ORIGINAL ARTICLE

Anti-Ro52/TRIM21 antibodies are associated with aberrant inflammatory circuits in patients with systemic autoimmune rheumatic diseases

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Abstract

Introduction: *Anti-Ro52/TRIM21 antibodies are markers for several systemic autoimmune rheumatic diseases (SARD).* **Objective:** *To assess whether anti-Ro52/TRIM21 antibodies are related to abnormalities in inflammatory circuits.* **Methods:** *Cross-sectional study of consecutive outpatients with SARD. Anti-Ro52/TRIM21 antibodies and serum amyloid A protein were measured by ELISA; panels for 18 cytokines and nine chemokines were analyzed on a Luminex reading platform, while high-sensitivity C-reactive protein (hs-CRP) and complement were measured by nephelometry.* **Results:** *Among 167 included patients, 143 had systemic lupus erythematosus (SLE), 16 had primary Sjögren's syndrome and eight had systemic sclerosis; 41 (24%) were positive for anti-Ro52/TRIM21 antibodies. Patients with anti-Ro52/TRIM21 antibodies had higher serum levels of IL-2, IL-4, IL-6, GM-CSF, IL-21, IL-22, hs-CRP and chemokines CCL4, CXCL8, CXCL10 and CXCL12, but lower levels of complement C4. Anti-Ro52/TRIM21 antibody titers were positively correlated with IL-2, IL-4, IL-6, IL-10, IL-21, IL-22, CXCL10, and hs-CRP, and negatively with complements C3 and C4. When only SLE patients were included, no association was identified between anti-Ro52/TRIM21 antibodies and disease activity or organ-specific involvement.* **Conclusions:** *Anti-Ro52/TRIM21 antibodies are associated with aberrant cytokine circuits and elevated levels of angiogenic molecules and neutrophil and monocyte chemoattractants, which suggests an active role for these antibodies in SARD.*

KEYWORDS: *Anti-Ro52/TRIM21 antibodies. Cytokines. Inflammation. Chemokines.*

Los anticuerpos anti-Ro52/TRIM21 están asociados a circuitos inflamatorios aberrantes en pacientes con enfermedades reumáticas autoinmunes sistémicas

Resumen

Introducción: *Los anticuerpos anti-Ro52/TRIM21 son marcadores de varias enfermedades reumáticas autoinmunes sistémicas (ERAS).* **Objetivo:** *Evaluar si los anticuerpos anti-Ro52/TRIM21 están relacionados con anomalías en los circuitos inflamatorios.* **Métodos:** *Estudio transversal de pacientes consecutivos y ambulatorios con ERAS. Los anticuerpos anti-Ro52/TRIM21 y la proteína amiloide sérica se midieron mediante ELISA; los paneles para 18 citocinas y nueve quimiocinas se analizaron en una plataforma de lectura Luminex; la proteína C reactiva (hs-CRP) y el complemento se midieron mediante nefelometría.* **Resultados:** *Se incluyeron 167 pacientes, 143 con lupus eritematoso sistémico (LES), 16 con síndrome de*

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Date of reception: 08-09-2022
Date of acceptance: 20-09-2022
DOI: 10.24875/GMM.M22000739

Gac Med Mex. 2023;159:55-64
Contents available at PubMed
www.gacetamedicademexico.com

Sjögren primario y ocho con esclerosis sistémica; 41 fueron positivos para anticuerpos anti-Ro52/TRIM21 (24 %). Los pacientes con anticuerpos anti-Ro52/TRIM21 tuvieron niveles séricos más altos de IL-2, IL-4, IL-6, GM-CSF, IL-21, IL-22, hs-CRP y quimiocinas CCL4, CXCL8, CXCL10 y CXCL12; y más bajos de complemento C4. Los títulos de anticuerpos anti-Ro52/TRIM21 correlacionaron positivamente con IL-2, IL-4, IL-6, IL-10, IL-21, IL-22, CXCL10 y hs-CRP; y negativamente con complemento C3 y C4. Al incluir solo LES, no se identificó asociación entre los anticuerpos anti-Ro52/TRIM21 y la actividad de la enfermedad o la afectación específica de órganos. **Conclusiones:** *Los anticuerpos anti-Ro52/TRIM21 se asocian a circuitos aberrantes de citocinas y niveles elevados de moléculas angiogénicas y quimioatrayentes de neutrófilos y monocitos, lo que sugiere un papel activo de esos anticuerpos en las ERAS.*

PALABRAS CLAVE: *Anticuerpos anti-Ro52/TRIM21. Citocinas. Inflamación. Quimiocinas.*

Introduction

Systemic autoimmune rheumatic diseases (SARDs) are complex disorders in which factors such as ultraviolet light, infections, hormones, and adjuvants act as triggers for aberrant immune mechanisms to be initiated in genetically susceptible individuals.[1] SARDs are characterized by a loss of immune checkpoints and effector cells uncontrolled activation. This aberrant immune functioning translates into abnormalities in cytokine circuits and into the production of autoantibodies, which form the pathophysiological substrate of tissue and organ damage that defines said diseases.[1]

