosphatidylethanolamine [22] as GW/PB autoantigens. However, this is the first report of the relative frequency of autoantibodies directed to specific GW/PB components and then studied clinical features associated with anti-GW/PB.

Materials and Methods

Serum, antibodies and clinical information

All sera used in the present study were obtained from the serum bank stored in the Mitogen Advanced Diagnostics Laboratory (University of Calgary, Calgary, AB). Sera were selected for study based upon a cytoplasmic discrete speckled (CDS) pattern of staining that was then confirmed to be anti-GW/PB by co-localization with established markers of GW/PB (see below). Patient diagnoses were obtained by retrospective chart review as approved by the Conjoint Medical Ethics Review Board at the University of Calgary.

Indirect immunofluorescence

Patient sera with reactivity to GW/PBs were initially identified as the CDS pattern observed by indirect immunofluorescence (IIF) using Hep-2 cell substrates (ImmunoConcepts, Sacramento, CA) using a protocol as previously described [11]. Reactivity with GW/PB was confirmed by colocalization using a murine monoclonal to Ago2 [23], chicken polyclonal antibodies to LSm4 (Abcam, Cambridge, MA) and/or murine monoclonal anti-GW182 (4B6;

CytoStore, Calgary, AB). The secondary antibodies were AlexaFluor conjugated anti-mouse/chicken/human IgG, (Molecular Probes, Eugene, OR). Nuclei in the cell substrates were stained with 4',6-diamidino-2-phenylindole (DAPI), which was included in the glycerol mounting medium (VectaShield, Vector Laboratories, Burlingame, CA).

In vitro transcription/translation and immunoprecipitation

Immunoprecipitation (IP) using various recombinant proteins was performed to test and confirm the reactivity of patient sera to GW182, Ago2, Ge-1 and RAP55. The full-length cDNA of GW182 [11], Ago2 (gift from Dr. Tom Hobman, University of Alberta); Ge-1 and RAP55 cDNAs [18;20] were used as templates to synthesize the respective proteins using an *in vitro* transcription and translation (TnT) rabbit reticulocyte lysate kit (TnT, Promega Biotec, Madison, WI) in the presence of ³⁵S methionine at 30°C for 4 hours as previously described [17]. A 2 - 5 µl the labeled sample was separated using SDS-PAGE and analyzed by autoradiography to confirm the presence of the TnT product. The TnT product was then used in IP reactions as described previously [17]. To ascertain the specificity of the individual recombinant proteins, *in vitro* translated luciferase protein was added to the IP mix to serve as a control for nonspecific co-precipitation.

Recombinant Protein and Addressable Laser Bead Immunoassay (ALBIA)

Recombinant GW proteins GW182, GW2, GW3 were prepared and purified as previously described [17;24]. Briefly, the respective cDNAs were subcloned into the expression vector pET28 (Novagen, WI) and transformed to *E. coli* JM109 (DE3) for recombinant protein production. The N-terminal 6X histidine fusion recombinant protein was purified from a 1-L culture using Ni²⁺ affinity chromatography as per the manufacturer's instructions (Qiagen, Valencia, CA). A set of addressable beads bearing laser reactive dyes (Luminex, Austin, TX) were coupled to the purified GW182, GW2, and GW3 as previously described [17;25]. The sera were diluted in HRP diluent (INOVA, San Diego, CA) to a final concentration of 1:100. Thirty microlitres of HRP diluent was added to each well followed by 10 µl of the diluted patient serum and then incubated on an orbital shaker for 30 minutes at room temperature. This was followed by addition of 40µl of phycoerythrin-conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1:50 to each well and incubated on the orbital shaker for an additional half hour. The reactivity of the antigen-coated beads was determined on a Luminex 100 dual-laser flow cytometer (Luminex). Each assay included negative and positive controls. The tests were semi-quantitative, and the results were expressed as median fluorescent units of the test sample.

Enzyme-linked immunosorbent assay (ELISA)

Patient sera were tested for reactivity to each of SSA/Ro60 and Ro52 using commercially available QUANTA Lite kits (INOVA, San Diego, CA) and the protocol provided by the manufacturer. Results were calculated and interpreted using manufacturer's recommended formula.

