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Restricted specificity of anti-ribosomal P antibodies to SLE patients in Israel

O. Shovman[1*], G. Zandman-Goddard[1,2*], B. Gilburd[1], M. Blank[1], M. Ehrenfeld[3], S. Bardechevski[1], L. Stojanovich[1], P. Langevitz[3], Y. Shoenfeld[1,4]

[1]Center for Autoimmune Diseases and Dept. of Medicine B, [2]Lupus Clinic, [3]Rheumatology Unit, Sheba Medical Center; Sackler Faculty of Medicine, [4]Incumbent of the Laura Schwartz-Kipp Chair for Autoimmunity, Tel Aviv University, Israel.

Ora Shovman, MD; Gisele Zandman-Goddard, MD; Boris Gilburd, PhD; Miri Blank, PhD; Michael Ehrenfeld, MD; Svetlana Bardechevski, MD; Ludmilla Stojanovich, MD; Pnina Langevitz, MD; Yehuda Shoenfeld, MD.

[*]These authors contributed equally to this work.

Please address correspondence to: Yehuda Shoenfeld, MD, FRCP, Department of Medicine B, Sheba Medical Center, Tel-Hashomer 52621, Israel.
E-mail: shoenfel@post.tau.ac.il

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ABSTRACT

Objective. *Anti-ribosomal P antibodies (aRib-P Ab) are highly specific for systemic lupus erythematosus (SLE), but their correlatation with disease activity and manifestations including renal, hepatic and central nervous system (CNS) involvement is still controversial. The aim of our study was to evaluate the prevalence of aRib-P Ab and their correlation with clinical manifestations and anti-dsDNA antibodies in SLE patients from Israel.*

Methods. *Elevated titers of aRib-P Ab utilizing the ELISA method were analyzed in 141 sera samples from 44 SLE patients, 20 Familial Mediterranean Fever (FMF) patients, 22 primary antiphospholipid syndrome (PAPS) patients, 12 patients with infections, and 43 healthy individuals. The SLEDAI score was utilized for assessing SLE disease activity.*

Results. *Elevated titers of aRib-P Ab were present in 11% of SLE patients (n = 6). The mean SLEDAI was 7 (range: 3-10). No statistically significant association was observed between the presence of aRib-P Ab and disease manifestations present in the SLEDAI. The 6 SLE patients had renal disease (n = 1), leucopenia (n = 1), rash (n = 3), and CNS involvement manifested as psychosis (n = 1) or depression (n = 1). Elevated titers of anti-dsDNA antibodies were found in 50% of patients with elevated titers of aRib- P Ab. Patients with PAPS, FMF, infections or healthy controls did not harbor elevated titers of aRib-P Ab.*

Conclusion. *Elevated titers of aRib-P Ab were restricted to SLE patients. We confirm previously reported associations of aRib-P Ab reactivity with disease activity and elevated anti-dsDNA Ab titers. No significant correlation with a specific manifestation described on the SLEDAI score was established in this small cohort of patients.*

Introduction

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease accompanied by an excessive autoantibody production, where more than 100 reveals that autoantibodies to the ribosomal phosphoproteins (anti-Rib-P Ab) are present in 6-46% of subjects with SLE (2). These antibodies recognize three specific ribosomal proteins termed P0, P1 and P2 that carry molecular weights of 35, 19 and 17KD (3). Furthermore, anti-Rib-P antibodies are highly specific for SLE, their presence in other connective tissue disease being only sporadic (4-5). Elevated titers of anti-Rib-P Ab are mainly detected in SLE patients during active disease and may be associated with particular clinical manifestations such as lupus nephritis, hepatitis (6-9) and central system involvement (CNS) manifested by neuropsychiatric involvement (2, 10, 12-15). Clinical and serological manifestations may be influenced by ethnic and environmental factors (16).

The objective of the present study was to assess the frequency of elevated titers of anti-Rib- P Ab in a cohort of Israeli SLE patients, where ethnic diversity is common due to immigration from many countries. In addition, we evaluated the correlation with disease activity and clinical manifestations of SLE, utilizing the SLEDAI score, and with the presence of elevated titers of anti-ds DNA Ab. Autoantibodies are detected in infections and hence this subgroup was also tested in the present study. FMF is an autoinflammatory disease with clinical manifestations found in autoimmune diseases. Repeated search for ANA, as well as other autoantibodies has led to negative or inconclusive results. Elevated titers of anti-dsDNA Ab correlating with disease activity were reported in FMF patients. In a larger study, screening for the presence of 8 autoantibodies in FMF, no significant differences were encountered for the presence, incidence, and frequency of autoantibody between FMF patients and normal individuals (17). We assessed the specificity of anti-Rib-P Ab in SLE by comparing the prevalence of these autoantibodies in infections and normal healthy individuals.

