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Original Article

Extraordinary antigenicity of the human Ro52 autoantigen

Peter D. Burbelo[1], Kathryn H. Ching[1], Brian L. Han[1], Emily R. Bush[1], Westley H. Reeves[2] and Michael J. Iadarola[1]

[1]Neurobiology and Pain Therapeutics Section, Laboratory of Sensory Biology, National Institute of Craniofacial Research, National Institutes of Health, Bethesda, Maryland, US; [2]Division of Rheumatology and Clinical Immunology and Center for Autoimmune Diseases, University of Florida, Gainesville, Florida, USA.

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Abstract: Autoantibody levels to the SSA complex, composed of Ro52 and Ro60 proteins, are commonly measured in the diagnoses of Sjögren's Syndrome (SjS), as well as other rheumatological diseases. One of these proteins, Ro52, is an interferon-inducible member of the tripartite motif family bearing a RING motif functioning as an E3 ligase that ubiquitinates interferon regulatory factor 8 and other proteins. Using Luciferase Immunoprecipitation Systems (LIPS) we explored the antigenicity of Ro52 in detail. Analysis of antibody responses against Ro52 and 20 other established antigens revealed that Ro52 had the highest antibody titers and most likely represents one of the most immunogenic human proteins. While the antibody titers in many of the SjS patients were significantly and substantially higher than the controls, all healthy individuals had anti-Ro52 autoantibodies. N- and C-terminal fragments of Ro52 showed immunoreactivity in these serum samples, but the sums of these antibody titers were significantly lower than the antibody titers directed against the full-length Ro52. Antibody profiling of controls and SjS patients with three different N-terminal fragments of Ro52 revealed that the coiled-coil region was the most useful diagnostic (66% sensitivity), followed by the B-box (31% sensitivity), and then the RING-finger (24% sensitivity). The C-terminal region of Ro52, containing the B30.2 domain, showed higher antibody titers in SjS patients compared to controls and this region was responsible for the high level of Ro52 immunoreactivity in healthy individuals. Analysis of immunoreactivity to TRIM5, a Ro52-related protein, and the B30.2 domain from BTN1 and pyrin, failed to show significant antibody titers with the control or SjS patient serum. These results highlight the unusually high level of Ro52 antigenicity and demonstrate that autoantibodies are directed at both linear and conformational epitopes spanning the entire molecule.

Keywords: Autoantibody, autoantigen, Sjögren's Syndrome, Luciferase Immunoprecipitation Systems (LIPS), and Ro52

Introduction

Sjögren's Syndrome (SjS) is an autoimmune disease involving immune damage to the salivary and lacrimal glands, which produce saliva and tears, respectively [1]. Manifestations of this disease can range from the sicca symptoms of dry mouth and eyes, to much more widespread symptoms involving the lungs, liver, and peripheral nervous system. Currently, classification of primary SjS is based on six criteria, including oral and ocular dryness, minor salivary gland inflammation, and the presence of certain autoantibodies [2]. The major autoantibodies measured are directed against the extractable nuclear antigen SSA, composed of a mixture of two distinct proteins, Ro52 (also called TRIM21) and Ro60 (also called TROVE2) [3]. In addition to SjS, autoantibodies to Ro52 and Ro60 are also found in a variety of other rheumatological diseases including systemic lupus erythematosis, myositis and systemic sclerosis. Although Ro52 and Ro60 show no sequence homology and do not interact, the autoantibodies against these proteins strongly correlate with each other for reasons that remain obscure.

Ro52 is a member of the tripartite motif (TRIM) family of proteins. Ro52 contains multiple domains including two zinc-finger motifs comprising the RING-finger, a B-box, a coiled-coil region and a C-terminal B30.2 domain (also called PRY/SPRY) [4]. Recent studies demonstrate that Ro52 is a ubiquitin ligase involved in the

proteosomal destruction of a variety of proteins [5-9]. The ubiquitin ligase activity of Ro52 maps to the N-terminus and requires a RING-finger motif [5, 8, 9]. Ro52 is also an immunoglobulin-binding protein [10-12], and its binding to the Fc region of IgG1 immunoglobulins requires its C-terminal B30.2 domain [13-15]. Takahata et al. found that Ro52 plays a role in the proteosomal destruction of misfolded IgG1, suggesting that it is involved in quality control of immunoglobulins [10]. Despite these studies, little is known about the relationship between Ro52's immunoglobulin-binding activity and its role as a major human autoantigen.

