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ORIGINAL ARTICLE

Ribosomal P autoantibodies are present before SLE onset and are directed against non-C-terminal peptides

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Abstract Autoantibodies to ribosomal P (ribo P) are found in 15–30% of systemic lupus erythematosus (SLE) patients and are highly specific for SLE. The goal of this study is to assess the temporal association of anti-ribosomal P (anti-P) responses with SLE disease onset, as well as to characterize select humoral ribo P epitopes targeted in early, pre-diagnostic SLE samples. Patients with stored serial serum samples available prior to SLE diagnosis were identified from a military cohort. Each sample was tested for antibodies against ribo P utilizing standard C terminus ribo P enzyme-linked immunosorbent assays (ELISA) and a solid phase, bead-based assay with affinity-purified ribo P proteins. In this study, antibodies to ribo P were more common in African American SLE patients (p=0.026), and anti-P-positive patients comprised a group with more measured autoantibody specificities than did other SLE patients (3.5 vs 2.2, p<0.05). Antibodies against ribo P were present on average 1.7 years before SLE diagnosis and were detected an average of 1.08 years earlier in pre-diagnostic SLE samples using affinity-purified whole protein rather than C-terminal peptide alone (p=0.0019). Furthermore, 61% of anti-P-positive patients initially had antibodies to aa 99–113, a known ribosomal P0 antigenic target, at a time point when no antibodies to the clinically used C terminus were detected. Our findings provide evidence that antibodies against ribosomal P frequently develop before clinical SLE diagnosis and are more broadly reactive than previously thought by targeting regions outside of the C terminus.

Keywords Lupus · Antibodies · Autoimmunity · Ribosomal P · Epitope

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by the production of autoantibodies directed against a variety of nuclear and cytoplasmic antigens. Among the SLE-specific autoantibodies, one subset is directed against ribosomal P (ribo P) phosphoproteins. Anti-ribosomal P (anti-P) occur almost exclusively in SLE [1] and have been associated with a number of clinical presentations including neuropsychiatric symptoms [2–6], nephritis [7], photosensitivity [8], malar rash [4, 8], and hepatic involvement [9]. Anti-ribosomal P have also variably been found to be associated with a more severe disease course [8], but this has not been confirmed in all studies [1]. Anti-P prevalence in SLE varies widely by race, ranging

from 10% to 40%, with approximately 20% of African American patients having anti-P responses [10–16]. These autoantibodies are far more prevalent in patients with juvenile SLE than in patients with adult-onset SLE [17, 18].

Antibodies to ribosomal P are directed against a family of phosphoproteins that are associated with the 60s ribosomal subunit. Ribosomal P proteins share a highly conserved 22 amino acid carboxyl-terminal sequence that is thought to comprise the immunodominant epitope [14, 19–21]. Autoantibodies against ribosomal P are directed against three proteins: P0 (38 kDa), P1 (19 kDa), and P2 (17 kDa). Initially, autoantibodies were thought to primarily target only the shared C-terminal sequence that is common to all three proteins [20], and clinical laboratories have historically used the C-terminal peptide ELISA (aa 296–317) to determine anti-ribosomal P reactivity in patient sera [22]. The 22-amino acid carboxyl-terminal peptide that is shared by the ribosomal P proteins is constructed on a 4-mer branching polylysine backbone (MAP) and used as the antigen for this assay [22]. However, Mahler et al. have recently shown that the anti-P response is not restricted to the C terminus and that patient antibodies also targeted other regions of this protein [14]. Fine specificity humoral epitope mapping of ribosomal P antibodies in patients with established SLE has identified additional antigenic targets, with one of the most common being a sequence containing amino acids 99–113 of P0 which is bound by 92% of patients studied [23]. Notably, anti-ribosomal P antibodies found in one SLE patient serum were found to target multiple regions of the P0 protein while conspicuously avoiding the C-terminal region [23].

Autoantibodies in SLE can usually be detected in patient sera many years before SLE diagnosis and tend to accumulate in the years leading up to diagnosis while patients are still clinically asymptomatic [24]. Furthermore, SLE-specific autoantibodies such as anti-Sm and anti-nRNP appear closer to the time of diagnosis than non-specific autoantibodies such as anti-Ro and anti-La [24, 25]. However, the accumulation and timing of anti-ribosomal P antibodies have not previously been analyzed.