Patients and methods

We analyzed the presence of elevated titers of anti-Rib-P Ab in 141 sera samples obtained from Israeli patients: 44 SLE patients, 20 patients with FMF, 22

patients with PAPS, 12 patients with infections, and 43 healthy individuals, during 2003.

The SLE and APS patients were recruited from the Lupus Clinic, 20 patients with FMF were examined in the Rheumatology Unit, 12 patients with infections were examined in the Department of Medicine and sera from 43 healthy volunteers were obtained from the Israel National Blood Bank. All patients with SLE fulfilled the ACR criteria for SLE (18). All patients with APS fulfilled the Sapporo criteria for APS (19). FMF was diagnosed by a certified rheumatologist. SLEDAI score on SLE patients was performed at the same visit as the blood sampling. All data regarding the SLE patients was retrieved from the Lupus Clinic Database (Microsoft Excel).

Detection of anti-Rib-P Ab

To achieve a highly sensitive screening assay for anti-Rib-P Ab, equimolar mixtures of the recombinant Rib-P proteins (P0, P1 and P2) in varying concentrations were absorbed onto ELISA plates (Maxisorb, Nunc, Denmark). Furthermore, the recombinant proteins were combined at the molar ratio of the native, pentameric Rib-P complex, one copy of P0 and each two copies of P1 and P2 in phosphate buffered saline (pH 7.6) by overnight incubation at 15°C followed by blocking with 0.5% BSA for 30min at RT. Results were expressed as the ratio (optical density of patient sample divided by the optical density of cut-off). A ratio < 1.0 was interpreted as negative, a ratio 1.0 to 1.4 was equivocal, and a ratio > 1.4 was positive. Positive samples were all above 3 standard deviations of the normal controls.

Detection of anti-dsDNA antibody titers

The detection of anti-dsDNA antibodies was by the Farr method.

Results

Six of 44 patients with SLE (11%) harbored elevated titers of anti-Rib-P Ab. Five of these patients were females, and the mean age was 44.3 years (range: 18-73 years old). There was no specific ethnicity among the patients, and HLA typing was not performed. The SLEDAI score range was 3-10, indicating that all patients had active disease because the score was above 0. Patients with severe disease indicated by a high SLEDAI score also had the highest titers of anti-ribosomal P Ab. Secondary APS was evident in only one patient from the SLE group. No significant correlation with clinical manifestations of SLE determined by the SLEDAI score was found. All of the other SLE patients and all healthy controls did not harbor elevated anti-Rib-P Ab titers.

Of the 6 patients with elevated anti-Rib-P Ab titers, one had renal disease, two had hematological manifestations (leucopenia, thrombocytopenia) and 3 had a rash. Two patients (33%) had evidence of CNS involvement; one patient had psychosis, the other suffered from cognitive decline and depression. Elevated titers of anti-dsDNA antibodies were found in 50% of SLE patients with elevated titers of Anti-Rib- P Ab, and 80% had low complement levels (Table I).

Elevated titers of anti-Rib-P Ab were not present in patients with PAPS, FMF, infections, or in the healthy controls.

Table I. Clinical and laboratory characteristics of SLE patients with elevated titers of anti-ribosomal P antibodies.

Patient/manifestation	1	2	3	4	5	6
OD 405 nm	1.096	0.31	2.82	1.89	0.79	0.60
CNS	-	-	Psychosis	Cognitive decline/DPR	-	-
Rash	+	+	-	-	+	-
Nephritis	-	-	-	-	+	-
Hematological	-	-	-	-	Low PLT	Leukopenia
Low complement level	-	+	-	+	+	+
Anti-dsDNA Ab	+	+	-	-	+	-
SLEDAI score	4	6	8	10	10	3

OD-0.23 was the cut-off of normal controls. DPR: depression; LAC: lupus anticoagulant; PLT: platelet; ND: not determined.

Discussion

In the present study the frequency of anti-Rib-P Ab was 11% in a group of 44 unselected adult SLE patients from a Lupus Clinic in Israel similar to that reported in the literature. The presence of elevated titers of anti-Rib- P Ab was restricted to SLE patients confirming the high specificity of this serological marker for the disease. All patients that harbored these antibodies had active disease, determined by the SLEDAI score, but the significant correlation with disease duration or specific clinical manifestations was not established.