Although Ro52 is a well-established autoantigen, almost all of the published studies have employed solid-phase immunoassays, such as ELISA, using immobilized peptides and recombinant proteins [16]. These approaches poorly detect conformational epitopes and show a limited dynamic range of detection [17]. As an alternative, we have measured antibodies using the solution-phase Luciferase Immunoprecipitation Systems (LIPS) technology, which harnesses light-emitting *Renilla* luciferase recombinant proteins to efficiently detect antibody responses to both linear and conformational epitopes [18]. Due to the highly linear light output of Ruc in the LIPS assay, most antibodies can be measured without serum dilution in a dynamic range of detection often spanning seven orders of magnitude. In our previous studies, LIPS profiling of autoantibodies against Ro52 and other autoantigens showed important diagnostic utility [19, 20]. Here we have used LIPS to assess the antigenicity of Ro52 and map important conformational epitopes. In addition, several Ro52-related proteins and protein domains were evaluated for immunoreactivity in control and SjS patient samples.

Materials and methods

Patients

A cohort collected at the University of Florida under Institutional Review Board-approved protocols consisted of 104 SjS and 30 control sera. The diagnosis of SjS was established using the European-American consensus criteria [2]. As previously described, anti-Ro60 and anti-La (SSB) seropositive status in these samples was also previously evaluated in the clinical laboratory of the Division of Rheumatology and Clinical Immunology and Center for Autoimmune Diseases, University of Florida and showed 56% sensitivity for detecting SjS in this cohort [19].

Renilla luciferase antigen constructs

A mammalian *Renilla* luciferase (Ruc) expression vector, pREN2, containing an N-terminal FLAG epitope tag was used for all Ruc-antigen constructs [21]. Previously, a deletion fragment of Ro52 was used in LIPS for the diagnosis of SjS [19, 20]. Although in our above mentioned papers the diagnostic performance of the Ro52 fragment was correct, we have now found that the described fragments used in this study were flipped. The correct deletion fragment nomenclature should be as follows: Ro52-Δ1 (spanning amino acid residues 2-273) and Ro52-Δ2 (spanning amino acid residues 277-475). Three new deletion constructs derived from the N-terminus of Ro52 were generated including Ro52-Δ3 (spanning amino acid residues 2-62), Ro52-Δ4 (spanning amino acid residues 70-128), and Ro52-Δ5 (spanning amino acid residues 129-273). In addition, the B30.2 domains of BTN1 and pyrin and the full-length TRIM5α protein were also generated as Ruc fusions and tested in the LIPS. The adapter primers used for PCR and the DNA/ protein sequences are available upon request.

LIPS assays

LIPS was used as described in a publication and technical video from the Journal of Visualized Experiments (http://www.jove.com/index/details.stp?ID=1549) [18]. In these assays, sera were processed in a 96-well format. A "master plate" was first constructed by diluting patient sera 1:10 in assay buffer A (50 mM Tris, pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100) in a 96-well polypropylene microtiter plate. For evaluating antibody titers by LIPS, 40 ml of buffer A, 10 ml of diluted human sera (1 ml equivalent), and 1 ´ 10^7 light units (LU) of Ruc-antigen Cos1 cell extract, diluted in buffer A to a volume of 50 ml, were added to each well of a polypropylene plate and incubated for 60 minutes at room temperature on a rotary shaker. Next, 5 ml of a 30% suspension of Ultralink protein A/G beads (Pierce Biotechnology, Rockford, IL) in PBS were added to the bottom of each well of a 96-well filter HTS plate (Millipore, Bedford, MA). To this filter plate, the 100 ml antigen-antibody reaction mixture was

transferred and incubated for 60 minutes at room temperature on a rotary shaker. The washing steps of the retained protein A/G beads were performed on a Tecan plate washer with a vacuum manifold. After the final wash, LU were measured in a Berthold LB 960 Centro microplate luminometer (Berthold Technologies, Bad Wilbad, Germany) using coelenterazine substrate mix (Promega, Madison, WI). All LU data was obtained from the average of at least two independent experiments and the resulting LU values were used without subtracting the buffer blank. The heatmap, shown in **Figure 2**, represents $\log_{10}$ transformed data values that are color coded ranging from red to green indicating high and low antibody titers, respectively.

Statistical analysis

For statistical analyses, the GraphPad Prism software (San Diego, CA) was used. Unless otherwise stated, antibody titers for the control and SjS serum samples are reported in the text and figures as the mean ± 95% confidence interval. Correlations among antibody responses to two of the Ro52 antigen fragments were assessed by the Spearman correlation coefficient. For determining the cut-off values for each of the LIPS tests, the mean value of the 30 control samples plus 3 standard deviations (SD) was used and is indicated in the figures.