This study evaluates the onset and progression of ribosomal P antibodies in SLE patients before the clinical onset of disease and investigates those regions of the ribosomal P protein that are antigenic early in the disease course. We have described the prevalence and demographics of anti-ribosomal P-positive SLE patients in a large, multi-racial cohort that is enriched for male lupus. Additionally, we characterize potential associations between anti-P and clinical manifestations and other autoantibody specificities. Together, these data provide a new, more detailed picture of the development of early, pre-diagnostic ribosomal P antibodies and their role in the development of SLE.

Patients and methods

Patients Experiments were performed in accordance with the Helsinki Declaration and approved by the Oklahoma Medical Research Foundation, National Naval Medical Center, and Walter Reed Army Medical Center Institutional Review Boards. Previously, we identified 130 patients who were classified with SLE according to ACR criteria [26, 27] while serving in the US military; details of inclusion criteria were reported previously [24]. Sufficient samples remained from 129 patients for anti-ribosomal P testing. Originally, four controls were matched to each patient based on gender, ethnicity, age (within 1 year), length of military service, and availability of stored serum samples. Of these four controls for each patient, one was randomly selected to be used as a control in the present study. Thus, samples from 129 controls were also tested as described below. Additionally, sera from rheumatoid arthritis patients (n=36), multiple sclerosis patients (n=18), and scleroderma patients (n=19) were also tested as described below. Demographic information, including self-reported ethnicity, gender and age at diagnosis, is reported in Table 1. The average age (SD) of diagnosis for all cases was 30.6 (6.8) years (range 18.5–46.9 years). The clinical characteristics of the patients including ACR criteria and autoantibody specificities are also reported (Table 1).

Patient sera A total of 625 serum samples were retrieved from the 129 identified SLE cases. Factor XIIIb levels were tested in all samples to ensure sample integrity and appropriate sample storage and handling [28]. After removal of samples with factor XIIIb errors from the analysis, 534 serum samples were available from the 129 cases, with an average of 4.1±2.2 serum samples (ranging from 1 to 12) available per patient (median 4.0 samples per patient). The earliest serum sample available for each individual patient was on average 4.1±2.7 years before SLE classification (ranging from 9.4 years before classification to within the same month as classification, median 4.0 years). Availability of early samples was not significantly different among anti-ribosomal P-positive (first serum sample collected before SLE diagnosis, mean=4.0 years) and anti-ribosomal P-negative (mean=4.1 years) patients.

Peptide synthesis and quality control The Fmoc-synthesis method was used to construct the C-22 (aa 296–317; accession # NP 444505) and Epitope 3 (aa 99–113; accession # NP 444505) peptides by the Molecular Biology Proteomics Facility at the University of Oklahoma Health Sciences Center on a 4-mer branching polylysine backbone (MAP^{TM}). The method consists of formation of an activated HOBT ester of the Fmoc-protected amino acid with the

Table 1 Demographic, ACR criteria, and autoantibody specificities of anti-ribosomal P positive and negative SLE patients

	Anti-ribo P (+; n=38)	Anti-ribo P (−; n=91)
Gender		
Male (n=45)	39%	33%
Female (n=84)	61%	67%
Ethnicity		
African American (n=80)	76%	56%
European American (n=34)	13%	31%
Malar rash	18%	27%
Discoid rash	21%	12%
Photosensitivity	29%	36%
Oral ulcers	29%	30%
Arthritis	76%	66%
Serositis	37%	45%
Pericarditis	24%	10%
Pleuritis	29%	44%
Nephritis	42%	40%
CNS	8%	3%
Hematologic disorder	76%	74%
Hemolytic anemia	3%	3%
Leukopenia	42%	55%
Lymphopenia	63%	55%
Thrombocytopenia	11%	11%
Immunologic disorder	84%	71%
ANA	100%	100%
Total SLE criteria	5.21	5.04
Average onset of first criteria (years before diagnosis)	1.51	1.66
Average age at diagnosis	30.5	30.7
Autoantibody specificities		
dsDNA	92%	56%
Sm	84%	69%
nRNP	50%	24%
Ro	68%	40%
La	50%	29%
Average Number of autoantibody specificities	3.45	2.18

