Pathology (December 2019) **51(7)**, pp. 723–726

IMMUNOPATHOLOGY

Autoantibodies to mRNA processing pathways (glycine and tryptophan-rich bodies antibodies): prevalence and clinical utility in a South Australian cohort

Mahmood Al Kindi[1,*], John Cassidy[1], Dianne Gilham[1], Marvin Fritzler[2], Pravin Hissaria[1]

[1]*SA Pathology/IMVS, Adelaide, SA, Australia;* [2]*University of Calgary, Calgary, Canada;* [*]*Current affiliation: Department of Microbiology and Immunology, Sultan Qaboos University Hospital, Muscat, Oman*

Summary

Autoantibodies to glycine and tryptophan-rich bodies (GWB) can be detected on routine antinuclear antibodies (ANA) testing and might have important disease associations. The aim of this study was to investigate the prevalence of anti-GWB antibodies identified on routine ANA testing, define their antigenic specificities and describe their clinical association. Anti-GWB antibodies were identified by distinct cytoplasmic staining pattern on all samples referred for ANA testing over a 6-month period. All positive anti-GWB samples were further tested on a multiplex addressable bead immunoassay (ALBIA) with known GWB antigens. Extractable nuclear antigens (ENA) were characterised by line immunoblot assay. Clinical details were collected retrospectively by contacting patients and the requesting clinicians. Eleven patients (7 females, 4 males) out of a total of 2136 positive ANAs requested on 11,265 samples had the classical GWB pattern (0.5%). The median age of patients was 66 years (range 39–92). There was no consistent disease association. Ten were confirmed to have distinct antigenic specificity for known GWB antigens. Ge-1/Hedls and RAP55 were the most common antigenic specificity targets [seen in 7 patients (64%) and in 5 patients (45%), respectively]. Ro52 was positive in 5/9 (56%) patients, SSB in 2/9 (22%) patients and Ro60 in 1/9 (11%) patient. The clinical association of anti-GWB antibodies is uncertain but might point towards autoimmune origin of certain non-specific musculoskeletal symptoms. The antigenic specificity of anti-GWB reactivity could point towards specific clinical associations: anti-RAP55 and Ge-1 in non-specific musculoskeletal conditions versus anti-GW182 in neurological diseases.

Key words: Glycine and tryptophan-rich bodies; anti-GWB; ANA; ALBIA; autoantibodies.

Received 17 March, revised 15 July, accepted 29 July 2019
Available online 17 October 2019

INTRODUCTION

Antinuclear antibodies (ANA) are autoantibodies detectable in various autoimmune diseases and are directed against different cellular components.[1] Indirect immunofluorescence (IIF) using HEp-2 cell is a highly sensitive, widely utilised assay for detection and distinguishing different antibodies targeting nuclear, mitotic and cytoplasmic antigens.[2–8] Although IIF is sensitive in determining antibodies against nuclear antigens, it is not optimum to antibodies against cytoplasmic antigens.[9] The International Consensus on ANA Pattern (ICAP) recommended to report nuclear, mitotic and cytoplasmic morphological patterns.[7,8] In recent years, these antibodies binding to cytoplasmic constituents like Golgi complex, mitochondria, ribosome, endosome, lysosomes, glycine and tryptophan-rich bodies (GWB) and others have been the focus of many studies. They are not a rare finding and their presence has been reported in up to 15% of all ANA requests in a tertiary diagnostic laboratory.[10]

GWB also known as mammalian processing (P)-bodies or Dcp-containing bodies are discrete cytoplasmic structures involved in mRNA turnover.[11–13] Antibody to these structures, specifically to GW182, was first described in serum of a patient with motor and sensory neuropathy.[14] They play a role in mRNA processing and RNA interference as part of RNA-induced silencing complex (RISC) and in the process of post-transcriptional gene silencing.[15] They are involved in short-interfering RNA (si-RNA) and microRNA (miRNA)-mediated mRNA degradation and/or translation repression. They have sites where aberrant mRNAs are recognised and degraded by decapping and 5′-to-3′ decay mechanism as compared to 3′-to-5′ exonucleases degradation in exosomes.[15]

GWBs are dynamic structures and vary in size during cell cycle with the largest during late S and G2 phase and higher expression in proliferating cells.[15] They contain different ribonucleoproteins implicated in mRNA degradation and silencing. GW182 was the first to be described and gave GWB its name.[14,16–18] Others include Ge-1 also known as Hedls, GW2, GW3, RAP55, hDcap1, hDcp2, LSm1, LSm4, Xrn1, and Su/Ago2 among others.[15,19–21] GWBs interact with other cytoplasmic RNP-rich granules which could explain their diverse clinical associations.[22]

