Anti-Ribosomal-P Antibodies in Lupus Patients and Healthy Controls: Evaluation of Three ELISA Assays

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ABSTRACT: **Background:** Anti-ribosomal-P antibodies have been associated with central nervous system manifestations of systemic lupus erythematosus. However, inconsistencies in their prevalence and clinical correlations have become an obstacle to their use as a diagnostic marker of the disease. This lack of consistency might stem from several factors, such as the lag period between clinical manifestations and the time blood was drawn, or the different methods used for antibodies detection.

**Objectives:** To evaluate three different enzyme-linked immunosorbent assay tests for the detection of anti-Rib-P Abs in patients with SLE and in normal controls.

**Methods:** Sera from 50 SLE outpatients and 50 healthy subjects were tested with three ELISA kits: Kit-1, using synthetic peptide comprising the 22 C-terminal amino-acids; Kit-2, using native human ribosomal proteins (P0, P1, P2); and Kit-3, which is coated with affinity-purified human ribosomal proteins. ELISA studies were performed according to the manufacturers’ instructions.

**Results:** The prevalence of anti-Rib-P Abs in SLE patients and controls was 30% vs. 0%, 17% vs. 21%, and 30% vs. 14% in kits 1-3 respectively. Anti-Rib-P Abs detected by Kit-1 correlated with the SLEDAI score (SLE Disease Activity Index). No correlation between prior CNS manifestations and anti-Rib-P Abs was observed.

**Conclusions:** A significant difference was documented between the ELISA kits used for the detection of anti-Rib-P Abs. A correlation was found between these antibodies (evaluated by Kit-1) and concurrent SLEDAI scores, in contrast to the lack of correlation with previous CNS manifestations. This supports the notion of “active serology” that is evaluated at the same time manifestations are present, as well as the need for standardization of laboratory assays in the future which will enable a better assessment of anti-Rib-P Abs presence and clinical significance.

KEY WORDS: anti-ribosomal-P antibodies, systemic lupus erythematosus, central nervous system, enzyme-linked immunosorbent assay, autoantibodies

Systemic lupus erythematosus is an autoimmune disease characterized by multi-organ involvement and the presence of more than 150 different antibodies [1]. Among SLE patients 12% to 95% are diagnosed with central nervous system involvement (CNS-SLE) as defined by the American College of Rheumatology [Unterman et al., submitted for publication] [2]. Yet, in many cases, diagnosing CNS-SLE remains a clinical challenge, and the presence of specific autoantibodies could be an important factor [3]. Twenty different brain-specific antibodies, such as anti-ribosomal-P antibodies, have been associated with CNS-SLE [4]. The anti-Rib-P Abs are highly specific for SLE, with a prevalence of 10–40%, in contrast to their rarity in healthy subjects and other autoimmune diseases [5-8]. The relationship between anti-Rib-P Abs and CNS-SLE was initially described by Eloisa Bonfa [9], who reported a correlation between antibody titers and psychotic events in SLE patients. These antibodies were found to be associated also with renal and hepatic manifestations of SLE [10-13]. Following Bonfa’s observation other studies yielded conflicting results, and despite substantial investigations the correlation between anti-Rib-P Abs and CNS-SLE manifestations remained controversial [4].

Anti-Rib-P Abs recognize 3 of the 80 identified ribosomal proteins that were defined as the target antigens in SLE. These three ribosomal proteins (P0, P1, P2) are located in the large ribosome subunit and are organized in a pentameric complex of one P0 and two copies of P1 and P2 [10]. Anti-Rib-P Abs are able to bind and penetrate certain cells (e.g., neuronal cells), then bind to ribosomal proteins, blocking protein synthesis [14]. Furthermore, a possible pathogenic role for these Abs was suggested in SLE due to their ability to induce apoptosis [15]. We recently studied the mechanism by which anti-Rib-P Abs mediated neuronal injury by injecting them directly into the brain ventricles of mice. Treated mice developed depressive-like behavior and a smell deficit. These...
neurological manifestations correlated with specific binding of anti-Rib-P Abs to the limbic system and the area of the brain that is associated with mood and olfaction [16-19].

The wide variations in prevalence of anti-Rib-P Abs, and the inconsistency regarding their clinical relevance in patients with SLE, might stem from differences between cohorts of patients since these antibodies are associated with genetic markers (i.e., HLA DQB) and geographic areas [8]. Furthermore, anti-Rib-P Abs titers correlate with SLE manifestations, activity and type of disease (i.e., juvenile SLE); therefore, the time interval between manifestation and the drawing of blood is important [5,10,20]. Finally, discrepancies in antibody prevalence may result from different methods of detection. Currently, detection of anti-Rib-P Abs is performed by enzyme-linked immunosorbent assay. Differences in antigens (i.e., purified proteins, synthetic or recombinant peptides) used for coating the ELISA plate as well as cross-reactivity of anti-Rib-P Abs with other autoantibodies [5,13,21] may yield different results.

In the current study we aimed to evaluate the prevalence of anti-Rib-P Abs determined by three different ELISA kits in 50 SLE patients and 50 healthy controls, as well as their clinical correlation.

