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ORIGINAL ARTICLE

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Clinical and serological associations of autoantibodies to GW bodies and a novel cytoplasmic autoantigen GW182

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Abstract A novel autoantigen named GW182 was recently identified when the serum from a patient with a sensory ataxic polyneuropathy was used to immunoscreen a HeLa cDNA library. Unique features of the GW182 protein include 39 repeats of glycine (G) and tryptophan (W) residues, binding to a subset of messenger RNA and localization to unique structures within the cytoplasm that were designated GW bodies (GWBs). The goal of the present study was to identify the clinical features of patients with anti-GW182 antibodies and to characterize the B cell anti-GW182 response by defining the epitopes bound by human autoantibodies. The most common clinical diagnosis of patients with anti-GW182 antibodies was Sjögren's syndrome followed by mixed motor/sensory neuropathy, and systemic lupus erythematosus. Of interest, 5 (28%), 9 (50%), and 3 (17%) of the 18 sera that react with GWBs had autoantibodies to the GW182 and the 52 kDa and 60 kDa SS-A/Ro autoantigens, respectively. Epitopes bound by the human autoantibodies were mapped to the GW-rich middle part of the protein, the non-GW rich region, and the C-terminus of GW182 protein. None of the GW182 epitopes had significant sequence similarities to other known proteins. GW182 represents a new category of ribonucleoprotein autoantigens.

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Keywords Autoantibodies · mRNA · Autoimmunity · Sjögren's syndrome · Neurological disease

Abbreviations *GWB:* Glycine tryptophan-rich cytoplasmic structure · *IIF:* Indirect immunofluorescence · *IP:* Immunoprecipitation · *NET2:* NaCl, EDTA, Tris buffer · *SDS:* Sodium dodecyl sulfate · *SjS:* Sjögren's syndrome · *SLE:* Systemic lupus erythematosus · *TBS:* Tris-buffered saline · *TnT:* Transcription and translation

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Introduction

The sera from patients with systemic autoimmune disease have been used to isolate and identify novel autoantigens that are components of macromolecular complexes that have a variety of cellular functions including transcription, translation, and ribosomal processing [1, 2, 3]. In addition, the identification of autoantigens and the characterization of their respective epitopes are used as diagnostic tools to assist in the clinical evaluation of autoimmune diseases [4, 5, 6]. For example, the presence of autoantibodies to double-stranded DNA and the Sm small nuclear ribonucleoproteins (RNPs) are highly specific serological markers for systemic lupus erythematosus (SLE) [7]. Sjögren's syndrome (SjS) is characterized by the presence of autoantibodies to SS-A/Ro, and/or SS-B/la [8]. In addition, the identification of autoantigens and their association with autoimmune disease is a key approach to understanding the autoimmune disease state [9, 10].

Recently a novel autoantigen named GW182 was discovered when the serum from a patient with ataxic sensory polyneuropathy was used to immunoscreen a HeLa cDNA library [11]. Interesting features of the GW182 protein include 39 repeats of glycine (G) and tryptophan (W) residues and its localization in unique cytoplasmic structures that have been designated as GW bodies (GWBs). The GW182 protein, which has an RNA recognition motif and binds specific mRNAs, is thought to be part of a mRNA-protein macromolecular complex. It has been postulated that GWBs provide an additional level of posttranscriptional gene regulation and function in mRNA processing in a cell compartment referred to as the ribosome or posttranscriptional operon [12, 13]. More recent evidence implicates the GW182 protein and GWBs in mRNA degradation pathways [14]. The goal of the present study was to characterize the B-cell immune response in patients with antibodies to GWBs and the GW182 protein which resides within the GWBs and to assess the clinical features of these patients. This is the first report of the clinical features of patients with anti-GWB antibodies and a description of the GW182 epitopes bound by these sera.

Materials and methods

Patient serum and antibodies

All human sera used in this study were obtained from serum banks at the Advanced Diagnostics Laboratory (University of Calgary, Calgary, Canada), the W.M. Keck Autoimmune Disease Center (Scripps Research Institute, La Jolla, Calif., USA), and Juntendo University (Tokyo, Japan). The index human serum used in this study was selected based on its reactivity to an apparently unique cytoplasmic domain and its reactivity with the native and recombinant GW182 protein [11]. Clinical information was obtained by contacting the referring physician and retrospective chart review.