activator HBTU. This activated ester is made in a 3.4-fold molar excess over the scale of the MAP peptide being synthesized. The 4-branch MAP resin is purchased from Applied Biosystems. After addition of the final amino acid, the peptide-bound synthesis support is washed with methanol and dried under vacuum overnight. The peptide is then cleaved from the synthesis support using 5.0 ml of a TFA cleavage cocktail which varies by peptide. Finally, the peptide was precipitated with ether. The cleaved MAP peptides were dissolved in 15% acetic acid–water and purified by reversed phase C18 chromatography. The HPLC fractions were monitored by MALDI-T of mass spectrometry. Fractions were pooled that consisted of a dominant peak of the correct mass. The measured masses for each peptide were within 1.5 Da of the theoretical average mass of the peptide. The purity of each peptide was confirmed by analytical HPLC analysis and by amino acid analysis following acid hydrolysis. The calculated amino acid values closely matched the theoretical values.

Ribosomal P0 carboxyl peptide ELISA The C-22 peptide (constructed as above) was used as the coating antigen on a 96-well polystyrene ELISA plate with 1 μg of antigen per well. Assays were performed as previously described [17, 23]. Briefly, the polystyrene plates were first coated with the ribosomal P0 carboxyl peptide MAP™ antigen, washed, and then blocked in 0.1% BSA solution. Next, sera, diluted at 1:100 and 1:1,000 in 0.1% bovine serum albumin–Tween solution, were added to the wells in duplicate and incubated. Plates were then washed and

incubated with anti-human IgG whole molecule secondary antibody conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, West Grove, PA). P-Nitrophenyl phosphate disodium solution was used as substrate, and the plates were then read at 410 nm on a microELISA plate reader. Positive controls with known specificity were used on each plate. This individual's serum at 1:100 was allowed to develop to an OD of 1.0. Each plate was standardized to have the positive control meet 1.0. Plates were deleted from analysis if positive control was above 1.15 or below 0.85, if the blank wells were over 0.05 or duplicate wells varied by >10%. The same methodology was used for the ribosomal P0 epitope 3 ELISA [23].

Solid phase bead-based autoantibody analyses The Bio-Plex ANA Screen kit (Bio-Rad, Hercules, CA) applies multiplex technology and dyed magnetic beads to simultaneously detect antibodies against the following antigens: dsDNA, chromatin, SSA-52 kDa, SSA-60 kDa, SSB, Sm, Sm/RNP, RNP-A, RNP-68 kDa, ribosomal P proteins, Scl70, centromere B, and Jo-1.

Undiluted serum samples from patients and controls were assayed by using the BioPlex 2200 system with the BioPlex ANA Screen kit. This enabled detection of antibodies against ribosomal P proteins that are affinity purified from calf thymus extract by the manufacturer using a minimal amount of patient sera. Factor XIIIb levels were tested in all samples to ensure sample integrity and appropriate sample storage and handling [28]. The BioPlex 2200 system, a fully automated random access analyzer, reports semi-quantitative values from 0 to 8, termed the antibody index (AI), for each autoantibody except anti-dsDNA. The positive cutoff for each assay is established by the manufacturer to equal 1.0 AI. For dsDNA, the BioPlex is able to perform a quantitative assay; the results of which are reported as international units per milliliter with a positive cutoff of 10 IU/mL. Samples with low factor XIIIb levels (original AI <0.2) were dropped from the analysis.

Western blot MOLT-4 extract (human mature T cell leukemia cell line) was electrophoresed through a 15% polyacrylamide gel, transferred to nitrocellulose and probed for anti-ribosomal P antibodies at a dilution of 1:100, and then incubated with secondary anti-human IgG conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, West Grove, PA). Finally, the nitrocellulose strips were exposed to an NBT/BCIP substrate to detect anti-ribosomal P antibodies. Assays were performed as previously described [17].