Results

Relative antigenicity of Ro52 and Ro60 compared to other human autoantigens and pathogen antigens

Our previously published LIPS studies showed extremely high anti-Ro52 autoantibody titers in SjS (and to a lesser extent in controls) requiring significant serum dilution to obtain values in the linear range [20]. To formally document the unusually high level of immunoreactivity against Ro52, the antibody titers for Ro52 in our standard LIPS format were compared against 21 other highly immunoreactive proteins, including human autoantigens and a variety of antigens from different pathogens. LIPS detection of antibodies to 20 of these antigens, including anti-La autoantibodies in patients with SjS, anti-GAD65 autoantibodies in type I diabetes patients and anti-p24 GAG antibodies in HIV-infected individuals, yielded geometric mean antibody titers (GMT) in the linear range of 10^4 to 10^6 LU with 1 µl of serum from different seropositive disease samples (**Table 1**). For these antigens, much lower levels of antibody titers were found in the corresponding control samples making all these LIPS tests highly useful for diagnostics (**Table 1**). Both Ro60 and Ro52 required a 1:200 (0.005 µl) and 1:400 (0.0025 µl) dilution of sera, respectively, to obtain values in the non-saturated linear range (**Table 1**). Despite this marked dilution of serum, high levels of anti-Ro52 autoantibodies were still present (GMT 1.2×10^7 LU) in healthy individuals, but patients with SjS showed markedly higher antibody titers with GMT of 4.0×10^8 LU (**Table 1**). Together these comparisons highlight the extraordinary immunogenicity of Ro52 and suggest that it may be the most antigenic protein recognized in humans. Based on these results, we further studied Ro52 to understand in detail its usually high antigenicity.

Mapping the highly immunogenic conformational epitopes within the N-terminus of Ro52

Previously the N-terminal fragment of Ro52, designated Ro52-Δ1, was found to be highly immunogenic with SjS samples and no longer required the sera to be diluted [20]. To further define the antigenicity of regions within the N-terminus of Ro52, three additional fragments: Ro52-Δ3 (spanning amino acids 2-62 containing the RING-finger), Ro52-Δ4 (spanning amino acids 70-128 containing the B-box), and Ro52-Δ5 (spanning amino acids 129-277 containing the coiled-coil domain) were tested by LIPS in a cohort of 104 SjS patients and 30 normal volunteers. Testing of Ro52-Δ3 by LIPS revealed that the mean of anti-Ro52-Δ3 autoantibodies in 104 SjS samples was 73,547 LU, which was over 30-fold higher than the mean of 2,327 LU of the 30 healthy controls (**Figure 1A**). Using a cut-off value 5,768 LU, the Ro52-Δ3 antibody test showed 24% sensitivity and 96% specificity in distinguishing the 104 SjS patients from the controls. Testing of Ro52-Δ4 by LIPS revealed that the mean antibody titer in 104 SjS samples was 157,319 LU, which was over 60-fold higher than the GMT of 2,488 LU of the controls (**Figure 1B**). Using a cut-off value of 4,760 LU, the Ro52-Δ4 antibody test showed 31% sensitivity and 96% specificity in distinguishing the 104 SjS patients from the controls. However, the Ro52-Δ5, containing the coiled-coil domain, was the most antigenic of the three fragments (**Figure 1C**). Testing of Ro52-Δ5 by LIPS

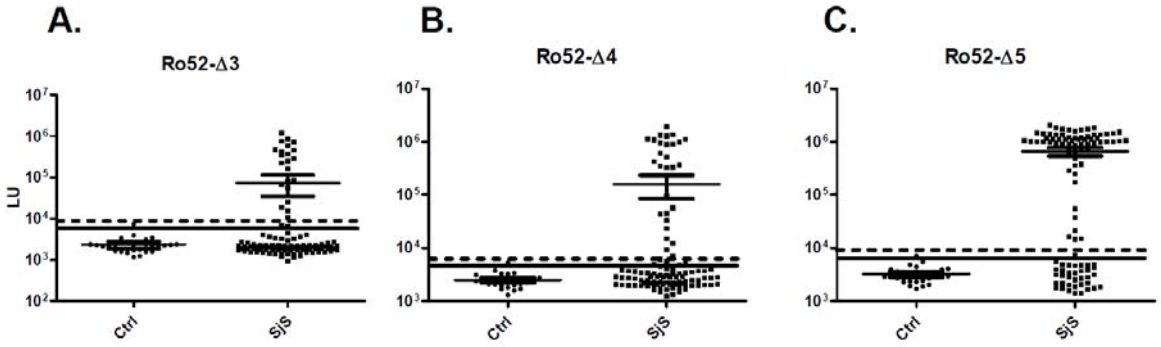

Figure 1. LIPS autoantibody detection to different regions of the N-terminus of Ro52 in normal controls and SjS. LIPS detection of autoantibodies against Ro52-Δ3 (**A**), Ro52-Δ4 (**B**), and Ro52-Δ5 (**C**) in 30 normal controls and 104 primary SjS patients. Each circle or square symbol represents individual normal controls or SjS patient samples, respectively. The short solid lines are the mean and 95% CI for each group. For determining sensitivity and specificity, the long solid lines and dashed lines represents the cut-off level derived from the control mean plus 3 SD and 5 SD, respectively.