Indirect immunofluorescence

The presence of anti-GW182 antibodies in the human sera were initially tested by Indirect immunofluorescence (IIF) using HEp-2 cell substrates (Immuno Concepts, Sacramento, Calif., USA) and had a cytoplasmic staining pattern that was characteristic of anti-GWB antibodies [11]. Reactivity with GWBs was confirmed by IIF colocalization studies on HEp-2 cells where a monoclonal antibody (4B6) to the recombinant GW182 protein which stains GWBs was used as the marker antibody [15]. Secondary antibodies for colocalization studies were fluorescein isothiocyanate conjugated anti-mouse IgG (Jackson ImmunoResearch, West Grove, Pa.,USA) and fluorescein isothiocyanate or Cy3-conjugated anti-human IgG (Jackson ImmunoResearch). Nuclei in the cell substrates were stained with 4′,6-diamidino-2-phenylindole that was included in the glycerol mounting medium (VectaShield, Vector, Burlingame, Calif., USA).

In vitro transcription/translation and immunoprecipitation

Reactivity of the sera with recombinant GW182 protein was confirmed by immunoprecipitation (IP) of the recombinant protein. The full-length GW182 cDNA was used as a template to synthesize the protein in an in vitro transcription and translation (TnT) protocol that used a rabbit reticulocyte lysate kit (TnT, Promega Biotec, Madison, Wis., USA) in the presence of [^{35}S]methionine at 30°C for 3–4 h as previously described [11, 16]. To confirm the presence of TnT products 2- to 5-µl samples were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and analyzed by autoradiography. The TnT products were then used in IP reactions by combining 100 µl of a 10% protein A Sepharose bead suspension (Sigma, catalog no. P-3391), 10 µl human serum, 500 µl NET2 buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 0.02% sodium azide), and 10 µl of radiolabeled protein product. After incubation for 1 h at 4–8°C the suspension was washed five times in NET2, the proteins eluted in 10 µl sample buffer, and analyzed by 10% gel SDS polyacrylamide gel electrophoresis as described [17].

Epitope mapping

Epitope mapping employed sequential peptides of 15 amino acids offset by five amino acids, representing the full-length GW182 protein, were synthesized on membranes using the SPOT technology as previously described [18, 19, 20]. The membranes containing the peptides were processed for immunoblotting by soaking the membrane in Tris-buffered saline (TBS; 10 mM Tris-HCl pH7.6, 150 mM NaCl) for 10 min and then blocking with 2% milk/TBS for 1 h at room temperature. The human sera were diluted 1/100 in 2% milk/TBS and applied to the membrane. After 2 h of incubation at room temperature the membrane was washed three times with TBS. A horseradish-peroxidase conjugated goat anti-human IgG (Jackson ImmunoResearch) was diluted according to the manufacturer's protocol, and reactivity was visualized using enhanced chemiluminescence western blotting detection reagents (Amersham International). After reactive epitopes were identified a BLAST search of the GenBank using the reactive sequences as the query was conducted to identify homologous sequences in other proteins.

Purified recombinant GW182

The GW182 cDNA insert encoding a partial length of the GW182 protein was subcloned into pET28 (Novagen, Madison, Wis., USA). *Escherichia coli* JM109 (DE3) was transformed with this subclone, and the recombinant protein produced was purified using Ni^{2+} affinity chromatography as per the manufacturer's instructions

(Qiagen, Valencia, Calif., USA). This recombinant protein was subsequently used in the laser bead immunoassay described below.

Laser bead immunoassay

A set of addressable beads bearing laser reactive dyes (Luminex, Austin, Tex.,USA) were selected to couple the recombinant purified GW182 protein. Unless otherwise specified, all incubations and reactions were conducted at room temperature. Ten micrograms of 1-ethyl-3-(3-dimethylaminopropyl) carboiimide hydrochloride (Pierce, Rockford, Ill., USA) and *N*-hydroxysuccinimide (Pierce) was placed in separate microcentrifuge tubes (USA Scientific) and dissolved in 200 µl activation buffer (0.1 M sodium phosphate, pH 7.2). Of the laser bead suspension 100 µl was placed into a microcentrifuge tube and centrifuged at 10,000 rpm in a microcentrifuge for 1 min, and the fluid was decanted. Forty microliters of activation buffer was added to the pelleted beads, and they were gently resuspended by brief sonication and vortexing. Five microliters of 1-ethyl-3-(3-dimethylaminopropyl) carboiimide hydrochloride and *N*-hydroxysuccinimide was added in sequence to the resuspended microspheres, followed by brief sonication and vortexing. The suspended spheres were incubated in the dark for 20 min before the purified recombinant GW182 protein, dissolved in coupling buffer (0.14 M NaCl, 0.01 M $NaPO_4$, pH approx. 7.2: PBS), was added to the mixture. After an additional incubation in the dark for 3 min, the suspension was centrifuged at 13,000 rpm for 3 min. The fluid was decanted and 125 µl coupling buffer added. The spheres were resuspended by sonication and vortexing as above before repelleting by centrifugation at 13,000 rpm for 3 min. The supernatant was decanted, 125 µl the protein solution (50 µg/ml) added, and the beads resuspended by sonication and vortexing. The protein and sphere suspension were incubated for 1 h at room temperature in the dark. The protein-coupled microspheres were pelleted by centrifugation at 10,000 rpm for 2 min and then resuspended in 125 µl washing buffer (PBS pH 7.2, 0.05% Tween 20). After two cycles of resuspension and pelleting in 125 µl blocking/storage buffer (0.5% BSA in PBS), the beads were stored as a suspension in 100 µl of blocking buffer at 2–8°C until required for use.