Statistical analysis Primary analyses were performed using the 129 SLE patients analyzed across time or separated into 38 ribosomal P-positive patients and 91 ribosomal P-negative patients frequency matched by ethnicity and gender. Changes in anti-ribosomal P concentrations were assessed using one-way ANOVA. Conditional logistic regression was used to analyze ACR criteria and autoantibody specificities (Table 2). Multivariate models were created using anti-ribosomal P-positive as the outcome variable. All variables significant at p value <0.05 in univariate analysis were considered for inclusion in the final model. Multiple conditional logistic regression analysis was used to simultaneously adjust for the various independent variables. Confounding and interaction were both assessed. Differences in time of positivity to ribo P between the Bioplex assay and the C terminus ELISA were assessed using Wilcoxon's signed rank test. The comparative receiver operator characteristic (ROC) analysis was performed as previously described [29]. All analyses were performed using SAS 9.0 (SAS Institute, Raleigh, NC, USA).

Results

Ribosomal P antibodies are frequently present before diagnosis and are more common in African American SLE patients

Thirty-eight SLE patients (29.5%) had antibodies directed against affinity-purified ribosomal P by a solid phase, bead-based assay. Antibodies to ribosomal P appeared on average 1.7 years prior to diagnosis (median 0.6 years prior, range 7.9 years prior to 2.2 years after) and are present before diagnosis in 84% of patients that eventually develop these antibodies. Anti-P appeared at a similar time on average as other SLE specific antibodies (anti-Sm and anti-dsDNA) and appeared closer to diagnosis than less SLE-specific autoantibodies such as anti-Ro and anti-La. The concentration of anti-ribosomal P antibodies was analyzed over time, and the concentration of anti-P antibodies in serum samples prior to 2 years before diagnosis was 3.3 (Bio-Rad Antibody Index; n=16); the concentration of anti-P in serum samples between 2 and 1 year prior to diagnosis was 4.6 (n=8), and the concentration of anti-P in the year prior to diagnosis was 5.4 (n=33). A significant increase in the concentration of antibodies to ribosomal P was seen during the time preceding diagnosis (p=0.049, one-way ANOVA) in anti-ribosomal P-positive patient samples with the concentrations reaching a plateau after diagnosis. Matched controls were also tested for antibodies against ribosomal P, and none were positive using the Bio-Rad assay. Anti-ribosomal P-positive patients were more likely to be African American (76% anti-P positive vs 56% anti-P negative; p=0.026), but no gender differences were detectable (Table 1). Interestingly, in this cohort, no significant

Table 2 Results of univariate and multivariate analyses of ACR criteria and autoantibody specificities of anti-ribosomal P-positive and age, race, and gender matched anti-ribosomal P-negative SLE patients

	p value	Odds Ratio (95% CI)
Univariate analysis		
Malar rash	0.664	0.81 (0.31–2.11)
Discoid rash	0.271	1.75 (0.65–4.74)
Photosensitivity	0.942	0.97 (0.39–2.42)
Oral ulcers	0.807	1.12 (0.46–2.72)
Arthritis	0.199	1.76 (0.74–4.18)
Serositis	0.320	0.67 (0.30–1.48)
Pericarditis	0.046	4.11 (1.02–16.53)
Pleuritis	0.069	0.44 (0.18–1.07)
Nephritis	0.988	0.99 (0.47–2.12)
CNS	0.229	3.10 (0.49–19.56)
Hematologic disorder	0.659	1.22 (0.50–2.99)
Hemolytic anemia	0.782	0.72 (0.07–7.44)
Leukopenia	0.124	0.54 (0.25–1.18)
Lymphopenia	0.201	1.70 (0.75–3.85)
Thrombocytopenia	0.747	1.25 (0.33–4.75)
Immunologic disorder	0.186	1.99 (0.72–5.50)
Total SLE criteria	0.166	1.27 (0.91–1.78)
Average onset of first criteria (years before diagnosis)	0.951	1.00 (0.87–1.16)
Average age at diagnosis	0.721	0.99 (0.94–1.05)
Autoantibody specificities		
dsDNA	0.001	8.74 (2.38–32.04)
Sm	0.090	2.42 (0.87–6.69)
nRNP	0.035	2.52 (1.07–5.93)
Ro	0.008	3.20 (1.36–7.56)
La	0.044	2.36 (1.02–5.42)
Average number of autoantibody specificities	0.003	2.27 (1.33–3.89)
Multivariate analysis		
Anti-dsDNA positive	0.035	4.49 (1.09–26.74)
Total autoantibody specificities >2 (not including anti-ribo P)	0.009	4.19 (1.38–14.52)