The TRIM21 molecule is a 52-kDa protein (Ro52) recognized by anti-Ro52/TRIM21 antibodies, which are serological markers often found in patients with SARD.[2] As a member of the tripartite-motif family of proteins, TRIM21 is composed of a RING domain, a type 2 B-box sequence and a helical region. TRIM21 is the E3 ubiquitin ligase enzyme, which participates in the post-translational modifications that lead to proteolysis in proteasomes.[3] After its activation by an activating enzyme (E1), ubiquitin is transferred to a conjugating enzyme (E2), from where it is transferred to the target protein by E3 ligase. This process results in poly-ubiquitination of the target protein, with the consequent capture and degradation by proteasomes (Fig. 1).[4] TRIM21 mediates ubiquitination of multiple proteins, including interferon regulatory factors (IRFs) and nuclear factor kappa B (NF-κB).[3,5] Therefore, TRIM21 is a potent regulator of cytokine production, and its functional interruption leads to a higher production of interleukins and interferons.[4,6,7]

Anti-Ro52/TRIM21 antibodies interaction with their antigenic target within living cells (cell lines and hybridomas) has been demonstrated.[2,8] This interaction leads to TRIM21 regulatory function loss, with a consequent increase in the production of immunoglobulins and soluble inflammatory mediators.[7] However, the cytokine circuits predominantly affected by the presence of circulating anti-Ro52/TRIM21 antibodies in patients with SARD have not yet been identified, and neither has it been elucidated if these antibodies can affect the serum levels of other inflammatory molecules such as chemokines and pentraxins.

Methods

The study was carried out at "Ignacio Chávez" National Institute of Cardiology, a tertiary care teaching hospital. Adult patients diagnosed with systemic lupus erythematosus (SLE),[9] primary Sjögren's syndrome (pSS),[10] or systemic sclerosis (SS) were included.[11] Patients with overlap syndromes and undifferentiated connective tissue disease or mixed connective tissue disease were excluded, although overlapping with antiphospholipid syndrome was allowed. No patient had clinically apparent infections. Pregnant or postpartum women, and individuals with a history of neoplasm, chronic kidney disease, or infection with hepatitis B or C virus, or human immunodeficiency virus were not included.

The study followed the guidelines of the Declaration of Helsinki, and the protocol (No. 16-960) was approved by the research and ethics committees of "Ignacio Chávez" National Institute of Cardiology. All patients signed an informed consent document and authorized the use of clinical data and biological samples for research purposes.

At recruitment, patients were examined by the same rheumatologist, and radiographic and laboratory test results were evaluated. In patients with SLE, disease activity was determined using the SLEDAI-2K index.[12] Specific organ involvement was evaluated according to pre-established definitions.[13]

Sera were obtained from venous blood by centrifugation (600 g, 15 minutes, 4 °C) and stored at –70 °C until their use. Each aliquot was thawed under standard conditions to measure the different mediators in independent processes. Anti-Ro52/TRIM21 antibodies