To analyze reactivity of the sera with the bound GW182, patient sera were diluted in Quanta Plex sample diluent (INOVA, San Diego, Calif., USA) to a final dilution of 1/1,000. To each well 40 µl of bead stock (1 part microspheres in blocking buffer to 40 parts Quanta Plex sample diluent) and 10 µl of diluted patient sera were added and incubated for 30 min on an orbital shaker. Then 50 µl phycoerythrin-conjugated goat anti-human IgG (Jackson ImmunoResearch) diluted 1/50 was added to each well and incubated on the orbital shaker for an additional 30 min. The reactivity of the antigen-coated beads was determined on a Luminex 100 dual-laser flow cytometer (Luminex). Control negative and standard positive sera were included in each assay. The tests were semiquantitative, and the results were expressed as median fluorescent intensity of the test sample.

Line immunoassay

The serum samples were tested for reactivity to other autoantigens using a “line” assay that includes recombinant and native SmB, SS-A/Ro52, SS-A/Ro60, SS-B/la, U1-RNP, Scl-70, ribo P antigens located on a solid phase strip (INNO-LIA, Innogenetics, Norcross, Ga., USA). The assays were performed according to the manufacturer’s instructions, and at the completion of the assay the strips were dried and were interpreted based on visual comparison of the intensity of the bands on the test strip to the cutoff control on another strip.

Results

IIF using the index human serum on HEp-2 cells showed a pattern of distinctive cytoplasmic dots and what was previously described as GWBs (Fig. 1a). The number of GWBs present in HEp-2 cells varied from zero in mitotic cells to more than 30 in interphase cells. Previously it was shown that GWBs containing the GW182 autoantigen are distinguished from other cytoplasmic organelles, including the Golgi complex, lysosomes, endosomes, and proteasomes [11]. Over a 14-month period the clinical reference laboratory (Advanced Diagnostics Laboratory, University of Calgary) received approximately 5,000 sera for autoantibody analysis as requested by physicians who were investigating the presence of autoimmune disease, such as SLE and SjS, in their patients. From these 5,000 serum samples approx. 200 sera showed a cytoplasmic speckled staining pattern on HEp-2 cells. Of these 200 sera 18 (9%) had autoantibodies to the GWBs as determined by colocalization with the monoclonal antibody 4B6 that reacts with the GW182 protein and stains