Table 1. Relative patient and control antibody titers determined by LIPS to different antigens

	Protein	Cohort	Seropositive GMT (LU)[1]	Seronegative GMT (LU)	Reference
Human	Ro52	SjS	4.0×10^8	1.2×10^7	[20]
Autoantigen	Ro60	SjS	2.0×10^8	6.0×10^6	[20]
	La	SjS	5.0×10^5	5.0×10^3	[20]
	TPO	SjS	1.5×10^4	5.0×10^3	[20]
	H+/K+ ATPase	SjS	5.0×10^5	1.0×10^0	[20]
	AQP4	SjS	3.0×10^4	3.0×10^3	[20]
	IA2	T1D	2.0×10^5	1.7×10^3	[34]
	GAD65	T1D	2.0×10^5	1.7×10^3	[35]
	p53	Cancer	2.0×10^5	1.7×10^3	[21]
Pathogen	EBV gp42	EBV	1.5×10^5	1.0×10^4	[36]
Antigen	CMV pp150-d1	CMV	3.0×10^5	1.5×10^1	[37]
	CMV pp65-d2	CMV	1.0×10^5	2.0×10^2	[37]
	HIV p24	HIV	6.0×10^4	4.0×10^2	[38]
	HIV ENV	HIV	6.0×10^4	4.0×10^2	[38]
	HSV1 gG-1	HSV-1	1.0×10^5	1.5×10^2	[39]
	HSV2 gG-2	HSV-2	3.0×10^5	1.5×10^2	[39]
	HTVL-I GAG	HTLV-1	1.0×10^5	1.0×10^0	[40]
	HTLV-I ENV	HTLV-1	3.5×10^5	1.0×10^0	[40]
	Loa Loa SXP-1	Loa	1.5×10^6	1.0×10^0	[41]
	Ov FAR-1	Oncho	3.0×10^5	4.0×10^2	[42]
	Ov API-1	Oncho	2.0×10^5	7.0×10^2	[42]
	Ov MSA-1	Oncho	6.0×10^4	7.0×10^2	[42]

[1] Only the seropositive samples above the cutoff were used.