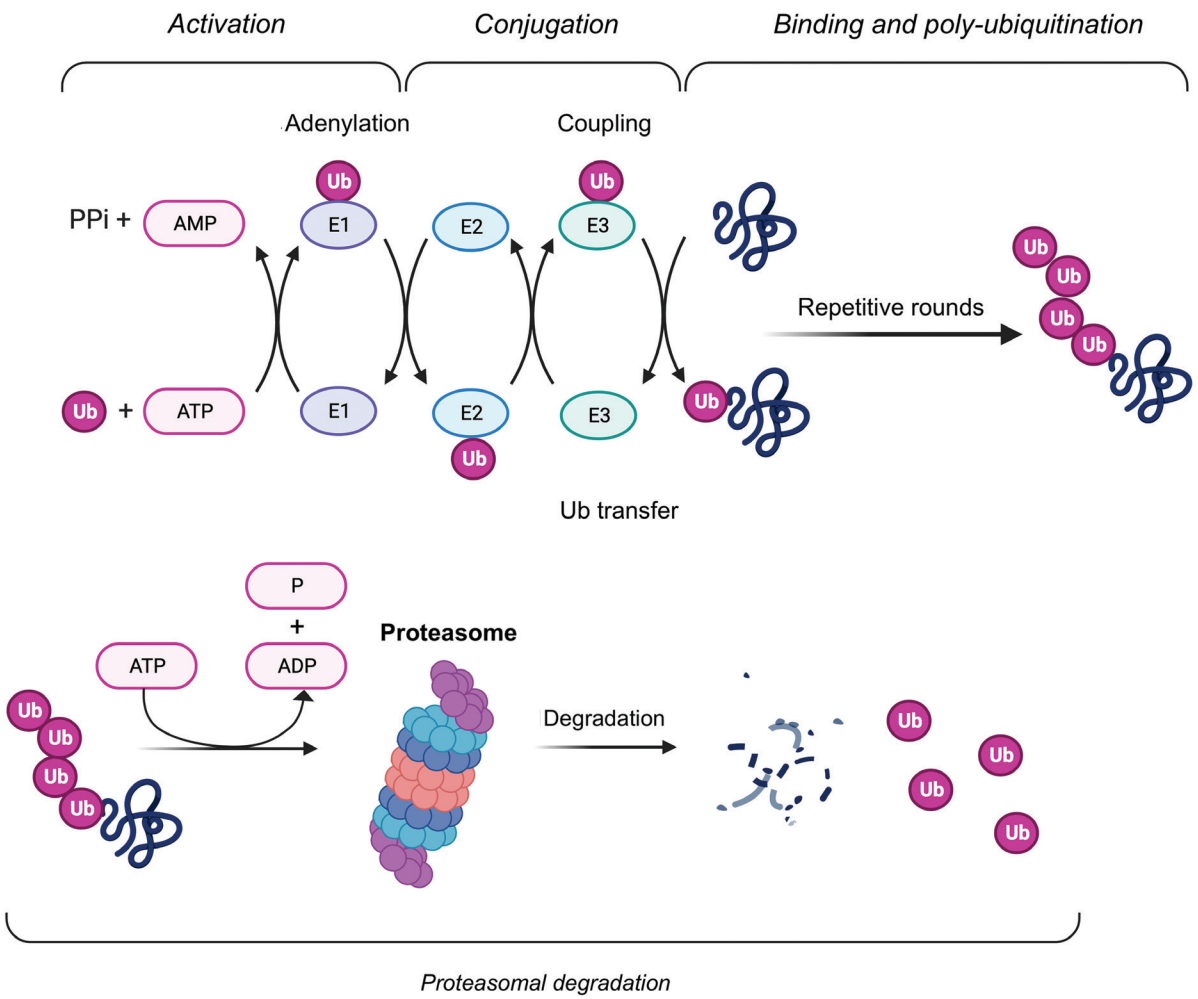

Figure 1. Protein ubiquitination system for proteasomal degradation. First, activating enzyme E1 activates ubiquitin using ATP and transfers it to conjugating enzyme E2. In turn, ligase enzyme E3 recognizes a protein substrate and recruits E2 enzyme to catalyze ubiquitin transfer from E2 to the protein substrate. After repeated binding and ubiquitination rounds, the poly-ubiquitinated protein substrate is transferred to an ATP-dependent protease complex, called proteasome, which degrades damaged or misfolded proteins into smaller polypeptides. Created with BioRender.com.

(Orgentec Diagnostika, Germany; cutoff for positivity at 25 U/mL) and serum amyloid A protein (ThermoFisher Scientific, USA; range: 9.4-600 ng/mL) were quantified by enzyme-linked immunosorbent assay. Cytokines were measured with an 18-Plex Human ProcartaPlex panel and chemokines with a 9-Plex Human ProcartaPlex panel (ThermoFisher) on a Luminex reading platform (MagPix, Luminex Corp., USA). High-sensitivity C-reactive protein (hs-CRP) and complement proteins C3 and C4 were measured by nephelometry (Beckman Coulter, USA).

Data distribution was evaluated using the D'Agostino-Pearson test. According to the observed distribution, medians (and minimum and maximum values) were used as descriptors for continuous variables; for discrete variables, proportions and percentages were used. Differences between groups (independent samples) were analyzed using Mann-Whitney's U-test; differences between more than two groups were evaluated with Kruskal-Wallis test (post-Dunn test). Differences between proportions were assessed using Fisher's exact test or the chi-square test, as appropriate. Associations were analyzed using Spearman's ρ-coefficient, with 95% confidence intervals (CI). Analyses were two-tailed, with a p-value < 0.05 indicating statistical significance. GraphPad Prism, version 9.3.1 (GraphPad Inc., USA), was used for calculations.

Protein-protein interaction networks with functional enrichment analysis for soluble mediators identified as being different based on anti-Ro52/TRIM21

Table 1. Main clinical and laboratory data of patients with systemic autoimmune rheumatic diseases

