Anti-RNA polymerase III antibody in lupus patients with proteinuria

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Abstract

Background: To investigate the relationship between serum anti-ribonucleic acid polymerase III (anti-RNAP3) autoantibodies (Abs) and proteinuria severity in lupus patients.

Methods: Serum antibodies reacting with anti-RNAP3 were measured in 49 systemic lupus erythematosus (SLE) patients (29 cases of SLE with proteinuria and 20 cases of SLE without proteinuria) and 10 healthy controls (HCs). For the patients, we recorded demographic data, daily urinary protein loss, serum anti-double strand deoxyribonucleic acid (anti-ds-DNA) antibodies, serum creatinine (Cr), estimated glomerular filtrating rate (eGFR), complement 3 (C3), and C4.

Results: Fewer anti-RNAP3 antibodies were found in the SLE patients than in the HCs (p = 0.061). In the SLE with proteinuria group, positive correlations were observed among anti-RNAP3 antibodies and daily urinary protein loss, serum C3, C4, and eGFR, and negative correlations were observed between anti-RNAP3-Abs and anti-ds-DNA-Abs and serum Cr levels. However, these correlations were nonsignificant (p > 0.05).

Conclusion: This study demonstrated the possible role of anti-RNAP3 antibodies in SLE patients with proteinuria, as evidenced by their positive and negative relationships with daily urinary protein loss, eGFR, C3, C4, serum Cr, and anti-ds-DNA-Abs. Although these correlations were nonsignificant, our study builds a foundation for future tailored studies, and more in-depth studies with larger samples are warranted to provide more information.

Keywords: Anti-RNA polymerase III (RNAP3) antibody; Biomarker; Disease activity; Lupus

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by cascades of tissue damage through cytokine/chemokine signaling by T and B lymphocytes. 1 Proteinuria is a common and severe manifestation of SLE. 2 Despite aggressive and tailored treatments for proteinuria, 20% of lupus nephritis (LN) patients still progress to end-stage renal disease. 3 Although several types of biomarkers have been used to reflect LN severity, distinct disease entities, and prognostic differences (eg, higher serum concentrations of anti-double strand deoxyribonucleic acid [anti-ds-DNA] antibodies [Abs], creatinine [Cr] levels, lower levels of serum complement [C], and estimated glomerular filtrating rate [eGFR] or renal biopsy), a satisfactory index for all aspects of LN remains lacking. 4–6 Antibodies reactive against ribonucleic acid (RNA) polymerase III (anti-RNAP3 Abs) were first described in systemic sclerosis (SSc) in 1993, 4 and have since been regarded as a marker highly predictive of renal involvement and renal crisis in SSc. 5–11 Anti-RNAP-Abs have been reported in SLE patients with and without scleroderma 12,11 and renal crisis in lupus-scleroderma overlap syndrome. 14 However, they have not been investigated in detail in pure lupus patients. Anti-RNAP-Abs can now be easily detected in serum by using commercially available enzyme-linked immunosorbent assay kits. Therefore, to identify an appropriate biomarker to reflect LN severity, we designed the present study to explore the relationships among serum anti-RNAP3-Abs, current commonly used biomarkers for LN activity (including serum levels of anti-ds-DNA-Abs, Cr, C3, C4, and eGFR), and proteinuria severity (daily amounts of urinary protein excretion) in patients with SLE.

2. METHODS

2.1. Patients and controls

This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (TVGH). Informed consent was obtained from all participants. We enrolled 49 consecutive Taiwanese SLE patients (29 with proteinuria and 20 without proteinuria) from the Outpatient Department of TVGH. All patients met the criteria for the 2012 Systemic Lupus International Collaborating Clinics revised and validated American College of Rheumatology SLE classification. 13 Blood samples were obtained from 10 age- and sex-matched patients to serve as healthy controls (HCs). These HCs were voluntary blood donors with healthy status and without any medical history of occult autoimmune or rheumatic diseases or abnormal
Abs (eg, ANA, anti-ds-DNA-Abs, rheumatoid factor, and anti-ENA-Abs). Clinical and laboratory assessments were performed on the day of blood sampling.