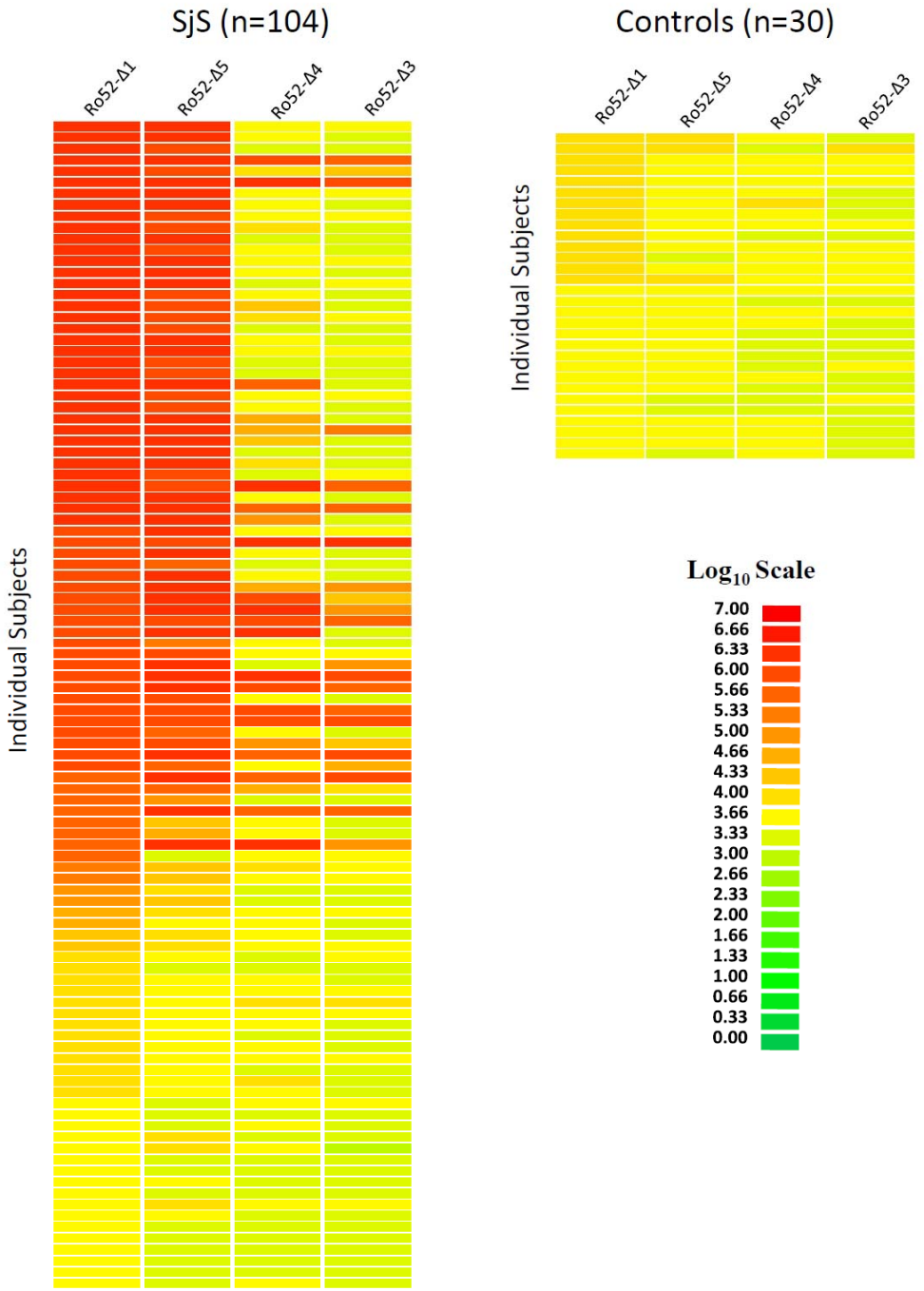

Figure 2. Heatmap representation of patient antibody responses to different protein fragments of Ro52. The antibody titer values for each control and SjS patient were $\log_{10}$ transformed, then color coded as indicated by the $\log_{10}$ scale of the heatmap, in which signal intensities range from red to green indicating high and low titers, respectively. Each row represents a single serum sample tested with the different antigens. The most informative antigen for SjS diagnosis by LIPS, Ro52-Δ1, is shown on the far left.

revealed that the mean of the anti-Ro52-Δ5 antibodies in 104 SjS samples was 652,390 LU, which was over 200-fold higher than the mean of 3,225 LU of the controls. Using a cut-off value of 6,684 LU, the Ro52-Δ5 antibody test showed 66% sensitivity and 96% specificity