differences were seen between anti-P-positive and anti-P-negative patients with respect to age at disease onset or with any particular clinical criteria, with the exception of pericarditis (p=0.046, univariate analysis without correction for multiple comparisons). Specifically, no statistically significant association was found in our patient collection between the presence of anti-ribosomal P antibodies and malar rash, photosensitivity, or nephritis as previously reported (Table 1). The incidence of neuropsychiatric symptoms was too low to accurately assess a trend toward increased prevalence in the anti-P positive population. Hepatic involvement was not specifically assessed in this cohort of SLE patients.

Ribosomal P antibodies are frequently associated with other autoantibody specificities

Anti-ribosomal P positive patients produce a higher number of autoantibody specificities than do other lupus patients. Anti-ribosomal P positive patients had an average of 3.45 additional autoantibody specificities (not including anti-ribosomal P) while anti-ribosomal P-negative patients had an average of 2.2 total autoantibody specificities (OR 2.27, p=0.003). Anti-ribosomal P positive patients more frequently had antibodies to nRNP (OR 2.52, p=0.035), 60 kD Ro (OR 3.20, p=0.008), La (OR 2.36, p=0.044), and dsDNA (OR 8.74, p=0.001), but interestingly did not have an increased frequency of Sm antibodies, which had been previously reported [14] (Tables 1 and 2).

African American SLE patients have more severe disease, as well as more autoantibodies, than other ethnic groups with SLE [30]. Therefore, to account for the significantly increased number of African American patients in the anti-ribosomal P positive group, we used ribosomal P-negative patients frequency matched for ethnicity and gender to compare the clinical characteristics in anti-P positive and anti-P-negative patients. Using this

univariate analysis, we found that anti-P positive patients were more likely to have pericarditis than their anti-ribosomal P-negative counterparts (p=0.046; Table 2). This analysis also confirmed that none of the previously reported clinical associations with anti-ribosomal P were present in this patient cohort. Multivariate analysis showed that being anti-dsDNA positive as well as having a total number of autoantibody specificities >2 (not including anti-ribosomal P) were independent predictors of being anti-ribosomal P positive as no confounding factors or interactions were present (odds ratios $= 4.49[1.09 - 26.74] p = 0.035$ and $4.19[1.38 - 14.52] p = 0.009$, respectively).

Ribosomal P antibodies in pre-clinical SLE commonly target non-C-terminal epitopes

Thirty-eight patients (29%) had anti-ribosomal P antibodies directed against affinity-purified proteins in a solid phase bead-based assay, and only 15 of these patients had detectable anti-ribosomal P antibodies against the C-terminal peptide (Fig. 1a). Therefore, 23 patients had antibodies to ribosomal P detected using whole protein that were not detected using the C-terminal peptide assay, making anti-ribosomal P antibodies in pre-clinical SLE more frequently detected by affinity-purified ribosomal proteins than by the C-terminal peptide alone. In patients with samples positive for anti-ribosomal P by either the Bioplex assay or C-terminal peptide ELISA, the Bioplex assay showed a positive result an average of 1.08 years prior to a positive test by C terminus ELISA (p=0.0019 by Wilcoxon's Signed Rank Test; Fig. 2); cases either had positive results reached with both assays in the same sample or no detectable anti-ribo P by either assay were assigned a difference of zero. In the 23 cases where a positive test was reached only by the Bioplex assay, the difference was assigned as time between the positive test and the last sample from that patient. A comparative ROC analysis was done to assess the ability of the C terminus ELISA and the Bioplex assay to discriminate between SLE patients and controls. The area under the curve (AUC) for the Bioplex assay was 0.721, and the AUC for the C terminus ELISA was 0.671. The curves were not significantly different (p=0.165). The Bioplex assay was more sensitive (38.6% vs 17.5%) but less specific (89.7% vs 98.6%) for discrimination between SLE patients and controls. Samples from eight discordant patients that were positive for antibodies against the C-terminal peptide by ELISA but negative to the affinity-purified ribosomal P proteins using the solid phase-based assay were also tested by western blotting and were found to be negative by this method as well (data not shown); additional samples were not able to be tested by western blot due to the small amount of sera we have available for individuals within this cohort. Additionally, sera from rheumatoid arthritis (n=36), multiple sclerosis patients (n=18), and scleroderma patients (n=19) were tested on the C-terminal peptide ELISA, and none were found to be positive.