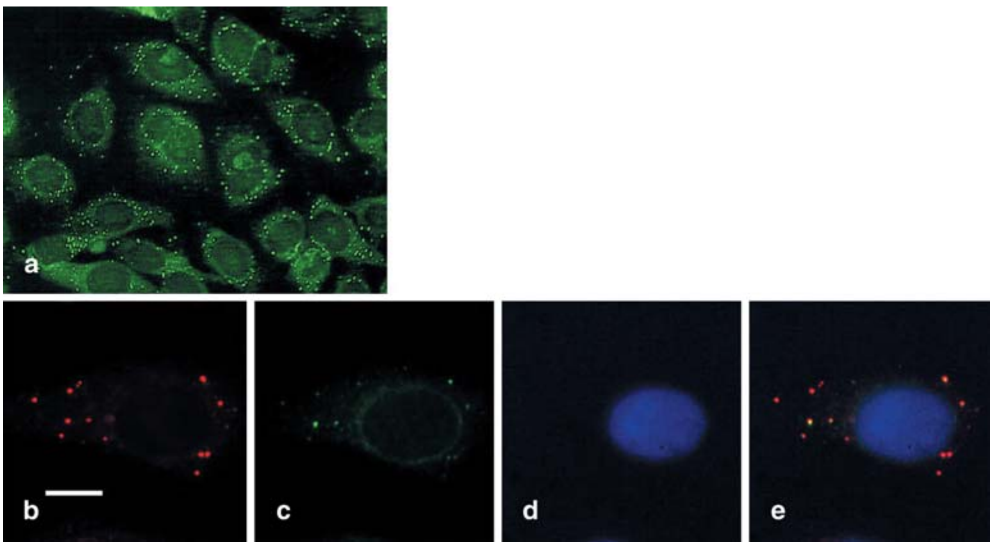

Fig. 1a–e IIF colocalization studies using human and murine monoclonal anti-GWB antibodies. Cytoplasmic bodies in HEp-2 cells detected with the index patient serum diluted 1/100 (**a**, **b**) colocalized with the staining of a monoclonal antibody 4B6 to GW182 (**c**). The nuclei are stained blue with 4′,6-diamidino-2-phenylindole (**d**) and the merged images are shown in **e**. *White bar* (**b**) 5 µm

Table 1 Demographic, clinical, and serological features of patients with anti-GWB antibodies (*AMA* anti-mitochondrial antibodies, *mAb* monoclonal antibody, *NHS* normal human serum, *PBC* primary biliary cirrhosis, *Pt* patient serum, *IP* immunoprecipitation, *SjS* Sjögren's syndrome, *SLE*, systemic lupus erythematosus, *SS-A/B* Sjögren's syndrome antigen A/B, *TnT* in vitro transcription and translation, *UCTD* undifferentiated connective tissue disease)

Patient no.	Age (years)	Sex	Diagnosis	IIF colocalized with mAb 4B6	Line assay				Laser bead assay GW182 antibodies[a]	TnT IP
					SmB	SS-A 52 kDa	SS-A 60 kDa	SS-B		
Group A										
1	73	F	Ataxic sensory polyneuropathy	+	–	+	–	–	7766[b]	+
2	75	F	Sensory neuropathy, arthritis, granuloma, silicon breast implants	+	–	+	–	–	72	–
3	38	F	Sensory neuropathy, granulomatous lymph nodes	+	–	+	–	–	1209[b]	+
4	46	F	Malar rash, arthralgia	+	+	–	–	–	189	–
5	64	M	AMA negative PBC	+	–	+	–	–	209	–
6	43	F	UCTD	+	–	–	–	–	123	–
7	51	F	Lymphoma	+	–	–	–	–	161	–
8	67	F	Renal failure, hypergammaglobulinemia	+	–	–	–	–	98	+
9	85	F	Diabetes, heart block	+	–	–	–	–	1014[b]	–
Group B										
10	77	F	SjS, ataxia	+	–	+	–	–	5999[b]	+
11	54	F	SjS, ataxia	+	–	+	–	–	123	–
12	48	F	SjS, motor neuropathy	+	–	+	–	–	287	–
Group C										
13	51	F	SLE	+	–	–	–	–	226	–
14	46	F	SLE	+	–	–	–	–	139	–
15	47	F	SLE, SjS	+	–	–	+	–	112	–
16	51	F	SLE, SjS	+	+	+	+	+	84	–
17	70	F	SjS	+	–	–	–	–	109	–
18	57	F	SjS, interstitial pneumonitis	+	–	+	+	+	116	–

[a] The results of the addressable laser bead immunoassay for antibodies to GW182 are expressed as median fluorescence units
[b] Sera with a positive test

the GWBs (Fig. 1). The other sera had antibodies to early endosome antigen 1 [21], ribosomal RNP [22], mitochondria [23], cytoplasmic linker protein (CLIP-170) [24], and other as yet unknown endosome or lysosome antigens. None of the 18 sera that bound GWBs had antibodies to dsDNA, chromatin, U1-RNP, topoisomerase I (Scl-70), fibrillarin (U3 RNP), or centromeres/kinetochores [10]. The immunoglobulin isotype of all sera with antibodies to GWBs was IgG as shown by isotype-specific staining of HEp-2 cells, immunoblotting, and protein A Sepharose immunoprecipitation of recombinant GW182 protein. The anti-GWB titers as determined by IIF on HEp-2 cell substrates ranged from 1/320–1/5,120. A study of 2500 healthy female blood donors showed that none of these samples contained anti-GWB antibodies as determined by IIF using HEp-2 cells [25].

Although all 18 sera had antibodies to the GW body, the multiplexed laser bead assay indicated that 4 of the 18 sera (nos. 1, 3, 9, 10) recognized the recombinant GW182 protein which is one of several proteins found within GWBs (Table 1). When the reactivity of the 18 sera was also tested by IP using in vitro transcribed/translated protein, it was observed that 4 sera (nos. 1, 3, 8, 10) IP the GW182 protein (Fig. 2). Therefore when the data of the two assays that used recombinant protein are combined, 5 of the 18 sera recognized GW182.