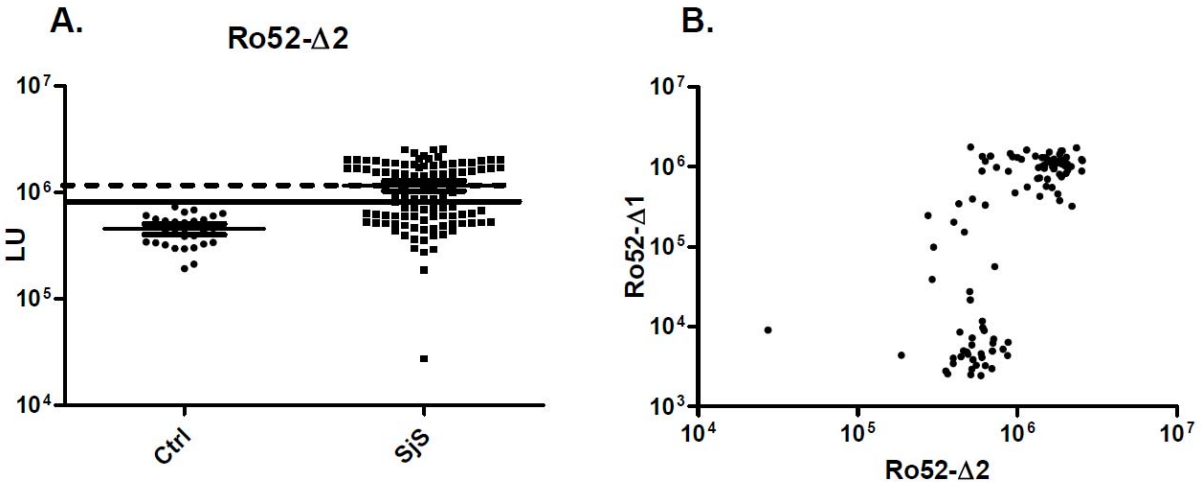

Figure 3. LIPS detection of autoantibodies to the B30.2 domain of Ro52. **A**. LIPS detection of autoantibodies to the B30.2 domain of Ro52 is shown with Ro52-Δ2 fragment. Each circle or square symbol represents individual normal controls or SjS patient samples, respectively. The short solid lines are the mean and 95% CI for each group. The solid and dashed lines represent the mean plus 3 SD and 5 SD, respectively, of the antibody titer for the normal volunteers. **B**. Correlation of autoantibody responses to Ro52-Δ1 and Ro52-Δ2. Spearman rank correlation showed that they weakly correlated (*R*=0.65).

in distinguishing the 104 SjS patients from the controls and showed similar antigenicity as the entire N-terminus of Ro52.

To easily visualize immunoreactivity to each individual with the different Ro52 fragments we employed a heatmap to graphically display the antibody responses. As a reference, we also determined the autoantibody titers against the entire N-terminus (Ro52-D1) in the samples and present this data in the heatmap. Using a $\log_{10}$ scale transformation of the different Ro52 protein fragments in this heatmap, there were obvious differences in immunoreactivity of these fragments in the different SjS patients (**Figure 2**). For example, the Ro52-Δ5 piece spanning the coiled-coil region showed almost an identical profile to the anti-Ro52-Δ1 antibody profile in the SjS patients, but showed less immunoreactivity in several of the controls. The two other sub-fragments, Ro52-Δ4 and Ro52-Δ3, were less informative and reacted only with a subset of the Ro52-Δ5 SjS positive serum. As shown in the heatmap, there was relatively little immunoreactivity seen in all four Ro52 fragments when tested in the control group (**Figure 2**). Together these results highlight the fact that the coiled-coil region shows the highest region of immunoreactivity within the N-terminus of Ro52 and that the other regions of the molecule are much less immunoreactive and informative.

The C-terminus of Ro52 is highly immunogenic with healthy human sera but is still a specific autoantibody target in SjS

The antigenicity of the C-terminus of Ro52-Δ2 containing the B30.2 domain was also examined in detail and also no longer required dilution of the serum. Testing of the 104 SjS patients and 30 normal controls revealed that C-terminal fragment showed immunoreactivity with all sera tested, but the SjS samples showed much higher titer values than the control sera (**Figure 3A**). Using a mean plus 3 SD cut-off based on the controls, this Ro52-Δ2 showed 54% sensitivity and 100% specificity. Remarkably, the absolute antibody titer values seen in the controls were higher than the values obtained in seropositive samples for other autoantigens (**Table 1**). Comparison of the antibody titers in the N-terminus with that of the C-terminus revealed many of the same SjS samples were also positive and analysis by Spearman rank correlation showed that they weakly correlated (*R*=0.65, **Figure 3B**).

To formally rule out that the immunoreactivity associated with this fragment might be due to direct binding to immunoglobulins in the serum

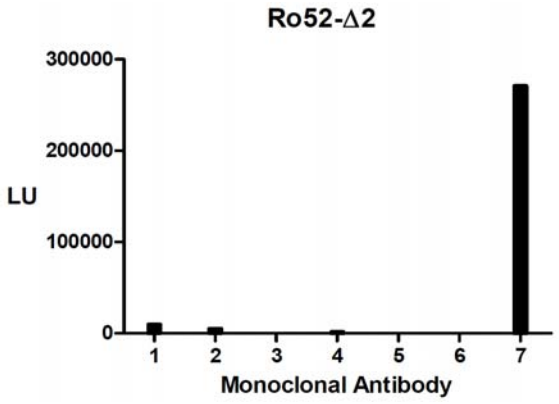

Figure 4. C-terminal Ro52-Δ2 is an autoantigen in SjS. Several different mouse monoclonal antibodies were tested including anti-Myc (lane 1), anti-La (lane 2), anti-AChRα3 (lane 3), anti-nAChRα7 (lane 4), anti-nAChRβ3 (lane 5), anti-IL4-Stat (lane 6) and anti-FLAG (lane 7). While the anti-FLAG monoclonal efficiently immunoprecipitated the FLAG-tagged Ro52-D2 protein, none of the other monoclonal showed significant activity.

of normal control and SjS patients, an additional experiment using defined monoclonal antibodies was used to further study the possibility that the C-terminal B30.2 domain might contain an immunoglobulin-binding domain. Several mouse monoclonal antibodies containing Fab regions to other targets not related to Ro52 were tested for their ability to immunopr cipitate Ro52. As a positive control, the anti-FLAG monoclonal antibody successfully immunoprecitatated Ro52-Δ2 because of the N-terminal FLAG tag on Ruc (**Figure 4**). In contrast, all the other monoclonal antibodies against targets such as Myc, La and the acetylcholine receptor subunits did not immunoprecipitate significant amounts of the Ro52-Δ2 target (**Figure 4**). These results demonstrate that the immunoreactivity of this C-terminal Ro52 fragment represents its true behavior as an autoantigen in SjS, but still shows immunoreactivity in healthy individuals.