Epitope mapping

Membranes containing *in situ* synthesized sequential peptides of 15 amino acids offset by five amino acids, representing full-length GW182, GW2, Ago2 and Ge-1 proteins were prepared (Eve Technologies, Calgary, AB) as previously described [17;25] and then used to map the epitopes on the respective proteins. The membranes were prepared for immunoblotting by soaking in 100% ethanol for 10 minutes followed by rehydration in Tris-buffered saline (TBS; 10mM Tris-HCl pH 7.6, 150 mM NaCl) for 10 minutes at room temperature. The membranes were then blocked in a solution of 2% milk/TBS at room temperature for one hour. Human sera were diluted 1:100 in 2% milk/TBS and overlaid on the membrane for 2 hrs at room

temperature after which the membranes were washed three times with 2% milk/TBS. A horseradish-peroxidase (HRP) conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1:12000 according to the manufacturer's protocol was used as the secondary antibody, and reactivity was visualized using enhanced chemiluminescence reagents (Amersham Biosciences, Piscataway, NJ). The intensity of each reactive peptide on the membrane was scored from 0 to 4 (0 being negative, 1 being weakly reactive and 4 the highest intensity). The assignment of a peptide as being reactive or non-reactive was determined after subtraction of the reactivity by a pooled normal human serum (NHS) control.

Results

Sera were identified with a CDS pattern of staining and the presence of anti-GW/PB antibodies was confirmed by IIF studies on HEp-2 cells using each patient serum in a colocalization reaction with Ago2, chicken polyclonal antibodies to LSm4 (Figure 1) and/or murine monoclonal anti-GW182 [24]. In a typical six month audit period at Mitogen Advanced Diagnostics Laboratory, 2500 samples are received for autoantibody analysis and of these 240 (9.6%) display a CDS pattern. Further verification that these sera had anti-GW/PB antibodies using the approach described above showed that 14/240 (5.8%) co-localized with these GW/PB markers. The frequency of anti-GW/PB is equivalent to antibodies to endosomes (i.e. early endosome antigen 1 - EEA-1), Sm and centromere proteins in this same cohort and more common than antibodies to proliferating cell nuclear antigen or the Golgi complex[25;26]. Using this approach, over four years 55 patient sera with anti-GW/PB antibodies were obtained for further study. The other sera displaying a CDS pattern had antibodies to endosome and lysosome autoantigens as previously reported [25;26], while other sera had antibodies to autoantigens yet to be identified.

Retrospective inquiry and chart review indicated that clinical and demographic information was available on 42/55 patients (Table 1). The age range of the patients was 36 to 90 yrs and the mean age was 60 and 69 yrs for the 39 female and 3 male patients, respectively. The demographic and clinical characteristics for these patients are summarized in Table 2. The most common diagnosis for this cohort of patients was neuropathies (33%) (motor and sensory neuropathies, ataxia) and Sjögren's syndrome (SjS) (31%). The other disease groups for patients with anti-GW/PB antibodies included SSc (14%), arthritis (14%), SLE (12%), primary biliary cirrhosis (PBC) (10%), rheumatoid arthritis (RA) (7%), cancer (5%), multiple sclerosis (2%) and other diagnoses (10%).

To determine which antigens were the primary targets of the GW/PB autoantibodies we chose GW/PB proteins that included the GW family proteins GW182, GW2, GW3, the decapping activating protein Ge-1, the putative decapping coactivator RAP55, and the RNAi associated protein Ago2 for further analysis. Two assays were employed to determine reactivity to the GW/PB proteins: addressable laser bead immunoassay [27] for only the GW family of proteins, and IP of recombinant GW182, Ago2, Ge-1, RAP55 generated by TnT using the respective cDNAs (Table 3). No reactivity was obtained with the pooled normal human serum in TnT/IP, ALBIA or ELISA. Analysis of antibody reactivity to GW182, GW2, GW3, Ago2, Ge-1, and RAP55 indicated that the two most common autoantigens were Ge-1 (58%) and GW182 (40%) (Table 3 and Figure 2). Recognition of the Ago2, RAP55, GW2, GW3 proteins by the anti-GW/PB sera was 16%, 18%, 16%, and 9%, respectively (Table 3 and Figure 2). Only one patient (#3) reacted with all the GW/PB proteins and eight (#s 8, 26, 27, 28, 33, 36, 52, 53) did not react with any of the proteins employed in the assays. Sera that did not react with any of the tested antigens had a broad range of anti-GW/PB titers ranging from 1/320 - 1/5120 (Table 3).