2.2. Serum level of anti-RNAP3-Abs
Serum samples were collected from all patients as they entered the study. Samples of peripheral venous blood were allowed to clot and centrifuged at 2000 rpm for 15 minutes to obtain sera, which were then snap-frozen at -80°C and stored until use. Serum levels of anti-RNAP3 were measured using a fluorochromeimunonassay (FEIA) (RNA Polymerase III FEIA Kit; Phadia AB Co., Ltd., Uppsala, Sweden). The serum concentration of anti-RNAP3-Abs was presented as fluorescent intensity in response units (RUs), according to the manufacturer’s protocol.

2.3. Measurement of anti-ds-DNA-antibody, Cr, eGFR, C3, and C4
The amount of anti-ds-DNA-Abs in each serum sample was quantified using a FEIA (ds-DNA FEIA Kit; Phadia AB Co., Ltd.). The complement C3 and C4 levels were measured using an immunoturbidimetric assay (Clinical Chemistry Complement Kit; Abbott, IL, USA). Serum Cr was checked using the kinetic alkaline picrate method (Clinical Chemistry Creatinine Kits; Abbott, IL, USA) and eGFR was also calculated according to the manufacturer’s standard protocol.

2.4. Statistical analysis
Statistical calculations were performed by using the Statistical Package for the Social Sciences for Windows (version 17; SPSS, IL, USA). Data were represented as the mean ± SD for continuous variables and as proportions for categorical variables. A p-value was provisionally regarded as significant if it was <0.05. The Mann–Whitney U test, Fisher’s exact test, and Spearman’s rank correlation (ρ) were used to analyze group differences, associations, and correlations.

3. RESULTS

3.1. Clinical demographic and laboratorial characteristics
The clinical and laboratory characteristics of 49 SLE patients are shown in Table. We divided the SLE patients into a proteinuria group (daily urinary protein loss > 0.5 g/d) and a nonproteinuria group (spot urine protein to urine creatinine was <0.01). The proteinuria group comprised 20 patients (F:M = 19:1). The average disease duration was similar in both groups, at 13.78 ± 10.1 years and 16.80 ± 12.6 years in the proteinuria and nonproteinuria groups, respectively (p = 0.612). The mean age in the proteinuria group was 40.10 ± 13.08 years and that of the nonproteinuria group was 46.25 ± 8.89 years (p = 0.087). Compared with the nonproteinuria group, the proteinuria group exhibited significantly higher serum levels of anti-ds-DNA-Abs and Cr, but lower C3 and eGFR in lupus patients (p < 0.05). No significant difference in anti-RNAP3-Abs was noted between the proteinuria and nonproteinuria groups (Table) or among the proteinuria, nonproteinuria, and HC groups (Fig. 1A) but anti-RNAP3-Abs was lower in the total SLE patients than in the HCs (Fig. 1B).

3.2. Correlation of anti-RNAP3-Abs to daily urinary protein loss, serum C3, C4, anti-ds-DNA-Abs, creatinine, and eGFR in SLE patients with proteinuria
Correlations between anti-RNAP3-Abs and various parameters in the proteinuria group and individual correlation coefficients are shown in Fig. 2. The serum anti-RNAP3-Abs were positively correlated with daily urinary protein loss (Fig. 2A), levels serum C3 (Fig. 2B), C4 (Fig. 2C), and eGFR (Fig. 2F) but negatively correlated with anti-ds-DNA-Abs (Fig. 2D) and serum Cr levels (Fig. 2E), although the correlations were nonsignificant (p > 0.05).