The Ro52-related proteins and protein domains are not autoantigens in SjS

There are several other human proteins that contain Ro52-related B30.2 domains, but perform other functions. For example, the B30.2 domains in BTN1 and pyrin bind xanthine oxidase [22] and caspase-1 [23], respectively. Due to the high level of immunoreactivity seen with the B30.2 domain of Ro52, both the B30.2 domain of BTN1 and pyrin were tested as targets of autoantibodies. LIPS testing of the B30.2 domain of BTN1 showed weak autoantibody titers in the samples and only one SjS patient sample was above the mean plus 5 SD cut-off of the control samples (**Figure 5A**). Similarly, testing of the B30.2 domain of pyrin also revealed no significant immunoreactivity with any of the samples (data not shown). Taken together, these results suggest that the high level of immunoreactivity found with the B30.2 domain reflects Ro52-specific conformational epi-

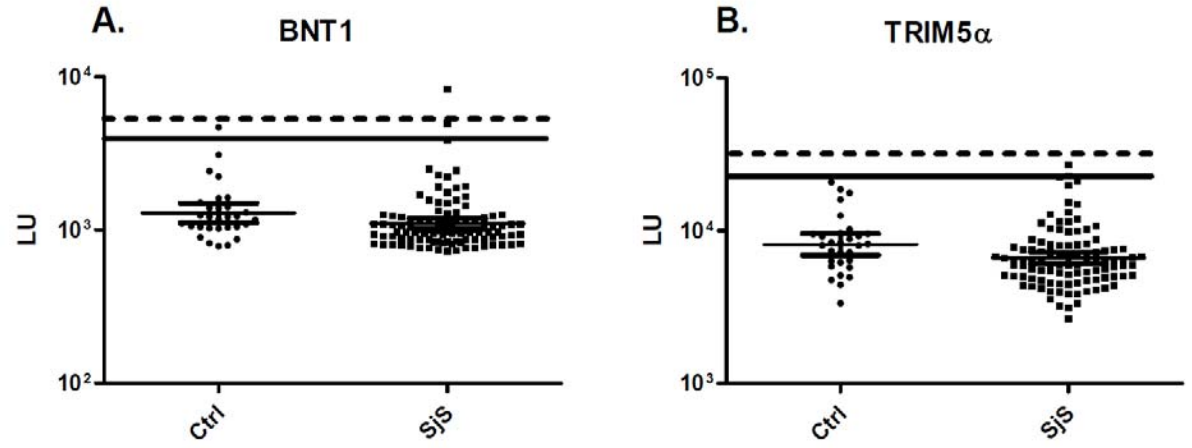

Figure 5. BTN1, a B30.2 Domain-Containing Protein, and TRIM5, are not autoantigens in SjS. Antibodies were evaluated by LIPS against BTN1 (**A**) and TRIM5 (**B**) with 107 SjS and 30 control sera. Each circle or square symbol represents individual normal controls or SjS patient samples, respectively. The short solid lines are the mean and 95% CI for each group. The solid and dashed lines represent the mean plus 3 SD and 5 SD, respectively, of the antibody titer for the normal volunteers. No significant difference in immunoreactivity was observed between the controls and SjS samples with either antigen.

topes and or unique peptides rather than short shared linear epitopes.

In addition, we also tested the antigenicity of entire Ro52-related protein TRIM5α. TRIM5α showed 40% identity and 58% similarity with the amino acids sequence spanning the entire length of the Ro52 including the RING-finger, B-box, a coiled-coil and the C-terminal B30.2 domain. From LIPS testing, none of the SjS samples showed anti-TRIM5α antibody titers above the cut-off of the controls (**Figure 5B**). These results suggest that this related protein, TRIM5α, is not a target of autoantibodies in normal controls or SjS patients.