To determine the reactive epitopes in Ago2 and Ge-1, epitope mapping was conducted with an established strategy and sera with autoantibodies to these proteins [17]. Since the epitopes on GW182 were previously reported using this technology [17], we report here the epitopes on other GW/PB autoantigens, Ge-1 and Ago2 by probing membranes containing spots of overlapping peptides spanning the full-length sequence of the Ago2 and Ge-1 proteins with the patient sera. Figure 2A illustrates that the reactive epitopes obtained for 3 patient sera containing autoantibodies to Ago2 were predominantly localized to the N- terminal region (Figure 3). When the peptide membrane containing Ge-1 peptides was probed, (8/9, 89%) of patients recognized the region represented as amino acids 890-939 and referred to as the NLS containing region (NLS-C), but also reacted with the WD40, Ser and Psi domains (Figure 3).

It was noted that some sera containing anti-GW/PB had antibodies to other nuclear and cytoplasmic antigens. By ELISA, 44% of the GW/PB positive sera had reactivity to Ro52, and 9% to SSA/Ro60. Moreover, 100% and 64% of the SjS patients had reactivity to SSA/Ro60 and Ro52, respectively. Since 10/20 of the patients with anti-Ro52 did not have SjS, the presence of anti-Ro52 was not always related to SjS. In addition, the presence of anti-Ro52 was not correlated with any of the other diagnostic categories. All 4 PBC patients had anti-mitochondrial antibodies and one had anti-CENP (Table 3). All 3 patients with limited cutaneous SSc had anti-CENP and 1/3 patients with diffuse cutaneous SSc had anti-topoisomerase I antibodies.

Discussion

In this report we studied a cohort of 55 anti-GW/PB sera and extend previous findings in smaller or other disease cohorts [17-22] to include several target antigens recognized by human anti-GW/PB autoantibodies that included GW182, GW2, GW3, Ge-1, Ago2, RAP55 and Ro52. The most common clinical diagnoses in patients with anti-GW/PB antibodies were neuropathies that included ataxia, sensory and/or motor neuropathies (33%), and Sjögren's syndrome (31%). At this time we can find no evidence that the spectrum of neurological signs and symptoms constitute a unique neurological syndrome or that SjS patients have occult neurological features or autoimmune liver disease. This study relied on retrospective clinical data but, in the future, prospective studies in which a uniform clinical evaluation for SjS, neurological disease, autoimmune liver disease (e.g. PBC) and other features of autoimmunity would be desirable.

Among the proteins surveyed, the most common target autoantigens for anti-GW/PB antibodies were Ge-1 and GW182 with a frequency of 58% and 40%, respectively. Only one serum reacted with all GW/PB proteins and eight did not react with any. Because even sera with high titer anti-GW/PB by IIF did not react with any of the antigens employed in our assay, it is unlikely that this finding is due to low titer autoantibodies. More likely, this finding suggests that there are other target autoantigens or that the assay conditions employed may not have contained the reactive epitopes on some proteins. In this study we used three different assays to identify GWB and other autoantibody reactivity: IIF on HEp-2 cells, TnT/IP, ALBIA and ELISA. Studies conducted on a large number of autoantigen systems have shown that there is high correlation between ALBIA and ELISA (Dr. R. Burlingame, INOVA Diagnostics and our unpublished observations). In addition, we have found good correlation of ALBIA with the TnT/IP assay and for most systems studied (including GW182 here) ALBIA has a slightly higher sensitivity than TnT/IP. Hence, if anything, in this study the frequency of anti-Ge-1 in this cohort could be even higher if we had the full length recombinant protein to use in an ALBIA.

The finding that Ge-1 is the most common autoantigen target in GW/PB was an unexpected finding since most of our attention had focused on GW182 and Ago2. It is notable that none

of the sera in this study targeted one 'universal' GW/PB autoantigen and, therefore, it does not appear that the human anti-GW/PB response is initiated toward a single component in these macromolecular structures. Perhaps this finding is not surprising considering the variety of diagnoses and clinical findings. In other studies some serological cohorts (e.g. anti-centromere, anti-fibrillarin) are associated with specific MHC markers but are also seen in a narrower diagnostic framework (anti-centromere with limited cutaneous SSc; anti-fibrillarin with diffuse cutaneous SSc) [28;29]. Genetic studies of patients with GW/PB antibodies may shed light their association with a variety of conditions, most notably SjS and neurological diseases.