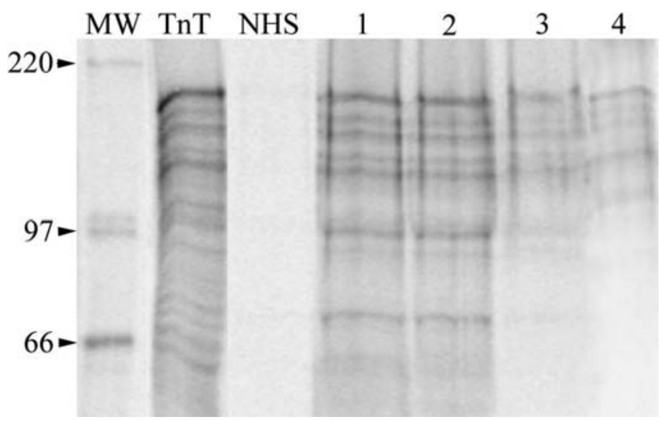

Fig. 2 Immunoprecipitation of the ^{35}S-labeled GW182 TnT recombinant GW182 protein with patient sera. Four sera (*lanes 1–4*) that stained GWBs, immunoprecipitation IP the recombinant GW182 (*TnT*), but normal human serum (*NHS*) did not. *MW* ^{14}C molecular weight markers

The clinical data obtained on the 18 patients who had the GWB staining pattern are summarized in Table 1. Of the 18 patients 17 (39%) with autoantibodies to the GWBs were women and ranged in age from 46 to 85 years (mean 58). The clinical diagnoses could be stratified into three groups: group A composed of 9 patients had predomi-

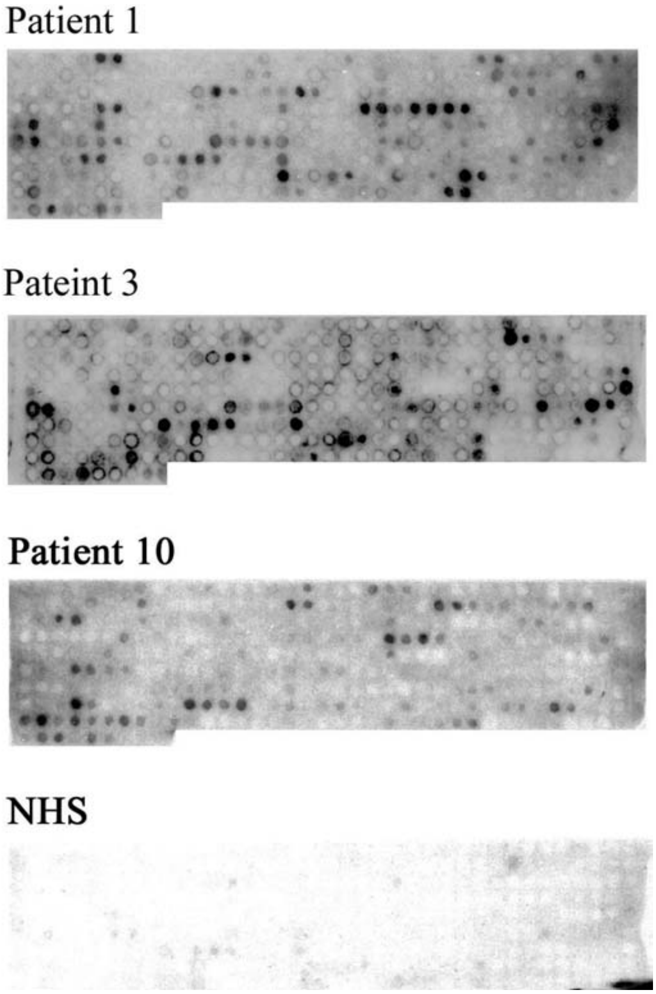

Fig. 3 Epitope mapping obtained using sequential 15mer peptides offset by five amino acids that represented the full-length GW182 protein were spotted on membranes and then probed with a normal human serum (*NHS*) and three sera with anti-GWB antibodies (*patients 1*, *3*, *10*)

nantly mixed motor and/or sensory neuropathy, although other disease manifestations were also noted; 3 patients in group B had SjS in addition to some neurological features that overlapped with group A; in group C there were 6 patients who had SLE and/or SjS without documented evidence of neurological disease. When the various diagnoses or clinical conditions were tabulated individually, SjS was the most common, seen in 7 of 18 (39%), followed by patients with neurological disease (motor and sensory neuropathy and/or ataxia) in 6 (33%), followed by SLE in 4 (22%).