Clinical and laboratory data	Total (n = 167)		Anti-Ro52/TRIM21 antibodies				p
			Positive (n = 41)		Negative (n = 126)		
	Median	Min-max	Median	Min-max	Median	Min-max	
Age in years	41	18–81	45	19-79	39	18-81	0.100
Evolution years	6	3-12	4	1-18	6	1-41	0.028
Body mass index (kg/m^2)	24.7	15.2-40.4	24.9	17.9-40.4	24.4	15.2-37.3	0.816
Laboratory tests							
Leukocytes (x 10^3/mm^3)	5.7	2.6-14.8	4.9	2.6-14.8	5.8	2.7-13.0	0.090
Neutrophils (x 10^3/mm^3)	3.3	1.3-13.3	2.7	1.3-13.3	3.3	1.3-9.8	0.201
Platelets (x 10^3/mm^3)	227	32-595	227	87-595	226	32-400	0.878
Hemoglobin (g/dL)	13.6	6.0-20.3	13.5	7.6-20.3	13.7	6.0-19.1	0.324
Creatinine (mg/dL)	0.7	0.3-13.9	0.7	0.3-6.9	0.7	0.3-13.9	0.564
Blood urea nitrogen (mg/dL)	14.3	4.8-81.9	12.9	4.8-75.8	14.5	6.6-81.9	0.423
ESR (mm/h)	10	1-140	14	4-119	10	1-140	0.166
C3 complement (mg/L)	94.4	11.9-150.1	92.7	11.9-135.9	94.9	23.1-150.1	0.062
C4 complement (mg/L)	15.7	1.5-43.8	13.4	1.7-29.0	17.0	1.5-43.8	0.003
	n	%	n	%	n	%	
Female gender	149	89	38	92	111	88	0.566
Autoimmune disease							
Systemic lupus erythematosus	143	85	28	68	115	91	0.001
Primary Sjögren syndrome	16	9	8	19	8	6	
Systemic sclerosis	8	4	5	12	3	2	
Hypertension	59	35	13	31	46	36	0.707
Diabetes	12	7	2	4	10	7	0.732
APLS	31	18	4	9	27	21	0.109
Treatment							
Glucocorticoids	80	119	18	43	62	49	0.592
Hydroxychloroquine	121	72	23	56	98	77	0.009
Cyclophosphamide	42	25	9	21	33	26	0.681
Mycophenolate mofetil	47	28	14	34	33	26	0.325
Azathioprine	37	22	5	12	32	25	0.086
Methotrexate	35	20	10	24	25	19	0.516
Antibodies							
Antinuclear antibodies	167	100	41	100	126	100	1.0
Anti-dsDNA	112	67	28	68	84		1.0
Anti-Ro/SS-A	70	41	41	100	29	2663	< 0.0001
Anti-La/SS-B	28	16	17	41	11	8	< 0.0001
Anti-Sm	23	13	6	14	17	13	0.800
Anti-RNP	55	32	11	26	44	34	0.444
Anti-nucleosomes	80	47	19	46	61	48	0.858

APLS: antiphospholipid syndrome; ESR: erythrocyte sedimentation rate.

antibodies expression were created using the STRING program, version 11.5, which is freely accessible online at https://string-db.org/[14] (accessed between January and March 2022).

Results

One-hundred and sixty-seven patients with SARD were recruited, with primary diagnoses being SLE in 143 (85%), pSS in 16 (9%), and SS in eight (4%). Median age was 41 years (18-81), and 149 were women (89%). Table 1 summarizes clinical and laboratory data.

As for anti-Ro52/TRIM21 antibodies, 41 patients (24%) were positive. Seropositivity was higher in SS (62%) and pSS (50%) in comparison with SLE (19%) (p = 0.028. Posttest: SS vs. SLE, p = 0.012; pSS vs. SLE, p = 0.010; SS vs. pSS, p = 0.679). In addition,

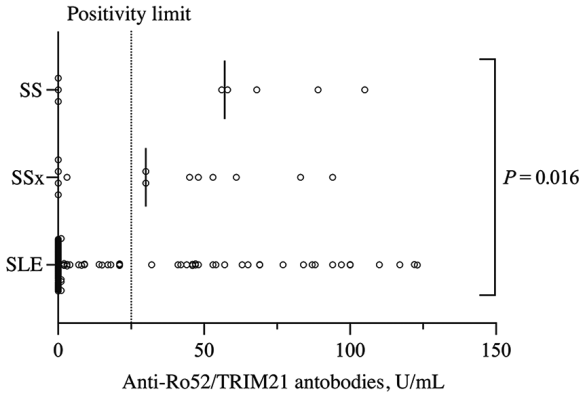

Figure 2. *Anti-Ro52/TRIM21 antibodies serum levels. Anti-Ro52/TRIM21 antibody levels were higher in patients with systemic sclerosis (SS) than in those with Sjögren's syndrome (SSx) or systemic lupus erythematosus (SLE). Median: 57 U/mL vs. 30 U/mL vs. 0 U/mL, respectively; p = 0.016 for multiple comparisons and p-value not significant on post-test analysis. Vertical lines indicate median values. Cutoff point for positivity = 25 U/mL.*

patients with anti-Ro52/TRIM21 antibodies had lower C4 complement levels (13.4, 1.7-29.0 mg/L vs. 17.0, 1.5-43.8 mg/L, p = 0.003) and higher seropositivity for anti-Ro/SSA (100% vs. 23%, p < 0.0001) and anti-La/SSB (41% vs. 8%, p < 0.0001). Other variables are presented in table 1. Anti-Ro52/TRIM21 antibodies titers (Fig. 2) were higher in patients with SS (57, 0-105 U/mL), intermediate in pSS (30, 0-94 U/mL) and lower in SLE (0, 0-123 U/mL, p = 0.016 for multiple comparisons; p-value not significant in posttest analyses).

Table 2 shows the levels of soluble mediators according to the presence of anti-Ro52/TRIM21 antibodies. There were significant differences in the levels of interleukin (IL)-2, IL-4, IL-6, granulocyte-monocyte colony-stimulating factor (GM-CSF), IL-21, and IL-22 between patients who were positive and negative for anti-Ro52/TRIM21 antibodies. While several cytokines were undetectable, analyzed chemokines were consistently detected in nearly all samples. In this sense, the highest levels of chemokines CCL4, CXCL8, CXCL10 and CXCL12 were found in patients with anti-Ro52/TRIM21 antibodies. Finally, hs-CRP concentration was higher in patients who were positive for anti-TRIM21 antibodies, while there was no difference in serum amyloid A protein levels.