Anti-GWB antibodies can be detected on ANA testing as a distinct cytoplasmic (discrete speckles) pattern (Fig. 1). The frequency of their detection in routine ANA testing is about 0.4 %.[10] It can be obscured by higher titres of other anti-cytoplasmic antibodies such as anti-mitochondrial antibodies. Their recognition also depends on the type of cell line

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2019.07.008

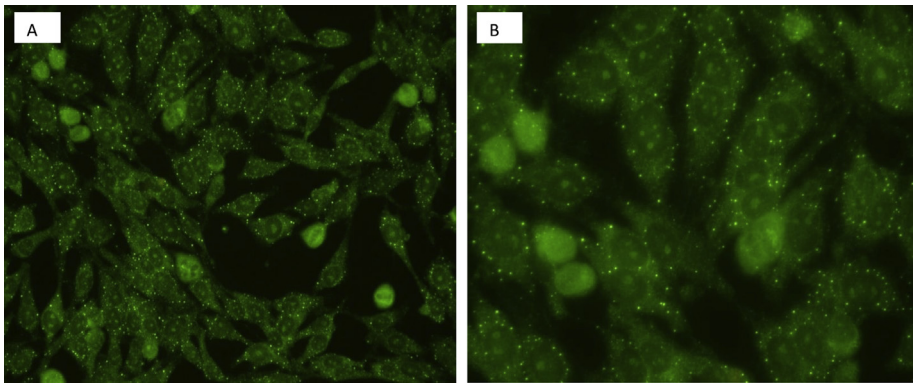

Fig. 1 Characteristic cytoplasmic discrete speckled pattern of anti-GWB antibodies on HEp2000 slides. (A) Low power and (B) higher power magnification.

and tissue used for assay. Confirmation can be by different methodology including bead immunoassays and immunoprecipitation. In a cohort of 55 patients Ge-1 was the major autoantigen target (58%), followed by GW182 (40%).[20] Anti-GWB antibodies have been associated with autoimmune diseases including Sjögren's syndrome, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and primary biliary cirrhosis (PBC), neurological diseases, and cancers.[20,23,24]

We found a distinct cytoplasmic staining pattern consistent with the presence of anti-GWB antibodies in few specimens submitted for routine ANA testing and decided to investigate the antigenic reactivity pattern and clinical significance in our cohort.

MATERIALS AND METHODS

Patients and laboratory analysis

Anti-GWB antibodies were identified by a distinct cytoplasmic staining pattern on all samples referred for ANA testing at Royal Adelaide Hospital using HEp2000 substrate (ImmunoConcepts, USA) over a 6-month period (September 2009 to March 2010). All positive anti-GWB samples were further tested on a multiplex addressable laser bead immunoassay (ALBIA) in the Mitogen Advanced Diagnostics Laboratory (University of Calgary, Calgary, Canada) with known GWB antigens (GW182, GW2, GW3, Ge-1/Hedls, and RAP55) as described previously.[20] Extractable nuclear antigens (ENA) were characterised by line immunoblot assay (Euroimmun, Germany) as per the manufacturer's instructions.

Clinical data and study approval

The clinical details of the patients were collected retrospectively by contacting patients and the requesting clinicians and asking them to complete a questionnaire. The study was approved by the medical ethics committee of the Royal Adelaide Hospital and written consent was obtained from all participants.

RESULTS

Demographic and clinical details

Eleven sera out of 2136 positive ANAs (0.5%) requested over the 6-month period showed anti-GWB pattern on HEp2000. The median age of subjects was 66 years (range 39–92 years) with seven (64%) females and four (36%) males as shown in Table 1. Clinical details are outlined in Table 2. There was no consistent disease association; three patients had a history of arthalgias, two had back pain, one patient each had SLE, RA, monoclonal gammopathy, ankylosing spondylitis and autoimmune thyroid disease, AMA-negative PBC, and non-specific rash.

Table 1 Frequency of cytoplasmic GWB pattern in our laboratory over a 6-month period (Sep 2009 – Mar 2010)

Demographic details	*n*
Total ANA requests	11,265
Positive ANA	2136
Anti-GWB pattern	
% of total ANA requests	11/11,265 (0.1%)
% of positive ANAs	11/2136 (0.5%)
Female : Male	7 : 4
Median age, years	66 (range 39–92)

Characterisation of GWB antigenic specificity

Testing for antigenic specificity to different GWB antigens was performed by ALBIA assay (Table 3). Ten were positive for GWB antigens. Although one was negative in the patient with SLE, the ANA pattern was typical of GWB. Ge-1/Hedls was the most common GWB antigenic target seen in seven patients (64%) followed by RAP55 detected in five patients (45%), Gw2 in two (18%), and GW182 only seen in one patient (9%) with no definite neurological symptoms. Two samples were positive for more than one GWB antigen.