When it was observed that some of the patients had SLE and SjS, we were interested to determine whether autoantibodies to known autoantigens that are typical markers of SLE and SjS were present. Autoantibodies to SS-A/Ro and SS-B/la were correlated with the diagnosis of SjS in 6/7 patients diagnosed with SjS (Table 1). However, four patients in group A had anti-SSA/Ro52 antibodies but did not have a clinical diagnosis of SjS or SLE. Interestingly, 9 sera had antibodies to the 52-kDa SS-A/Ro antigen, but 7 did not have coexisting antibodies to the 60 kDa SS-A/Ro antigen. One patient (no. 4) had a malar rash, arthralgia, and antibodies to the SmB protein but did not fulfill criteria [26] for classification as definite SLE.

Only 4 of the 18 patient sera (22%; nos. 1, 3, 8, 10) with anti-GWB antibodies as defined by colocalization, IP the GW182 protein. Three of these four sera (nos. 1, 3, 10; Table 1) were used for epitope mapping due to limited quantity available for the fourth serum (no. 8). Multiple epitopes over the entire length of GW182 were recognized by the patient sera (Figs. 3, 4). Four overlapping reactive peptides were shared between patient no. 1 and patient no. 10: amino acids 666–695, 951–970, 1676–1690, 1691–1705. Several peptides were in common between patient no. 1 and patient no. 3: amino acids 431–450, 766–780, 921–945, 951–970, 1101–1115, 1161–1185, 1191–1205, 1391–1410, 1431–1445, 1616–1630. Interestingly, only one peptide (1511–1525) was bound by both patient no. 10 and patient no. 3. The reactive epitopes mapped to the GW-rich, the middle portion, the non-GW rich, and the C-terminal domains of the GW182 protein (Fig. 4). When the reactive peptides were subjected to a BLAST analysis, only the published GW182 protein and related EST clones, KIAA1460, KIAA1582, and KIAA1093 showed more than 60% amino acid sequence identity. The KIAA1460 EST is known to be partial-length GW182 [11].

Discussion

In this study we report the clinical features of 18 patients who have autoantibodies to a unique structure within the cytoplasm which we previously named GW bodies or GWBs. Five of the 18 sera that colocalized with the murine monoclonal antibody to GW182 (monoclonal antibody 4B6) which stains the GW bodies, reacted with the GW182 protein in two different immunoassays. This suggests that either the epitopes reactive by IIF are not present in the recombinant proteins used in these assays, or that GWBs contain target autoantigens that remain to be defined. Studies are underway to define additional autoantigens in GWBs.

The incidence of anti-GWB antibodies reported in this study was 0.36%. In the Advanced Diagnostics Laboratory at the University of Calgary, this approaches the incidence of autoantibodies to Sm and Golgi antigens (0.4% and 0.5%, respectively) and was higher than antibodies to proliferating cell nuclear antigen (0.1%), Jo-1 (histidyl t-RNA synthetase (0.1%) and Scl-70 (topoisomerase I, 0.3%; unpublished data).

It is not clear whether the autoantibodies directed against GWBs and GW182 are pathogenic. The clinical diagnosis of patients with GWB autoantibodies included SjS, motor or sensory neuropathies, SLE, and a variety of other clinical conditions. Observations of mRNA processing may be relevant because particles containing