All anti-ribosomal P antibody positive patients that did not have detectable antibodies against the C-terminal peptide were tested for antibodies to another common ribosomal P0 antigenic target, spanning amino acids 99–113, previously termed Epitope 3 [23]. Ribosomal P0 Epitope 3 (AA99–113, RDMLLANKVPAAARA) has been shown to be antigenic in approximately 92% of anti-ribosomal P-positive established SLE patients [23]. As

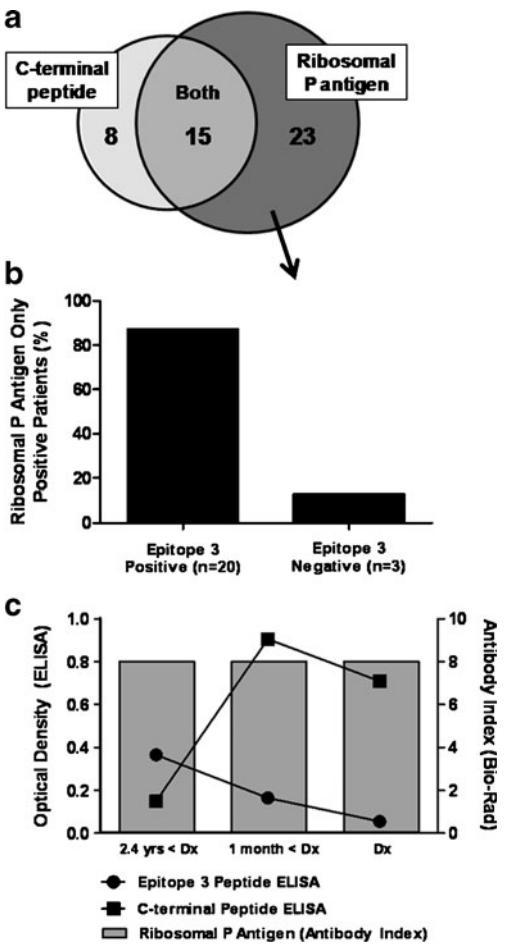

Fig. 1 Anti-ribosomal P antibodies commonly target non-C-terminal epitopes. **a** Venn diagram depicting the percentage of patients positive during the pre-clinical and early disease period for antibodies to affinity-purified ribosomal P only, C-terminal peptide only, and both. Numbers shown represent absolute number of patients within each group. **b** Twenty patients positive for antibodies to ribosomal P protein but not to the C-terminal peptide had antibodies directed against Epitope 3. Four of these 20 patients later developed antibodies to the C-terminal region while the patients were under observation. **c** A representative patient is shown with the ELISA data shown in the line graphs (corresponding to the y-axis on the left) and the Bio-Rad data shown in the bar graph (corresponding to the y-axis on the right). Dx denotes SLE diagnosis

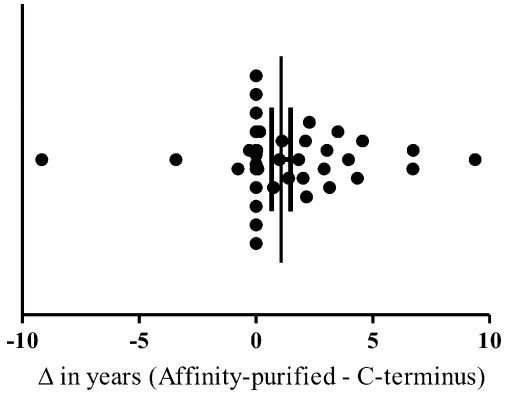

Fig. 2 SLE patients are positive by affinity-purified ribosomal P earlier than by the C terminus. In patients with samples positive for anti-ribosomal P by either the Bioplex assay or C-terminal peptide ELISA, the Bioplex assay showed a positive result an average of 1.08 years prior to a positive test by C terminus ELISA (p=0.0019 by Wilcoxon's signed rank test)