Ge-1 is important for GW/PB integrity and is required for functional interaction between Dcp1 and decapping catalyst Dcp2 [18;30-34]. Of note, autoantibody reactivity to Ge-1 did not coincide with reactivity to GW182 for the majority of samples tested (only 8/32 as determined by TnT/IP analysis). This might be explained by evidence that the decapping complex may only transiently interact with GW182 bound mRNA (see review[12]) thereby, limiting intermolecular epitope spreading. In other autoantigen systems, intermolecular epitope spreading is most usually observed with the components of "stable" macromolecular complexes such as snRNPs (i.e. Sm) and hY RNP (i.e. Ro) [35]. In support of this conclusion it is noted that 89% patients with antibodies to Ago2 also had antibodies to GW182 (see also [21]) a finding that might be expected considering that GW182 has been shown to associate/interact with Ago2 [36;37].

The frequency of antibodies to Ro52 in the present study was 44% suggesting that Ro52 is a common target in patients with anti-GW/PB autoantibodies, but the presence of antibodies to Ro52 did not correlate with the diagnosis of SjS or SLE. It has been reported that Ro52 localized to "dot-like" structures in the cytoplasm [38], however it has not yet been confirmed whether these dot-like structures are GW/PBs. Ro52 is known to be a RING-dependent E3 ligase [39; 40], and the presence of an ubiquitin-associated (UBA) domain in GW3 and a putative UBA domain in GW182 [12] may be related to the generation of antibodies to Ro52 as a result of interaction between Ro52 and GW182 during GW protein degradation in the proteasome pathway [26;41].

It is of interest that the Ago2 epitopes were localized to the N-terminal region because analysis of the crystal structure of *P. furiosus* Ago protein [42] indicates that the N-terminus is exposed and, hence, could be readily accessible during antigen processing making it a likely target for autoantibody reactivity. Further, the connection between RNAi components, specifically Ago2 and Dicer [21;43], and autoimmune reactivity to GW/PBs is intriguing. Recent data has suggested that mammalian viruses interact with and may indeed suppress the RNAi pathway and therefore, RNAi may function as an innate anti-viral response mechanism [44-46]. The association of viral infection and the development of autoimmunity have been proposed [47] and an association between viral infection and the development of autoimmunity has been proposed to be due to the presence of autoantibodies to components of the RNAi pathway and GW/PBs [21;43].

Since previous studies mapped the reactive epitopes of GW182 to the GW rich, the mid-portion, the non-GW rich and the C-terminal domains of the GW182 protein [17] our focus in this study was the other major target autoantigens Ge-1 and Ago-2. We showed that the Ge-1 amino acid residues (890-939) containing the NLS were the most reactive epitopes. This is in agreement with a previous report of Bloch et al. [48] who identified the immunoreactive region localized to amino acids 877-934. In addition, we identified other reactive regions of Ge-1 that included the N-terminal WD40 repeats, the Ser rich domain and the second Psi repeat.

Although Ge-1 antibodies were found in 5% of PBC sera [18] our study indicated that in addition to PBC these antibodies are seen in patients with other clinical diagnoses including

those with neuropathies, SjS, RA, SLE and various malignancies. Ge-1 was first discovered using serum from a patient that was initially thought to have SjS [48] but subsequent evaluation and follow-up showed that she had anti-mitochondrial antibodies and sicca complex associated with PBC [18]. This highlights the importance of longitudinal studies and clinical evaluation of all patients with various autoantibodies. For example, patient 26 in our study with disabling headaches and high titers of anti-mitochondrial and nuclear envelope antibodies, both of which are associated with PBC [49], could have sub-clinical autoimmune liver disease that may progress to PBS.

In summary, the present study reports the frequency of autoantigen targets among components of GW/PBs including GW182, Ago2, Ge-1 and RAP55. Human anti-GW/PB antibodies are mostly seen in patients diagnosed with neuropathies and Sjögren's syndrome. Though Ge-1 and GW182 are common targets of patient antibodies, there are yet unknown antigens to be discovered as 18% (10/55) patients do not react to any of the antigens tested.