PEPTIDE			PATIENT		
Number	Sequence	Position	#1	#3	#10
6	EKDGLRNSTGLGSQN	26-40			
7	RNSTGLGSQNKFVVG	31-45			
18	NNRMNAWGTVSSSSN	86-100			
22	STLNSASNHGAWPVL	106-120			
29	QCSTIGQMPNNQSIN	141-155			
30	GQMPNNQSINSKVSG	146-160			
36	SEVSGTQKVSFSGQP	176-190			
37	TQKVSFSGQPQNITT	181-195			
45	ELPSSNTGAWRVSTM	221-235			
54	GTTWGAYGSNYSGDK	266-280			
55	AYGSNYSGDKCSGPN	271-285			
63	QVNTNKGGGVWESGA	311-325			
64	KGGGVWESGAANSQS	316-330			
65	WESGAANSQSTSWGS	321-335			
67	TSWGSGNGANSGGSR	331-345			
68	GNGANSGGSRRGWGT	336-350			
77	GKTFTNGWKSTEEED	381-395			
78	NGWKSTEEEDQGSAT	386-400			
87	EKGTGESQSRDRRKI	431-445			
88	ESQSRDRRKIDQHTL	436-450			
92	NRTDLDPRVLSNSGW	456-470			
93	DPRVLSNSGWGQTPI	461-475			
97	WDTETSPRGERKTDN	481-495			
105	GNDTSSVSGWGDPKP	521-535			
106	SVSGWGDPKPALRWG	526-540			
117	KNKQGWGDGQKSSQG	581-595			
118	WGDGQKSSQGWSVSA	586-600			
133	SKPTPSQGWGDPPKS	661-675			
134	SQGWGDPPKSNQSLG	666-680			
135	DPPKSNQSLGWGDSS	671-685			
136	NQSLGWGDSSKPVSS	676-690			
137	WGDSSKPVSSPDWNK	681-695			
138	KPVSSPDWNKQQDIV	686-700			
139	PDWNKQQDIVGSWGI	691-705			
148	EPSPESIRRKMEIDD	736-750			
150	MEIDDGTSAWGDPSK	746-760			
154	VNMWNKNVPNGNSRS	766-780			
171	WGSSSVGPQALSKSG	851-865			
177	PGNRPTGWEEEEDVE	881-895			
185	SSKGLSGKKRRRERG	921-935			
187	RRERGMMKGGNKQEE	931-945			
189	NKQEEAWINPFVKQF	941-955			
190	AWINPFVKQFSNISF	946-960			
191	FVKQFSNISFSRDSP	951-965			
192	SNISFSRDSPEENVQ	956-970			
198	MEIDKHSLNIGDYNR	986-1000			
199	HSLNIGDYNRTVGKG	991-1005			
200	GDYNRTVGKGPGSRP	996-1010			
201	TVGKGPGSRPQISKE	1001-1015			
202	PGSRPQISKESSMER	1006-1020			
217	MFGVGNTAAQPRGMQ	1081-1095			
220	QPPAQPLSSSQPNLR	1096-1110			
221	PLSSSQPNLRAQVPP	1101-1115			
227	PNNGGLNPLFGPQQV	1131-1145			
228	LNPLFGPQQVAMLNQ	1136-1150			
231	LSQLNQLSQISQLQR	1151-1165			
233	SQLQRLLAQQQRAQS	1161-1175			
234	LLAQQQRAQSQRSVP	1166-1180			
235	QRAQSQRSVPSGNRP	1171-1185			
239	GRPLSVQQQMMQQSR	1191-1205			
256	KEPQSRLRKWTTVDS	1276-1290			
257	RLRKWTTVDSISVNT	1281-1295			
263	FRLEESPFVPYDFMN	1311-1325			
264	SPFVPYDFMNSSTSP	1316-1330			
270	SPNGSSSVNWPPEFR	1346-1360			
271	SSVNWPPEFRPGEPW	1351-1365			
272	PPEFRPGEPWKGYPN	1356-1370			
273	PGEPWKGYPNIDPET	1361-1375			
276	DPYVTPGSVINNLSI	1376-1390			
279	NTVREVDHLRDRNSG	1391-1405			
280	VDHLRDRNSGSSSSL	1396-1410			
287	PLSSTAQSTSARNSD	1431-1445			
288	AQSTSARNSDSKLTW	1436-1450			
292	TNTSLAHELWKVPLP	1456-1470			
293	AHELWKVPLPPKNIT	1461-1475			
294	KVPLPPKNITAPSRP	1466-1480			
297	PPGLTGQKPPLSTWD	1481-1495			
298	GQKPPLSTWDNSPLR	1486-1500			
300	NSPLRIGGGWGNSDA	1496-1510			
301	IGGGWGNSDARYTPG	1501-1515			
302	GNSDARYTPGSSWGE	1506-1520			
303	RYTPGSSWGESSSGR	1511-1525			
304	SSWGESSSGRITNWL	1516-1530			
313	LPHGNALVRYSSKEE	1561-1575			
314	ALVRYSSKEEVVKAQ	1566-1580			
323	QSLTPSPGWQSLGSS	1611-1625			
324	SPGWQSLGSSQSRLG	1616-1630			
335	YSTSLWGPPSSSDPR	1671-1685			
336	WGPPSSSDPRGISSP	1676-1690			
337	SSDPRGISSPSPINA	1681-1695			
338	GISSPSPINAFLSVD	1686-1700			
339	SPINAFLSVDHLGGG	1691-1705			
340	AFLSVDHLGGGGESM	1696-1710			