3.3. Amount of urinary protein in relation to the titer of anti-RNAP3-Abs
We assessed the correlation between the amount of daily urinary protein excretion of lupus patients with proteinuria and different serum titers of anti-RNAP3-Abs (Fig. 3). Regardless of the cut-value of serum anti-RNAP3-Abs concentration (Fig. 3A: 40 RU, Fig. 3B: 50 RU, and Fig. 3C: 60 RU), the anti-RNAP3-Ab titers showed no correlation with the amount of urinary protein excretion in lupus patients with proteinuria (p > 0.05).

3.4. Anti-RNAP3-Ab levels in relation to concentrations of urinary protein excretion in lupus patients with proteinuria
We stratified protein excretion concentration into four levels: 1.5 g/d, 2.0 g/d, 2.5 g/d, and 3.0 g/d. Higher anti-RNAP3-Ab levels were observed in the groups with a higher concentration of daily urinary protein excretion, although the difference was nonsignificant (Fig. 4).

4. DISCUSSION
This study revealed that serum anti-RNAP3-Abs levels were higher in the HCs than in the SLE patients. In SLE patients, cells are always under high oxidative stress.14 Reactive oxygen species (ROS) related to oxidative stress in terms of inflammation can induce DNA cleavage, cellular aging, and tissue damage,17 particularly in LN patients.18 Brf2, a RNAP3 core transcription factor found exclusively in vertebrates, comprises a redox-sensing module and regulates transcription in a redox-dependent manner. The Brf2- and RNAP3-related selenocysteine (SeCys) redox-sensing capability could act as a safety mechanism by limiting stress and maintaining cell survival by activating detoxification processes.

Table
Demographic and laboratorial characteristics of SLE patients with and without proteinuria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SLE with proteinuria (n = 29)</th>
<th>SLE without proteinuria (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>40.10 ± 13.08</td>
<td>46.25 ± 8.89</td>
<td>0.087</td>
</tr>
<tr>
<td>Female/male</td>
<td>27/2</td>
<td>19/1</td>
<td>0.813</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>13.78 ± 10.1</td>
<td>16.80 ± 12.6</td>
<td>0.612</td>
</tr>
<tr>
<td>Anti-RNAP3-Ab, RU</td>
<td>51.93 ± 36.36</td>
<td>47.70 ± 17.09</td>
<td>0.531</td>
</tr>
<tr>
<td>C3, mg/dl</td>
<td>70.35 ± 27.89</td>
<td>91.62 ± 29.83</td>
<td>0.014</td>
</tr>
<tr>
<td>C4, mg/dl</td>
<td>15.49 ± 10.23</td>
<td>19.638 ± 7.601</td>
<td>0.194</td>
</tr>
<tr>
<td>Anti-dsDNA-Abs, IU/mL</td>
<td>139.51 ± 153.29</td>
<td>19.72 ± 24.65</td>
<td>0.001</td>
</tr>
<tr>
<td>1Cr, mg/dl</td>
<td>1.68 ± 1.32</td>
<td>0.76 ± 0.18</td>
<td>0.013</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>63.31 ± 40.30</td>
<td>86.16 ± 15.33</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*p < 0.05 = significant; Data are presented as mean ± SD.
Anti-RNAP3-Ab = anti-ribonucleic acid polymerase III-antibodies; C = complement; Cr = creatinine; eGFR = estimation glomerular filtrating rate; RU = response unit; SLE = systemic lupus erythematosus.

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of enzymes under redox stress. Conversely, under conditions of oxidative stress, Nrf2-mRNA-related detoxification enzymes are truncated by proteosomal degradation, whereas RNAP3-related SeCys has no function. In SLE patients with high oxidative stress, RNAP3- and Brf2-related SeCys formation may be suppressed to maintain cell survival. Therefore, it is conceivable that anti-RNAP3-Abs formation may be more downregulated in SLE patients than in HCs.

We also found that higher serum levels of anti-RNAP3-Abs in patients with proteinuria was associated with higher C3, C4, and eGFR but lower anti-ds-DNA and serum Cr. These results implied that RNAP3 may act as a protector against or a suppressor of renal inflammation in SLE patients, and that RNAP3, Brf2, and SeCys may act in concert to regulate ROS, resulting in cell survival.