shown in Fig. 1b, 20 patients (87%) that were initially positive for antibodies to the ribosomal P protein but negative for anti-C-terminal peptide antibodies had antibodies to Epitope 3. Four of these 20 patients later developed antibodies under observation to the C-terminal region. A representative patient is shown in Fig. 1c. Additionally, sera from rheumatoid arthritis patients (n=36), multiple sclerosis patients (n=18), and scleroderma patients (n=19) were tested on the Epitope 3 ELISA, and sera from two RA patients were found to be positive, while no scleroderma or MS patient sera was found to have detectable anti-Epitope 3 binding. The two serum samples from RA patients that bound Epitope 3 could represent false positive results, or alternatively, these patients could potentially transition to SLE, as has been previously described [31]. These findings provide supportive evidence that early antibodies against ribosomal P frequently bind regions other than the C-terminal peptide.

Discussion

Herein, we present the first account of the early, pre-diagnostic accumulation of antibodies against the ribosomal P protein in patients that develop systemic lupus erythematosus. We show that ribosomal P antibodies appear prior to diagnosis in 84% of anti-P positive patients, and these antibodies develop on average 1.7 years prior to SLE diagnosis. Ribosomal P antibodies are specific to SLE and like other SLE-specific antibodies tend to appear closer in time to diagnosis than antibodies that are less specific to SLE such as anti-Ro.

The detection of ribosomal P antibodies is traditionally accomplished with ELISA using the C-terminal portion of the protein, and this is a relatively easy, cost-effective detection method. However, a large portion of the patient sera in this study did not contain antibodies directed against this region. Several patients who targeted different specificities in early serum samples eventually went on to make antibodies to the C terminus, which suggests an identifiable pattern of anti-P development. Initially, patients produce antibodies directed against a non-C-terminal region of the peptide. As the disease progresses toward diagnosis, patients develop antibodies against the C terminus, presumably through epitope spreading [32, 33]. Additionally, the concentration of ribosomal P antibodies increases as diagnosis is approached. This critical finding about the evolution of anti-P has important implications in identifying the initial inciting events of autoimmunity against the P protein. While our work is not comprehensive and does not look at all possible epitopes of ribosomal P, we have identified at least one epitope that is commonly targeted before diagnosis and in the absence of antibodies to the C terminus. This finding not only has implications for identifying the early events leading to the production of autoantibodies against the ribosomal P protein but also suggests that there may well be better diagnostic specificities to use than assays restricted to the C-terminal epitope. This observation differs somewhat from a previous report by Mahler et al. in which no patient sera reacted exclusively with regions outside the C terminus [14]. However, their data were restricted to patients with established disease; they did not evaluate any patient in the period before diagnosis. Based on the data presented herein, laboratories should consider a testing methodology that is sensitive for the detection of autoantibodies to ribosomal P in early stage, pre-diagnostic SLE patients.

Numerous associations have been reported between antibodies to ribosomal P and various clinical criteria [2–9]. We have not replicated these findings, possibly due to the unique cohort or sample size limitations inherent in this study. This study has a relatively low number of patients with the symptoms classically associated with ribosomal P (e.g., only four patients in our cohort had documented CNS disease). In addition, this unique cohort is >50% non-European American and is enriched with male lupus patients (35%). Furthermore, this study focuses on antibodies and clinical manifestations that are present very early on in the course of disease. The temporal relationship between ribosomal P antibodies and their associated clinical symptoms remains unknown; therefore, it is conceivable that these patients may go on to develop those clinical symptoms at a later date.

This work adds to our previous findings detailing autoantibodies and early clinical manifestations of SLE in patients prior to diagnosis. SLE is nearly always diagnosed months to years after the onset of clinical symptoms and as

we have previously shown, even longer after the onset of autoantibodies [24]. These early immunologic events have rarely been studied due to the difficulty of obtaining appropriate samples, and this military cohort provides us with the unique opportunity to address these questions. By understanding early events as autoimmunity progresses to SLE, we can hopefully develop a better understanding of the initial triggers as well as the accumulation of events leading to disease with the ultimate hope of diagnosing and modifying the disease process earlier in its course.

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