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Abbreviations

Ago2, Argonaute protein 2
 ALBIA, addressable laser bead immunoassay
 CDS, cytoplasmic discrete speckled
 GW/PB, glycine(G)-tryptophan(W) repeat/processing bodies
 Hedls, human enhancer of decapping large subunit
 HRP, horseradish-peroxidase
 IIF, indirect immunofluorescence
 IP, immunoprecipitation
 LSm, like Sm
 MFU, median fluorescence units
 miRNA, microRNA
 NHS, normal human serum
 NLS, nuclear localization signal
 PBC, primary biliary cirrhosis
 RA, rheumatoid arthritis
 RAP55, RNA associated protein of 55 kDa
 RNAi, RNA interference
 RNP, ribonucleoprotein
 RRM, RNA recognition motif
 SjS, Sjögren syndrome
 SLE, systemic lupus erythematosus
 snRNP, small nuclear ribonucleoprotein
 TnT, transcription and translation

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Figure 1.

Human anti-GW/PB sera that showed a CDS pattern of staining (left column) were identified as having anti-GW/PB on the basis of IIF colocalization studies using murine anti-Ago2, and chicken anti-LSm4 antibodies were performed using HEp-2000 cells. A human serum that contains both nuclear and CDS staining is shown to illustrate that some anti-GW/PB sera have separate antibodies directed to nuclear and cytoplasmic antigens. The secondary antibodies included Alexa Fluor 568 anti-human IgG (shown in the first column), Alexa Fluor 488 anti-mouse IgG (shown in the second column), and Alexa Fluor 647 anti-chicken IgG (shown in the third column). The arrows in the right column, where the images to the left have been merged, show that the CDS pattern of the human serum colocalized with the Ago2 and LSm4 markers. Nuclei were stained with DAPI (not shown) dissolved in glycerol mounting medium (VectaShield).

Figure 2.

Immunoprecipitation analysis using human sera positive for anti-GW/PB antibodies. These studies identified sera that recognized all, one or none of the *in vitro* translated proteins (Ago2, Ge-1, GW182 and RAP55). **A:** Sera that IP recombinant Ago2. **B:** Sera that IP recombinant Ge-1. **C:** Sera that IP recombinant GW182. **D:** Sera that IP recombinant RAP55. Normal human serum (NHS) did not precipitate any of the proteins (lane 1 in all panels). As described in Methods, the luciferase protein was included in the IP reaction to serve as a control for antibody binding specificity but none of the sera reacted with this protein.

Figure 3.

Epitope mapping obtained using membranes that contained spots of 15mer peptides overlapping by 5 amino acids representing the full-length Ago2 protein. **A)** Membranes probed using normal human serum, and sera from patients 1, 3 and 15. The asterisks highlight the reactive peptides. A positive signal was scored after reactivity by NHS was subtracted from the reactivity produced by the patient's sera. **B)** Table representing the peptide sequence, the amino acid positions and the reactivity of patient sera. Only the most reactive domain represented as the N-terminus, is included in the table. The normal human serum also reacted with two peptides that did not overlap with any of those of the anti-GW/PB sera. **C)** Bar graph with the y-axis indicating the number of patients with positive reactivity to the various domains

of the protein (x-axis). The numbers in parenthesis are the amino acid residues represented within each domain.

Figure 4.

Epitope mapping obtained using membranes that contained spots of 15mer peptides overlapping by 5 amino acids representing the full length Ge-1 protein. **A)** Membranes probed using normal human serum (NHS), and sera from patients 3, 13, and 14. A positive signal was scored after reactivity by NHS was subtracted from the reactivity produced by the patient's sera. **B)** Table representing the peptide sequence, the amino acid residues and the reactivity of patient sera to WD40, the Ser rich domain (S), the NLS containing (NLS-C) and the Psi domains. The Psi domain represents the second of four Psi domains in Ge-1 [48]. **C)** Bar graph with the y-axis indicating the number of patients with positive reactivity to the various domains of the protein (x-axis). The numbers in parenthesis are the amino acid residues contained within

each domain. Though patients have reactive epitopes within each domain, the NLS-C is the most reactive.