Discussion

Here we used a highly sensitive quantitative immunoassay to the study the antigenicity of Ro52. From these studies Ro52 appears to be one of the most antigenic proteins when compared to other well-known antigenic proteins. While side-by-side comparisons of the antigenicity of Ro52 and other proteins have never been done before, the findings of high immunoreactivity with Ro52 are consistent with the ability to diagnostically detect useful autoantibodies against native SSA by relatively simple immunoassays such as Oucterlony double immunodiffusion [24] and counterimmunoelectrophoresis [25]. These high LIPS antibody titers with Ro52 and Ro60 contrast with most other autoantigens and explain why sensitive solution-phase immunoassays rather than solid-phase immunoassays are needed to detect antibodies to many other autoantigens [17]. Ro52 and Ro60 proteins share no primary amino acid sequences, do not appear to interact *in vivo* and have different functional activities. Possible clues to Ro52's antigenicity in SjS and other rheumatological diseases may relate to high levels of expression in antigen-presenting cells such as B cells, macrophages and dendritic cells [26] and its up-regulated in response to interferons [7, 27, 28].

Although there have been many previous studies on antigenic epitopes of Ro52, the majority of these studies have used less than optimal immunoassays [16]. For example, Ottoson et al. recently could not to detect autoantibodies to the B30.2 domain of Ro52 by immunoblot or ELISA [29]. In contrast, autoantibodies against the B30.2 domain of Ro52 are readily detectable by LIPS. The dramatic loss of LIPS detectable autoantibodies when Ro52 was divided into two separate N- and C-terminal fragments is consistent with the notion that many of the ant-Ro52 autoantibodies are directed at conformational epitopes. Autoantibodies to the N-terminus showed high specificity with SjS patients but not with the controls. From testing three different N-terminal fragments, the coiled-coil region was found to be markedly more antigenic than the B-box or RING finger domain. That is, the coiled-coil region of Ro52 using the Ro52-D5 deletion fragment showed extraordinarily high titers in many of the SjS patients. The antibody titers in the Ro52 seropositive SjS patients were generally 1,000-fold higher than controls and showed a sensitivity of 66% and specificity of 96%. As proposed by others, it is likely that this coiled-coil region of Ro52 may be more accessible to recognition by autoantibodies than other regions of the molecule [29, 30]. While other studies using less than optimal immunoassays have found autoantibodies to this region in children with heart block [31], and SjS [32] , the relative difference in antibody titers between the patients and controls are much more modest. Since we found that the coiled-coil region of Ro52 discriminated controls from SjS patients the best, it is not surprising that these autoantibodies are the most informative for diagnosis in many other conditions.

Although the C-terminus of Ro52 is an immunoglobulin binding domain [13-15], our data using several monoclonal antibodies showed no evidence of Fc-mediated immunoprecipitation suggesting that the high levels of immunoprecipitation activity seen in human serum samples are not due to artifactual protein-protein interactions. In addition, the SjS samples that were seropositive with the C-terminal B30.1 domain were all also seropositive with the N-terminus of Ro52. However, the high level of immunoreactivity to the C-terminal B30.2 domain within control patients makes it possible that this region may be instrumental in driving the antigenicity of Ro52 in SjS and other rheumatological diseases. The lack of immunoreactivity of the B30.2-related domains of BTN1, pyrin and the full TRIM5α protein also strongly suggests that conformational epitopes are more important than short linear epitopes. Although more exhaustive studies are needed, these results suggest that autoantibodies seen in these SjS patients are highly specific for Ro52 and not other

related proteins. Furthermore, these findings are consistent with the results of two additional deletion fragments (Ro52-D6 and Ro52-D7) derived from the splitting the B30.2 fragment, which were found to be completely devoid of immunoreactivity (Burbelo et al., unpublished data). Taken together these results suggest that conformational epitopes within Ro52 are responsible for antigenicity. What drives antigenicity to these conformational epitopes still remains unanswered. In light of an interesting report showing that a region within Ro60 shows molecular mimicry with non-linear epitopes with the Epstein Bar Virus EBNA protein [33], it is possible that this region of Ro52 also shares epitopes with antibodies directed against a pathogen protein. Moreover, based on the finding that healthy controls also contained high titer antibodies to Ro52, Ro52 may be attractive target autoantigen to monitor environmental triggers that might induce the production of these autoantibodies in healthy individuals.

Acknowledgement

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Abbreviations: SjS, Sjögren's Syndrome; LIPS, Luciferase Immunoprecipitation Systems; LU, light Units; Ruc, *Renilla* luciferase; SD, standard deviations; TRIM, tripartite motif.

Please address correspondence to: Peter D. Burbelo, PhD, Building 49, Room 1C36, 49 Convent Drive, Bethesda, MD 20892. Phone: 301-402-0778. Fax: 301-402-0667. E-mail: (burbelop@nidcr.nih.gov).

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