The association between anti-Ro52/TRIM21 antibody titers and soluble mediators was evaluated (Table 3). As it can be observed in figure 3, anti-Ro52/TRIM21 showed a direct association with the levels of IL-2 (ρ = 0.21), IL-4 (ρ = 0.18), IL-6 (ρ = 0.18), IL- 10 (ρ = 0.18), IL-21 (ρ = 0.30), IL-22 (ρ = 0.23), CXCL10 (ρ = 0.22) and hs-CRP (ρ = 0.22), as well as an inverse association with C3 (ρ = −0.17) and C4 (ρ = −0.20).

The potential association between anti-Ro52/TRIM21 antibody levels and disease activity was evaluated in patients with SLE, given that they constituted the largest population. As it can be observed in figure 4A, disease activity showed no correlation with anti-Ro52/TRIM21 antibody levels (ρ = −0.003, p = 0.971). Similarly, we found no differences according to neuropsychiatric, vasculitic, articular, renal, hematological, mucocutaneous, or serous involvement (Figure 4B).

Finally, protein-protein interaction networks showed interactions between the TRIM21 molecule and different inflammatory mediators, mainly through transcription factors IRF3, IRF5, IRF7 and NF-κB (via its inhibitory system). These interactions were observed beyond those expected by chance and were based on relationships determined both experimentally and by text mining (Fig. 5).

Discussion

In this study, we found that anti-Ro52/TRIM21 antibodies are associated with an aberrant response of the Th17-phenotype cytokine circuit and high levels of molecules that are angiogenic and chemoattractant for neutrophils and monocytes. This abnormal response occurs regardless of disease activity or involvement of a specific organ.

Circulating autoantibodies are considered the hallmark of SARD, and the way they can bind to intracellular antigens for producing functional alterations has been the subject of intense research.[15] Alarcón Segovia et al. demonstrated for the first time the ability of some anti-ribonucleoprotein antibodies for penetrating cells through Fc-gamma receptors.[16] Subsequently, the capacity of other autoantibodies to penetrate living cells and produce functional alterations, even by mechanisms independent from those of Fc-gamma receptors.[17-20]

As for anti-TRIM21 antibodies, those obtained from patients with SLE or pSS have been observed to characteristically target the TRIM21 molecule functionally-active RING domain (domain with E3 ligase activity), and, therefore, they can inhibit TRIM21 ligase activity by sterically blocking E3/E2 interaction.[2,8] Ubiquitination-mediated protein degradation regulates multiple cellular processes, including cell cycle control, signal transduction, DNA repair, autophagy, and apoptosis.[4,21]

Table 2. Inflammatory markers serum levels

Marker	Anti-Ro52/TRIM21 antibodies				p
	Positive (n = 41)		Negative (n = 126)		
	Median	Min-max	Median	Min-max	
Cytokines (pg/mL)					
Interleukin-27	5	5-1393	5	5-487	0.176
Interleukin-1β	0	0-46	0	0-27	0.064
Interleukin-2	0	0-147	0	0-55	0.035
Interleukin-4	1	1-289	1	1-218	0.009
Interleukin-5	0	0-282	0	0-74	0.228
Interleukin-6	0	0-179	0	0-416	0.040
Interleukin-10	0	0-22	0	0-58	0.069
Interleukin-12p70	0	0-27	0	0-137	0.268
Interleukin-13	0	0-19	0	0-28	0.176
Interleukin-17A	0	0-32	0	0-92	0.547
Interferon-gamma	63	0-463	42	0-433	0.100
GM-CSF	1	1-273	1	1-160	0.035
Tumor necrosis factor	7	0-149	0	0-137	0.066
Interleukin-9	0	0-17	0	0-21	0.418
Interleukin-23	0	0-275	0	0-82	0.106
Interleukin-18	71	0-379	56	0-1063	0.326
Interleukin-21	0	0-2389	0	0-874	0.001
Interleukin-22	8	8-187	8	8-189	0.007
Chemokines (pg/mL)					
CCL2 (MCP-1)	214	41-1210	187	14-3943	0.301
CCL3 (MIP-1α)	72	13-423	49	4-560	0.263
CCL4 (MIP-1β)	326	170-739	263	130-716	0.034
CCL5 (RANTES)	248	75-1645	173	43-8870	0.168
CCL11 (eotaxin)	67	13-487	59	6-435	0.394
CXCL1 (GRO-α)	58	13-167	41	5-357	0.066
CXCL8 (interleukin-8)	16	3-88	12	0-179	0.032
CXCL10 (IP-10)	135	31-741	91	15-992	0.007
CXCL12 (SDF-1)	864	374-2727	718	0-3754	0.039
Pentraxins					
hs-CRP (mg/L)	2.9	0.5-14.6	1.7	0-131.0	0.007
Amyloid protein A (ng/mL)	63	0-146	66	0-147	0.719

GM-CSF: granulocyte-macrophage colony-stimulating factor; hs-CRP; high-sensitivity C-reactive protein.