Table 1

Demographic and Detailed Clinical Characteristics of 42 patients with GW/PB autoantibodies

Patient #	Age	Sex	Clinical Diagnosis
1	48	F	SjS, motor neuropathy
2	73	F	Motor neuropathy
3	77	F	SjS, ataxia
4	46	F	SjS, arthritis, celiac disease
5	68	F	SLE
6	44	F	Multiple sclerosis, myopathy
7	44	F	Multiple neurological symptoms
8	59	F	RA, SjS
9	63	F	Metastatic lung carcinoma, vasculitis
10	58	F	SjS, ataxia
11	62	F	Villous adenoma
12	82	F	SjS
13	64	F	RA, RP
14	73	F	RA, neuropathy
15	80	M	Prostate carcinoma, sensory neuropathy, gout
16	36	F	Limited cutaneous SSc, recurrent digital ulcers & infections
17	77	F	Dementia/cognitive deficit, breast & colon cancer
18	50	F	Interstitial lung disease
19	90	F	SjS, PBC, limited cutaneous SSC, diabetes, cataracts, mild pulmonary hypertension, hypothyroidism, psoriasis, mild aortic stenosis
20	75	F	Sensory neuropathy, arthritis, granuloma, renal disease, silicon breast implants
21	40	F	Sensory neuropathy, granuloma/lymphoma
22	53	F	SLE
23	46	F	Malar rash, arthritis
24	59	F	Overlap autoimmune disease (SLE, SjS, limited SSc, hepatitis)
25	48	F	Diffuse cutaneous SSc & SjS
26	47	F	Chronic disabling headache
27	76	F	Seronegative inflammatory polyarthritis
28	47	M	PBC
29	37	F	SjS, sensory polyneuropathy, general malaise
30	67	F	PBC, hypothyroidism, allergic rhinitis
31	66	F	PBC, idiopathic pulmonary fibrosis
32	75	F	Sensory neuropathy, heart failure, type II diabetes, high blood pressure, polyarthritis
33	60	F	SjS, RP, diffuse cutaneous SSc, neuropathy (sensitivity of hands)
34	36	F	Limited cutaneous SSc
35	58	F	Diffuse cutaneous SSc
36	79	F	Neuropathic pain & sensory changes, weakness affecting lower extremities
37	60	F	Gastritis, abnormal liver function tests
38	80	M	SjS, gastric dysmotility, severe weight loss, thyrotoxicosis, Grave's disease, proximal myopathy, cardiomyopathy
39	51	F	Probable SLE, multiple personality disorder
40	62	F	SLE, lupus nephritis, secondary SjS
41	50	F	Psoriatic arthritis
42	75	F	Multi-focal sensory peripheral neuropathy
Mean	61		
Sex		39F: 3M	

Abbreviations: MS, multiple sclerosis; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; RP, Raynaud's phenomenon; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Table 2

Summary of demographic and clinical characteristics of patients

Diagnosis	Number of Patients (%)	Mean Age (yrs)	Gender (F:M)
Neuropathy	14 (33%)	67	13F:1M
SjS	13 (31%)	63	12F:1M
SSc	6 (14%)	50	6F
Arthritis	6 (14%)	61	6F
SLE	5 (12%)	59	5F
PBC	4 (10%)	68	3F:1M
Cancer	4 (10%)	70	3F:1M
RA	3 (7%)	65	3F
MS	1 (2%)	44	1F
Other	3 (7%)	52	3F

Abbreviations: MS, multiple sclerosis; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; RP, Raynaud's phenomenon; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Table 3
Autoantibody Profile of 55 Patients with GW/PB autoantibodies*