Fig. 4 Amino acid sequence and position of the GW182 protein synthetic peptides and their reactivity with three patient sera with anti-GWB antibodies. *Gradient of white to black* Increasing intensity of reaction of antibodies with peptide

mRNA and the human protein staufen have been observed in neurons [27, 28]. Staufen binds double-stranded RNA and was visualized in RNA containing particles in rat hippocampal neurons after transient transfection experiments [28, 29]. It may be relevant that the GW182 autoantigen was also shown to bind mRNA through its RNA binding motif [11]. It is interesting to speculate that the storage of mRNA by GWBs may be an important process in maintenance of neurons and neurotransmission and that disruption of GW182 function by autoantibodies may affect neural integrity and subsequent motor/sensory neurological disease. This view is supported by preliminary data suggesting that GW182 is highly expressed in neural tissues (unpublished observations). Recent evidence suggests that GW182 and GW bodies are involved in mRNA decapping and subsequent mRNA degradation [14]. It is interesting to speculate that disruption of the GW182 protein and/or GW bodies by the presence of autoantibodies affect one aspect of the mRNA degradation pathway vital in the overall maintenance and function of the cell. Although we have not determined whether mRNA degradation in the GWBs is directly related GW182 function in nonstop [30] or nonsense-mediated mRNA decay [31, 32], the failure to degrade problematic mRNAs with no stop codons or premature termination codons may have pathological consequences on the function of the cell and subsequently be manifest as a disease state.

Our study shows that multiple epitopes of the GW182 protein are recognized by the human antibodies. The SPOT method of epitope mapping has been validated, and the majority of studies has shown that each patient displays an individual epitope pattern [6]. The diverse and heterogenic epitope recognition pattern among the patients observed in this study is not unlikely since the fine specificity of B-cell immune processes strongly depends on the MHC system. Epitope mapping followed by BLAST analysis confirmed that the autoantibody targets are unique to the GW182 protein because sequence similarity to other known eukaryotic or prokaryotic

Table 2 Ribonucleoprotein (*RNP*) autoantigens (*MCTD* mixed connective tissue disease, *PM* polymyositis, *RA* rheumatoid arthritis, *RNP* ribonucleoprotein, *SjS* Sjögren's syndrome, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis)

RNP antigen	RNA: protein antigens	Associated disease
Sm	U2-U6 snRNA: SmD	SLE
U1-RNP	U1 snRNA: U1-A, C, 70 kDa	MCTD
Ra33	hnRNA: A2 core protein	RA
SS-A/Ro	hY RNAs: 60 and 52-kDa proteins	SLE/SjS
SS-B/la	rRNA: P0/P1/P2 proteins	SjS
Ribosomal P proteins	rRNA: L12/S10/l5 proteins	SLE
Ribosome		SLE
Fibrillarin	U3-RNA: 35 kDa fibrillarin	SSc
Hu	mRNA: 37–45 kDa	Paraneoplastic neurological syndromes
GWB	mRNA, 182 kDa GW182	SLE/SjS/neuropathy

proteins or expressed sequence tags was not observed. This suggests that the GW182 protein drives the autoimmune response and reactivity to endogenous or exogenous proteins with similar sequence motifs and molecular mimicry is less likely. This also raises the possibility that, as with many other autoantibody systems, autoreactivity to GW182 demonstrates intramolecular epitope spreading [33, 34]. A study using more sera and different methods in an extended epitope mapping study should shed more light on the epitope distribution on GW182.

The association of anti-GWB antibodies with antibodies to the 52 kDa SS-A/Ro antigen, particularly in the patients with no evidence of SjS and SLE was an unexpected finding. Although the 52-kDa SS-A/Ro antigen has been localized to both the nucleus and cytoplasm, antibodies from a variety of sources directed to the 52-kDa SS-A/Ro autoantigen do not produce a GWB staining pattern [35, 36]. The function of the 52 kDa SS-A/Ro antigen is not clear [37], and the observation that it is associated with GWB antibodies may help clarify its function.

In summary, GWBs are a novel class of RNP autoantigens that are specifically recognized by human autoantibodies. Over the past three decades several autoantigens that are part of RNP macromolecular complexes have been described, and we propose that autoantibodies to GWBs and GW182 now join this growing list (Table 2). Some of these autoantigens, including Sm, U1-RNP, and Hu, have been shown to have a central role in mRNA splicing, mRNA processing, and mRNA translation [37, 38, 39]. In this study we observe that the diseases associated with autoantibodies to GWBs overlap with those associated with other RNPs but extend to patients who appear to have primary neurological disorders.

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