Association of anti-GWB with other cytoplasmic antigens

Antigenic specificity to other cytoplasmic antigens was tested by ALBIA and line immunoblot (Euroimmun). Ro52 was the most frequent non-GWB specificity (56%) seen solely in four patients and in combination with Ro60 in one patient. SSB was present in 2/9 (22%) patients with no history of primary Sjögren's syndrome (Table 3).

DISCUSSION

Antibodies to GWB were identified by the presence of specific cytoplasmic staining pattern on routine ANA testing and subsequently confirmed using specific immunoassays. Cytoplasmic patterns on ANA testing are not uncommon. In the last few years, there has been considerable attention given to cytoplasmic autoantibodies; among others, these include autoantibodies directed to mitochondria, lysosomes, the Golgi complex, ribosomes, etc. There have been very few studies looking at the clinical relevance of anti-GWB antibodies.[5,16,20,25] Since these antibodies give a very distinct staining pattern and can be easily identified, reporting of

Table 2 Demographic, clinical, and serological features of patients with anti-GWB antibodies

Patient no.	Age, years	Sex	Diagnosis	ALBIA[a]	ENA (immunoblot assay)[b]
1	54	M	Arthalgia	RAP55	Ro52
2	64	F	Arthalgia	Ge-1/Hedls	Ro52
3	48	F	SLE	Negative	SSB
4	71	M	Rash	RAP55	Negative
5	68	M	Back pain	RAP55, SRP	Not done
6	92	F	Back pain	RAP55, Ge-1/Hedls, Gw2	Not done
7	79	F	Sjögren's syndrome, MGUS	Ge-1/Hedls	Ro52
8	39	F	Ankylosing spondylitis, autoimmune thyroid disease	Ge-1/Hedls, GW182, RAP55, GW2	SSB
9	90	M	AMA negative PBC	Ge-1/Hedls, Jo-1	Negative
10	65	F	Arthalgia	Ge-1/Hedls	Ro52
11	60	F	RA, hypothyroidism	Ge-1/Hedls	Ro60, Ro52

AMA, anti-mitochondrial antibodies; MGUS, monoclonal gammopathy of undetermined significance; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.
[a] Addressable laser bead immunoassay (ALBIA) with known GWB antigens (GW182, GW2, GW3, Ge-1/Hedls, Ago 2, and RAP55) and other cytoplasmic antigens (SRP, CLIP-70, Jo-1, EEA1 and PDC).
[b] Extractable nuclear antigens (ENA) using Euroimmun immnoblot.

Table 3 Antigenic specificity in 11 patients with anti-GWB pattern on indirect immunofluorescence

Antigenic specificity	Patients *n* (%)
Ge-1/Hedls	7/11 (64%)
RAP55	5/11 (45%)
GW2	2/11 (18%)
GW182	1/11 (9%)
GW3	0
Negative for all tested GWB antigens	1/11 (9%)
Ro52	5/9 (56%)
Other cytoplasmic antigens	2/11 (18%)

these novel autoantibodies would increase our understanding and help in dissecting their clinical significance.

In our study, the clinical association of anti-GWB antibodies was not consistent with any specific autoimmune disease but might point towards autoimmune origin of certain non-specific musculoskeletal symptoms. Ge-1 was the most common antigenic target in our cohort which is similar to previously published findings.[20] RAP55 was the second most common GWB antigenic target found in 45% of patients. This is different to the Canadian study which found GW182 as the second most common antigen.[20] The difference in the antigenic specificities could be explained by the difference in the clinical conditions in the two patient cohorts. The Canadian cohort had neuropathy as the most common clinical feature and was found to be associated with the presence of anti-GW182 antibody.[14,20] In contrast, our cohort did not have any patient with neuropathy and anti-GW182 was identified only in one patient who had reactivity to multiple GWB antigens (Table 2). Thus, it is possible that autoantibody directed against GW182 antigen is more commonly associated with neuropathy. This hypothesis needs to be tested further in a prospective study.

The higher incidence of antibody to Ro52 in patients with anti-GWB antibodies in our study was in keeping with previous studies.[16,20] The presence of this antibody was found to be more commonly associated with anti-Ge-1 antibody and arthralgia. Ge-1 has been reported to be a central component of mammalian P-bodies responsible for mRNA degradation.[19] Ro52 antigen has been localised to both the nucleus and cytoplasm. The function of this antigen is not entirely clear. We can hypothesise from our findings that Ro52 antigen might function as a chaperone in transfer of RNA from nucleus to cytoplasm for its degradation. Further studies are necessary in order to validate this correlation. Our study was limited in that it was a single-centre study with a relatively small sample size.