Patient No.	CDS Titer/Other*	Ago2	TnT-IP Ge-1	RAP55	GW182	ALBIA ^a GW2	GW3	Ro52	ELISA Ro60
Normal	0/neg	-	-	-	125	51	53	2	2
1	>5120/NE	-	+	-	453 ^b	55	20	109 ^b	3
2	>5120/-	+	+	+	24582 ^b	150	118	107 ^b	5
3	>5120/-	+	+	+	8953 ^b	1412 ^b	88	110 ^b	4
4	1280/NE/NLR	-	-	+	1602 ^b	141	337 ^b	107 ^b	8
5	320/RNP	-	+	-	5417 ^b	624 ^b	40	16	4
6	640/NSp	-	-	-	208	2	36	5	5
7	1280/-	-	-	+	14	24	40	3	3
8	640/-	-	-	-	109	104	61	8	4
9	1280/-	-	-	+	485 ^b	92	131	107 ^b	4
10	1280/NuMA	-	+	-	443 ^b	534 ^b	48	4	3
11	1280/-	-	+	+	69	100	37	3	2
12	1280/Ro	-	+	-	352	8	4	108 ^b	128 ^b
13	160/-	-	+	-	135	10	0	38	3
14	>5120/-	-	+	-	245	23	63	106 ^b	3
15	2560/-	-	-	-	5360 ^b	321 ^b	134	5	4
16	1280/CENP	-	+	-	1386 ^b	1732 ^b	248 ^b	98 ^b	3
17	1280/-	-	+	-	148	38	67	107 ^b	2
18	5120/NSp	-	+	+	76	67	43	104 ^b	5
19	2560/CENP/M	-	+	-	582 ^b	180	7	108 ^b	2
20	1280/-	-	+	-	409	0	0	109 ^b	3
21	2560/-	+	+	-	1209 ^b	149	201 ^b	10	2
22	1280/NSp	-	-	-	435 ^b	123	69	6	2
23	640/-	-	+	-	518 ^b	118	90	9	7
24	320/CENP	-	+	+	86	52	106	5	3
25	1280/topo I	-	+	-	245	45	34	102 ^b	119 ^b
26	5120/M/NE	-	-	-	220	63	57	4	2
27	320/-	-	-	-	220	85	36	3	2
28	2560/M	-	-	-	90	383	45	6	2
29	1280/NSp	-	+	-	228	44	186	5	4
30	5120/M	-	+	-	1434 ^b	638 ^b	1054 ^b	31	6
31	5120/M	-	+	-	148	64	46	8	3
32	>5120/NLR/NSp	-	+	-	276	79	151	106 ^b	17
33	640/NSp	-	+	-	3	77	135	107 ^b	3
34	1280/CENP	-	-	-	443 ^b	435 ^b	60	98 ^b	3
35	1280/H. NSp	+	-	-	109	77	79	90 ^b	4
36	320/-	-	-	-	59	64	41	9	1
37	5120/-	-	+	-	520 ^b	55	41	15	8
38	1280/NSp	+	-	-	78	56	50	106 ^b	2
39	1280/NSp	-	-	-	290	92	50	6	3
40	640/NSp	-	+	-	50	77	68	77 ^b	2
41	640/-	+	-	-	73	34	29	3	2
42	2560/RNP	-	+	+	60	48	30	107 ^b	3
43	2560/NSp	-	+	+	115	24	59	107 ^b	124 ^b
44	5120/NSp	-	+	-	410 ^b	58	83	108 ^b	123 ^b
45	5120/cyt	-	+	+	84	51	51	107 ^b	3
46	1280/-	-	+	-	157	34	16	4	3

Patient No.	CDS Titer/Other *	Ag02	TnT-IP		RAP55	GW182	ALBIA ^a		ELISA	
			Ge-1				GW2	GW3	Ro52	Ro60
47	2560/cyt	+	+	+	+	644 ^b	55	21	106 ^b	123 ^b
48	320/-	+	-	-	-	42	64	60	2	3
49	1280/-	-	-	-	-	115	424 ^b	103	2	2
50	5120/-	-	+	+	-	499 ^b	22	2	10	3
51	1280/NSp	-	+	+	-	168	80	88	3	2
52	5120/NE/NSp	-	-	-	-	73	28	57	5	3
53	640/NSp	-	-	-	-	122	57	50	3	4
54	>5120/NSp	-	+	+	-	2369 ^b	94	77	5	4
55	640/-	+	-	-	-	989 ^b	83	214 ^b	6	3
Total pos. (%)		9 (16%)	32 (58%)	10 (18%)	22 (40%)	9 (16%)	5 (9%)	24 (44%)	5 (9%)	

Abbreviations: ALBIA, addressable laser bead immunoassay; CENP, centromere protein; cyt, cytoplasmic; ELISA, enzyme linked immunoassay; H, homogenous nuclear; M, mitochondria; NE, nuclear envelope; NLR, nucleolar; NSp, nuclear speckled; RNP, ribonucleoprotein; TnT-IP, transcription and translation immunoprecipitation assay; topo I, topoisomerase I (Scl-70).

* All sera were identified on the basis of indirect immunofluorescence (IIF) staining producing a cytoplasmic discrete speckled (CDS) pattern on HEp-2000 cells [50] and co-localization with marker GW/PB antibodies. The titer of the CDS antibodies and patterns of IIF or other antibodies other than CDS are indicated.

^aThe results of the ALBIA are expressed as median fluorescence units (MFU). Note that the MFU and the ELISA OD values are directly comparable. In general, MFUs >1500 and ELISA ODs of >100 are considered high binding values.

^bSera with a positive test.