Nevertheless, a high anti-RNAP3-Ab titer seemed to be correlated with high urinary protein excretion. This apparent contradictory phenomenon may have resulted from the highly inflammatory status of lupus kidney disease, which simultaneously triggers the activation of the RNAP3 cascade and protein leakage through the glomerular filtration membrane. The former then leads to the development of anti-RNAP3-Abs and itself presents phenotypically with high levels of Abs and severe proteinuria.

Despite the aforementioned findings, the present investigation had some limitations. First, the study involved only a single center and a relatively small number of SLE patients with or without LN. Thus, a definitive conclusion could not be drawn. Additional in-depth studies with larger sample sizes should be performed in the future. Second, because of the cross-sectional design, causality cannot be established. Further studies are needed to elucidate the underlying mechanisms of the observed associations.

Fig. 1 Serum levels of anti-ribonucleic acid polymerase III (RNAP3) antibodies (Abs) were measured using a fluoroenzymeimmunoassay, and the serum concentrations of anti-RNAP3-Abs are presented as fluorescent intensity in response unit (RU). A, Anti-RNAP3-Abs among systemic lupus erythematosus (SLE) patients with proteinuria, SLE patients without proteinuria, and healthy controls (HCs); B, Anti-RNAP3-Abs in all SLE patients and HCs.

Fig. 2 Serum levels of anti-ribonucleic acid polymerase III (RNAP3) antibodies (Abs) were measured using a fluoroenzymeimmunoassay (FEIA), and the serum concentrations of anti-RNAP3-Abs are presented as fluorescent intensity in response unit (RU). Serum anti-ds-DNA-Abs were also quantified using an FEIA. The correlation of anti-RNAP3-Abs with (A) daily urinary protein excretion, (B) serum complement 3 (C3), (C) serum C4, (D) anti-double strand deoxyribonucleic acid Abs, (E) creatinine, and (F) estimated glomerular filtrating rate was assessed in systemic lupus erythematosus patients with proteinuria. ρ: Spearman’s rank coefficient.
design of this study, data on the effects of anti-RNAP3-Abs during the clinical course of lupus disease and treatment outcomes with immunosuppressive or cytotoxic medication were not available for all patients. Finally, we only checked the serum levels of anti-RNAP3-Abs; data regarding Bf2, Nrf2, and SeCys tRNA were lacking. We will design additional studies to measure more molecules involved in redox homeostasis in the future.

Fig. 3 Serum levels of anti-ribonucleic acid polymerase III (RNAP3) antibodies (Abs) were measured using a fluoroenzymeimmunoassay, and the serum concentrations of anti-RNAP3-Abs are presented as fluorescent intensity in response unit (RU). Amounts of urinary protein excretion were assessed in relation to different serum concentrations of anti-RNAP3-Abs.

Fig. 4 Serum levels of anti-ribonucleic acid polymerase III (RNAP3) antibodies (Abs) were measured using a fluoroenzymeimmunoassay, and the serum concentrations of anti-RNAP3-Abs are presented as fluorescent intensity in response unit (RU). Serum levels of anti-RNAP3-Abs in systemic lupus erythematosus patients were assessed in relation to different amounts of urinary protein excretion.
In conclusion, we demonstrated that anti-RNAP3-Abs are positively correlated with C3, C4, and eGFR but negatively correlated with anti-ds-DNA-Abs and Cr. However, these correlations were nonsignificant. Whether anti-RNAP3-Abs can be used as a biomarker for monitoring renal function in lupus patients with proteinuria remains unclear and needs additional, larger studies to elucidate, for which the results of our study might provide a foundation.

ACKNOWLEDGMENTS
This is supported by Ministry of Science and Technology (NSC102-2314-B075-067-MY3) and Taipei Veterans General Hospital (V105C-114).

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