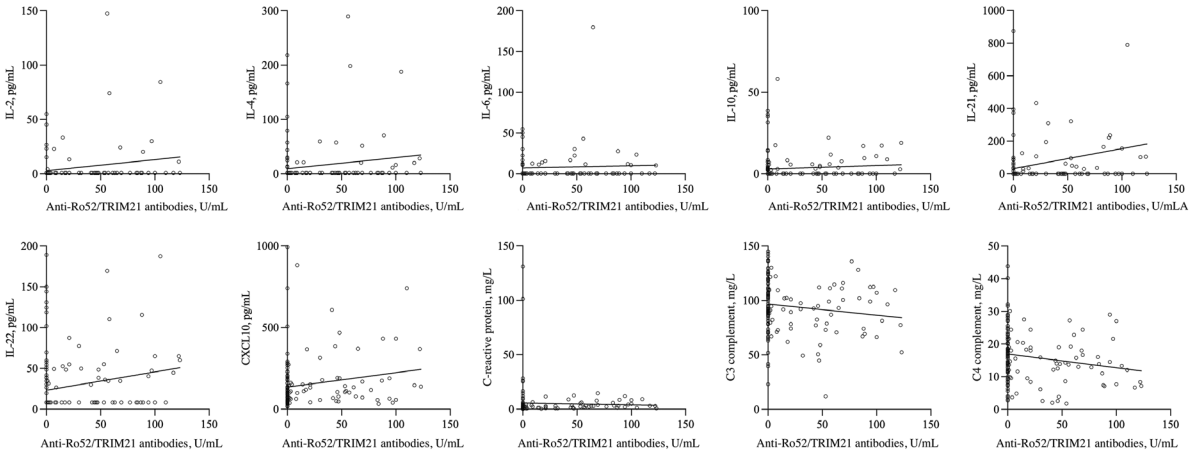

Figure 3. *Association between anti-Ro52/TRIM21 antibodies titer and inflammatory markers. Charts with the correlation analyses that showed significant association between anti-Ro52/TRIM21 antibody levels and different soluble inflammatory mediators. Spearman's ρ coefficient values are presented in the text. Regression line is plotted on each chart.*

Table 3. Correlation analysis between anti-Ro52/TRIM21 antibody titers and inflammatory markers serum levels

Marker	Spearman's ρ coefficient	95% CI	p
Interleukin -27	0.09	−0.08 to 0.25	0.288
Interleukin-1β	0.10	−0.06 to 0.26	0.208
Interleukin-2	0.21	0.04 to 0.36	0.012
Interleukin-4	0.18	0.01 to 0.34	0.026
Interleukin-5	0.12	−0.04 to 0.28	0.153
Interleukin-6	0.18	0.02 to 0.34	0.025
Interleukin-10	0.18	0.02 to 0.34	0.027
Interleukin-12p70	0.08	−0.08 to 0.24	0.351
Interleukin-13	0.09	−0.07 to 0.25	0.291
Interleukin-17A	0.03	−0.14 to 0.20	0.709
Interferon-gamma	0.09	−0.08 to 0.25	0.292
GM-CSF	0.15	−0.02 to 0.30	0.077
Tumor necrosis factor	0.11	−0.05 to 0.27	0.170
Interleukin-9	0.06	−0.10 to 0.23	0.433
Interleukin-23	0.14	−0.03 to 0.30	0.092
Interleukin-18	0.06	−0.11 to 0.22	0.504
Interleukin-21	0.30	0.14 to 0.44	0.0002
Interleukin-22	0.23	0.07 to 0.38	0.004
CCL3 (MIP-1α)	0.09	−0.08 to 0.26	0.287
CXCL12 (SDF-1)	0.12	−0.06 to 0.28	0.175
CXCL10 (IP-10)	0.22	0.05 to 0.38	0.008
CXCL8 (interleukin-8)	0.16	−0.01 to 0.32	0.059
CCL11 (eotaxin)	0.05	−0.13 to 0.22	0.583
CCL5 (RANTES)	0.05	−0.15 to 0.25	0.616
CCL4 (MIP-1β)	0.10	−0.07 to 0.26	0.241
CCL2 (MCP-1)	0.03	−0.14 to 0.20	0.733
CXCL1 (GRO-α)	0.12	−0.06 to 0.28	0.172
hs-CRP	0.22	0.05 to 0.38	0.008
Amyloid protein A	0	−0.17 to 0.17	0.978
ESR	0.14	−0.04 to 0.31	0.117
C3 complement	−0.17	−0.32 to 0	0.048
C4 complement	−0.20	−0.35 to -0.03	0.016

CI: confidence interval; ESR: erythrocyte sedimentation rate; GM-CSF: granulocyte-macrophage colony-stimulating factor; hs-CRP: high-sensitivity C-reactive protein.