CONCLUSION

In summary, antigenic specificity of anti-GWB reactivity could point towards specific clinical associations (e.g., anti-GW182 antibody with neuropathy, anti-Ge-1 and anti-RAP55 with musculoskeletal conditions). Prospective clinical studies will help in finding definite clinical associations, and biology of the GWB system may help in explaining the physiological role of Ro52 antigen.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Address for correspondence: Dr Mahmood Al Kindi, Department of Microbiology and Immunology, Sultan Qaboos University Hospital, Muscat, Oman. E-mail: dralkindi@squ.edu.om

References

1. Solomon DH, Kavanaugh AJ, Schur PH. American College of Rheumatology ad hoc committee on immunologic testing guidelines. Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum* 2002; 47: 434–44.
2. Tan EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv Immunol* 1982; 33: 167–240.
3. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989; 44: 93–151.
4. Bradwell AR, Hughes RG, Karim AR. Immunofluorescent antinuclear antibody tests. In: Detrick B, Hamilton RG, Folds JD, editors. *Manual of Molecular and Clinical Laboratory Immunology*. 7th ed. Washington DC: ASM Press, 2006; 101–11.
5. Satoh M, Chan EKL, Sobel ES, *et al*. Clinical implication of autoantibodies in patients with systemic rheumatic diseases. *Expert Rev Clin Immunol* 2007; 3: 721–38.
6. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. *Ann Rheum Dis* 2010; 69: 1420–2.
7. Chan EKL, Damoiseaux J, Carballo OG, *et al*. Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014-2015. *Front Immunol* 2015; 6: 412.

8. Chan EK, Damoiseaux J, de Melo Cruvinel W, *et al*. Report on the second international consensus on ANA pattern (ICAP) workshop in Dresden 2015. *Lupus* 2016; 25: 797–804.
9. Vermeersch P, Bossuyt X. Prevalence and clinical significance of rare antinuclear antibody patterns. *Autoimmun Rev* 2013; 12: 998–1003.
10. Stinton LM, Eystathioy T, Selak S, *et al*. Autoantibodies to protein transport and messenger RNA processing pathways: endosomes, lysosomes, Golgi complex, proteasomes, assemblyosomes, exosomes, and GW bodies. *Clin Immunol* 2004; 110: 30–44.
11. Behm-Ansmant I, Rehwinkel J, Doerks T. mRNA degradation by miRNAs and GW182 requires both CCR4: NOT deadenylase and DCP1: DCP2 decapping complexes. *Genes Dev* 2006; 20: 1885–98.
12. Jain S, Parker R. The discovery and analysis of P bodies. *Adv Exp Med Biol* 2013; 768: 23–43.
13. Yao B, Li S, Chan EK. Function of GW bodies in siRNA and miRNA pathways. *Adv Exp Med Biol* 2013; 768: 71–96.
14. Eystathioy T, Chan EK, Tenenbaum SA, *et al*. A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. *Mol Biol Cell* 2002; 13: 1338–51.
15. Jakymiw A, Pauley KM, Li S, *et al*. The role of GW/P-bodies in RNA processing and silencing. *J Cell Sci* 2007; 120: 1317–23.
16. Eystathioy T, Chan EK, Takeuchi K, *et al*. Clinical and serological associations of autoantibodies to GW bodies and a novel cytoplasmic autoantigen GW182. *J Mol Med* 2003; 81: 811–8.
17. Yang Z, Jakymiw A, Wood MR, *et al*. GW182 is critical for the stability of GW bodies expressed during the cell cycle and cell proliferation. *J Cell Sci* 2004; 117: 5567–78.
18. Lian S, Jakymiw A, Eystathioy T, *et al*. GW bodies, microRNAs and the cell cycle. *Cell Cycle* 2006; 5: 242–5.
19. Yu JH, Yang WH, Gulick T, *et al*. Ge-1 is a central component of the mammalian cytoplasmic mRNA processing body. *RNA* 2005; 11: 1795–802.
20. Bhanji RA, Eystathioy T, Chan EK, *et al*. Clinical and serological features of patients with autoantibodies to GW/P bodies. *Clin Immunol* 2007; 125: 247–56.
21. Jakymiw A, Ikeda K, Fritzler MJ, *et al*. Autoimmune targeting of key components of RNA interference. *Arthritis Res Ther* 2006; 8: R87.
22. Moser JJ, Fritzler MJ. Relationship of other cytoplasmic ribonucleoprotein bodies (cRNPB) to GW/P bodies. *Adv Exp Med Biol* 2013; 768: 213–42.
23. Bloch DB, Nobre RA, Yang WH. GW/P-bodies and autoimmune disease. *Adv Exp Med Biol* 2013; 768: 61–70.
24. Luft LM. *Characterization of GWBs in Breast Cancer*. PhD thesis dissertation. Calgary: University of Calgary, 2015.
25. Bloch DB, Yu JH, Yang WH, *et al*. The cytoplasmic dot staining pattern is detected in a subgroup of patients with primary biliary cirrhosis. *J Rheumatol* 2005; 32: 477–83.