To date, the reported prevalence of anti-Rib-P Ab for SLE ranges from 6-46%, being higher in Asian patients compared to Afro-American and Caucasian patients (2, 6, 10, 20). Moreover, the prevalence of anti-Rib-P Ab is higher in subjects with early disease onset (21). The observation that age and race may influence anti-Rib-P production may be related to the findings that the synthesis of these autoantibodies may be affected by certain MHC class 2 alleles (21, 22), particularly HLA-DQB1*0602 (14). In a previous study, significant differences between Israeli and European patients were reported in the occurrence of Raynaud's phenomena, elevated titers of antibodies to Sm, RNP, lupus anticoagulant false-positive VDRL, and low complement were more common in Israeli patients (16). Although ethnic diversity existed among the patients with elevated titers of anti-Rib-P Ab in this study, the prevalence of these Ab remained similar to that described in the literature.

The variation in the observed frequency may be dependent on the test system used to detect autoantibodies. Several

ELISA systems with different analytes, i.e. whole proteins, recombinant polypeptides, a synthetic peptide comprising the 22C-terminal amino acids or a multiple antigen (MAP) construct have been designed for research studies (6, 21, 23-27). Since low and various prevalences of anti-Rib-P Ab represent a limit for their utilization as a new diagnostic and/or serological criterion for SLE, international standardization of laboratory tests for anti-Rib-P Ab is necessary. Recently, the diagnostic accuracy and utility of a new sensitive Rib-P screening ELISA based on recombinant Rib-P polypeptides was confirmed in an extended international multi-center study (28). This novel approach could be applied to the identification of anti-Rib P Ab, especially in anti-dsDNA and anti-Sm negative patients with SLE.

Despite substantial investigations, the correlation between anti-Rib-P Ab and clinical disease manifestations is still controversial. A caveat in our study was the small sample size. In a recent multi-national study including 77 Israeli SLE patients, the frequency of elevated titers of anti-Rib-P Ab was 11.7%, similar to the results of this study (28). We could not confirm a statistically significant association of anti-Rib-P Ab with lupus related psychosis or nephritis.

The clinical association of elevated titers of anti-Rib P Ab and psychosis was originally described by Bonfa *et al.* (29). Since then, several studies have reported an association of anti-Rib-P Ab with organic CNS involvement particularly lupus psychosis (10, 11, 13, 14, 20, 21, 30) while other investigators did not confirm this finding (2, 24). In a recent review, 12 of 17 studies reported an association between elevated titers of anti-Rib-P Ab and neuropsychiatric lupus, predominantly psychosis and depression (2, 11, 20-22, 29-37). In an international meta-analysis combined standardized data from 1,537 SLE patients, anti-Rib-P Ab testing had limited diagnostic value for neuropsychiatric lupus and could not differentiate among various disease phenotypes (38).

An association of anti-Rib-P Ab with lupus nephritis was described by several groups of investigators (7, 8, 28, 39-41) but not by others (11, 23). An interesting finding is the positive association between elevated titers of anti-Rib-P Ab and anti dsDNA Ab, especially in patients with renal involvement (39, 40). It is intriguing that both these autoantibodies are specific markers of SLE and vary with disease activity. In our study a similar serological association was encountered in 50% of SLE patients who harbored both autoantibodies yet only one patient had lupus nephritis.

An interesting finding was that 3 patients with malar rash harbored these antibodies. The association of elevated anti-Rib-P Ab titers and skin involvement manifested as malar rash has been proposed in European series (21, 31) and in an extended international multicenter study (28).

Elevated titers of anti-Rib-P Ab are restricted to SLE and found rarely (< 5%) in patients with other autoimmune diseases including systemic sclerosis, primary Sjögren's syndrome, dermatomyositis, rheumatoid arthritis, undifferentiated connective tissue disease, overlap syndrome, and PAPS (36, 42-45). In a recent study, anti-Rib-P Ab titers were not detected in a subgroup of FMF patients and in only 1/140 patients with infections (28), similar to the findings in this study, and further accentuating the specificity in SLE.

Conclusion

The prevalence of anti-Rib-P Ab in Israeli SLE patients was reported and found to be similar to that in the literature. Furthermore, elevated anti-Rib-P Ab titers were restricted to SLE patients when compared to other diseases and healthy controls. We confirm previously reported associations of anti-Rib-P reactivity with disease activity and serological markers such as anti-dsDNA. No correlation with a specific manifestation of SLE was established in this small cohort of patients. The high specificity of anti-Rib-P Ab for SLE and their correlation with disease activity, young age of disease onset, race and clinical manifestations make these autoantibodies of a particular interest. Further research is necessary in order to determine the clinical significance of anti-Rib-P Ab in a large cohort of Israeli SLE patients.

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