Some transcription factors that are responsible for the production of type I interferons, such as IRF3, are recognized by the TRIM21 molecule for ubiquitin-mediated degradation, which results in type I interferons production downregulation.[4] In parallel, NF-κB is a critical transcription factor in a wide variety of cellular events. In resting cells, NF-κB is sequestered in the cytoplasm by inhibitory protein IkappaB (IκB). NF-κB can be transiently activated by cell surface receptors such as tumor necrosis factor (TNF) receptor, Toll-like receptor 4, or lipopolysaccharide receptor.[3] These inducers lead to IκB kinase complex (IKK) activation, which in turn phosphorylates IκB, making it susceptible to TRIM21-mediated ubiquitination with subsequent proteasomal degradation. IκB degradation allows free NF-κB to move to the nucleus, where it promotes the transcription of multiple genes involved in inflammation, oncogenesis, and apoptosis. Strangely enough, TRIM21 can also ubiquitinate IKK, which facilitates its degradation and, therefore, TRIM21 net effect is as a potent NF-κB signaling negative regulator by preventing IκB phosphorylation via IKK.[3]

Given the relevance of TRIM21 as a cytokine response regulator, it can be assumed that anti-Ro52/TRIM21 antibodies exert a facilitating effect on the production of inflammatory mediators. A recent study demonstrated that anti-Ro52/TRIM21 antibodies are associated with type I and II interferons overproduction, as well as with B-cell hyperactivation, with a consequent increase in immunoglobulin production.[7] This study also showed differences in IL-6, IL-10, and interferon-induced chemokines CXCL8 and CXCL10 serum concentration.[7] In addition, TRIM21 expression-deficient mononuclear cells from patients with inflammatory myopathies have been observed to show an inflammatory phenotype characterized by higher production of IL-6, interferon-α, and TNF.[6] Furthermore, TRIM21-deficient CD4+ cells are characterized by IL-17 overproduction, which is a prototypical cytokine of the Th17 phenotype.[6] This finding is relevant to the results of our study, since effector lymphocytes differentiation into Th17 has the function of activating neutrophils in order to maintain epithelial homeostasis in physical barriers such as the skin and mucous membranes. In addition, Th17 lymphocytes are potent inducers of tissue inflammation and autoimmunity.[22]

Differentiation into the Th17 phenotype is mediated by the RORγt factor, which in turn is induced by cytokines produced by dendritic cells such as IL-6 and

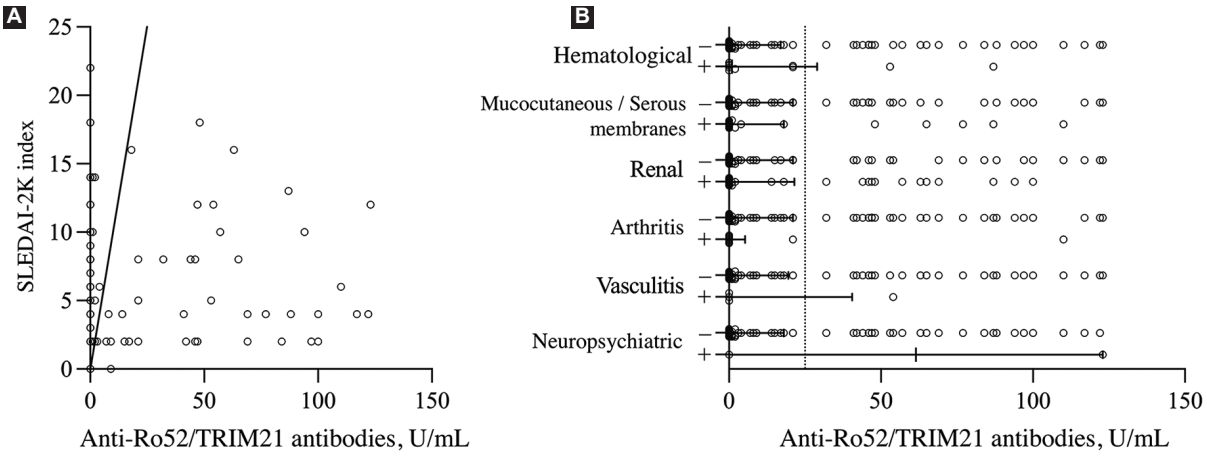

Figure 4. *Anti-Ro52/TRIM21 antibodies and disease activity in patients with systemic lupus erythematosus.* **A:** *Spearman's correlation analysis did not show a linear association between anti-Ro52/TRIM21 antibody levels and disease activity evaluated using SLEDAI-2K index (ρ -0.003, 95% CI = -0.181 to 0.175, p = 0.971).* **B:** *no significant differences were found in anti-Ro52/TRIM21 antibodies levels according to the presence of organ-specific damage in patients with SLE. Horizontal lines indicate median values with interquartile range.*

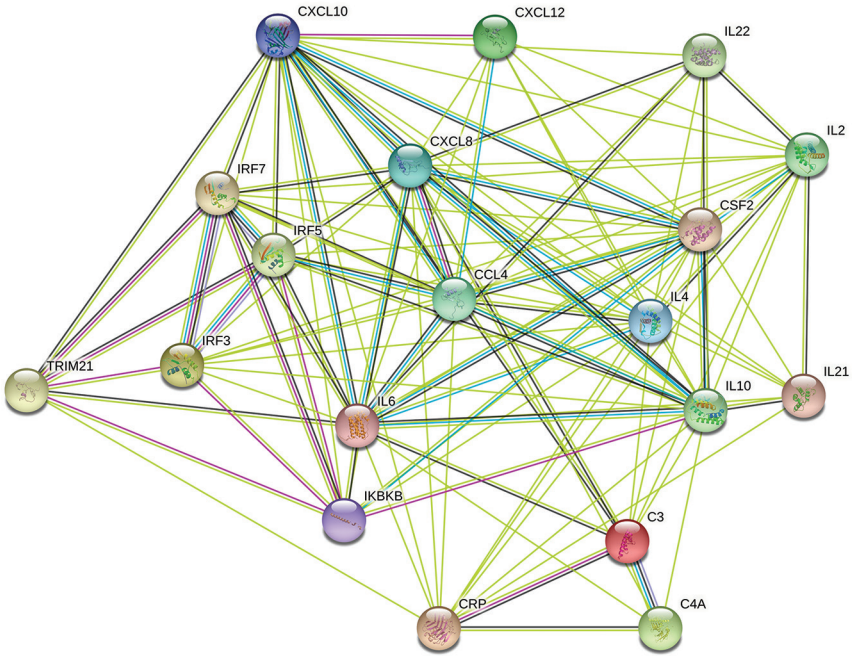

Figure 5. *Protein-protein interaction networks with functional enrichment analysis. Interactions between the TRIM21 molecule and cytokines, chemokines and pentraxins, using transcription factors IRF3, IRF5, IRF7 and NF-κB as intermediaries (via their inhibitory system, IKBKB). Interactions beyond those expected by chance were found both in experimentally demonstrated relationships (pink lines) and in relationships identified by text mining (green lines). Created with STRING: Functional Protein Association Networks.*

IL-21. Once polarized, Th17 cells exert their actions by producing cytokines of the IL-17 family, including IL-17A and IL-22.[22] Of note, patients in our study with anti-Ro52/TRIM21 antibodies also had the highest levels of all cytokines belonging to the Th17 phenotype, as well as of the proliferation factor that is common to all lymphocytes, IL-2.

In parallel with a Th17 phenotype is the activation of neutrophils as immune response final effector cells. In our study, patients with anti-Ro52/TRIM21 antibodies had the highest levels of CCL4 and CXCL8, which are neutrophil main chemoattractant and degranulation molecules, which facilitates acute neutrophilic inflammation.[23,24] CXCL10 and CXCL12 induce

powerful chemoattractant responses for monocytes and other leukocytes, while recruiting endothelial progenitor cells, thus regulating angiogenesis.[25,26]

Finally, in the analysis of protein-protein interaction networks, we propose how anti-Ro52/TRIM21 antibodies could block TRIM21 in vivo functioning by inhibiting its action as an E3 ubiquitin ligase. Once this function is lost, factors IRF3, IRF5 and IRF7, as well as the NF-κB pathway, would remain intact for regulating the transcription of genes that drive T-cell polarization into the Th17 phenotype, while facilitating the production of neutrophil and monocyte chemoattractant factors and angiogenesis inducers, thus completing the pathogenic circle of aberrant cytokine and chemokine circuits associated with the presence of anti-Ro52/TRIM21 antibodies.

Despite having biological support, we cannot overlook the lack of an association between anti-Ro52/TRIM21 antibodies and disease activity, at least in SLE. Although others have also failed to find clinical associations,[7] anti-Ro52/TRIM21 antibodies have been described in congenital heart block, neonatal lupus, valvular disease, inflammatory myopathy, and interstitial lung disease, which suggests that anti-Ro52/TRIM21 antibodies are indeed associated with autoimmune reactions.[27-31] In addition, whether the association between anti-Ro52/TRIM21 antibodies and abnormal immune response is a static process in which the mere presence of antibodies induces an aberrant cytokine production or, conversely, if it is a dynamic phenomenon where the latter fluctuate continuously in relation to disease activity, other antibodies functioning, the genetic substrate and other factors not directly related to self-reactivity against the TRIM21 molecule, remains to be defined.

Conclusion

Anti-Ro52/TRIM21 antibodies are often detected in patients with SARD, and their presence is associated with aberrant circuits of cytokines belonging to the Th17 phenotype, as well as with increased levels of neutrophil and monocyte chemoattractant molecules and angiogenic molecules, which suggests an active participation of these antibodies in the regulation of tissue damage mechanisms that are produced in SARDs.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

The authors declare that the study was funded by Instituto Nacional de Cardiología “Ignacio Chávez”.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures that were followed adhered to the ethical standards of the responsible committee for experimentation on human beings and were in agreement with the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained informed consent from the patients or subjects referred to in the article. This document is in the possession of the corresponding author.

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