PATIENTS AND METHODS

We analyzed the presence of elevated titers of anti-Rib-P Abs in 50 Israeli SLE outpatients and 50 healthy age and gender-matched controls. This study was approved by the local Helsinki Committee, and all participants signed an informed consent form. All patients with SLE fulfilled at least four of the American College of Rheumatology criteria for SLE.

On the day that blood was drawn, patients were evaluated for clinical data, including age, gender, duration of disease, current clinical manifestations, treatment and SLEDAI score. CNS manifestations were not observed in our group of SLE outpatients at the time of the study. However, patients’ clinical files were reviewed for previous CNS involvement (i.e., stroke, transient ischemic attack, epilepsy, transverse myelitis, and optic neuritis).

DETECTION OF ANTI-RIB-P ABS

Sera were kept at -20°C until evaluation, which was performed for all sera simultaneously by the same investigators. Three ELISA kits were evaluated: Kit-1, which uses synthetic peptide comprising the 22 C-terminal amino-acids; Kit-2, which uses native human ribosomal proteins (P0, P1, P2); and Kit-3, which is coated with affinity-purified human ribosomal proteins. All analyses were performed according to the manufacturers’ instructions.

STATISTICAL ANALYSIS

All the statistical calculations were done using the statistical software SAS. Normally distributed variables were summarized using the mean and SD. The median and range were used for non-normally distributed variables. Univariate comparisons between nominal variables were performed by chi-square test. Significance was determined at P < 0.05. For correlation between two continuous variables, we used Pearson’s or Spearman’s correlation coefficients for normal or non-normal variables, respectively.

RESULTS

We examined two groups of subjects: 50 SLE patients and 50 matched controls. Their mean age (± SD) was 41 ± 15 and 40 ± 12 years respectively. Females represented 82% of the SLE group and 78% of the controls. Among the SLE patients the duration of the disease was 8.57 ± 7.36 years (range 1–30 years) and the SLEDAI score was 5.9 ± 4.7 (range 0–21).

The prevalence of anti-Rib-P Abs in SLE patients and controls was 30% vs. 0%, 17% vs. 21%, and 30% vs. 14% in Kits-1, 2, and 3 respectively [Figure 1]. In five SLE patients the sera were found to be positive by all kits. The concordance between Kit-1 and Kit-3 was 90%, whereas between Kit-1 and Kit-2 it was only 36%. Regarding Kit-1, the sensitivity of the assay was 67% and the specificity 81%.

The presence of anti-Rib-P Abs utilizing Kit-1 correlated with SLEDAI score (P = 0.02). In other words, higher SLEDAI scores (mean ± SD) were documented in patients with detectable anti-Rib-P Abs (7.9 ± 4.6) compared with SLE patients with no detectable anti-Rib-P Abs (4.7 ± 3.9) [Figure 2]. Antibodies detected by Kit-2 did not correlate with SLEDAI or with any other clinical manifestation.

CNS manifestations were previously reported in 11 of our 50 SLE patients (22%). Nine of these 11 serum samples were analyzed for the presence of anti-Rib-P Abs and 3 (33%)
DISCUSSION

A significant difference was found between the three ELISA kits used to detect anti-Rib-P Abs. This discrepancy probably represents the different antigens used for coating these ELISAs.

In the three assays used in the current study the prevalence of anti-Rib-P Abs was within the expected range for SLE patients (17–30%). However, this is higher than previously reported for Israeli patients (11–12%), which could be the result of different cohorts examined or different methods of detection used in each study [5,8]. Elevated titers of anti-Rib-P Abs are considered to be restricted to SLE and are rarely (<5%) detected in normal subjects or in patients with other autoimmune diseases such as systemic sclerosis, Sjögren’s syndrome, dermatomyositis, rheumatoid arthritis, antiphospholipid syndrome, undifferentiated connective tissue disease, and familial Mediterranean fever [5,8]. In this study anti-Rib-P Abs were detected in 21% and 14% of healthy subjects utilizing Kit-2 and Kit-3 respectively, suggesting a low specificity of these kits. Cross-reactivity between other antibodies that are more prevalent in healthy subjects (such as anti-dsDNA, anti-Ro, anti-Sm, and anticardiolipin) and anti-Rib-P Abs has been reported and might be the cause of this low specificity [8].

In the current study, anti-Rib-P Abs detected by Kit-1 correlated with disease activity (SLEDAI), confirming previous studies [5,9,11,13]. In contrast, a lack of association between anti-Rib-P Abs and SLE-CNS manifestations was observed; this could be explained by the retrospective determination of CNS involvement in our study and the lack of information regarding mood disorder and non-focal CNS manifestations. Previously, 12 of 17 studies showed an association between elevated titers of serum anti-P Abs and CNS-SLE manifestations, predominantly psychosis and depression [4]. Interestingly, most reports that did not demonstrate such associations analyzed CNS manifestations at any time during follow-up, and not particularly at the time of antibodies determination. Three studies recently evaluated the clinical interaction with anti-Rib-P antibodies. The first was an international multicenter study that used ELISA to determine anti-Rib-P Abs in 947 SLE patients and 1113 control subjects (healthy or with other autoimmune diseases). A statistically significant correlation with CNS manifestations was observed (P < 0.04) [8]. The second was a quantitative evaluation of anti-Rib-P Abs detected via immunoblot assay and/or ELISA and CNS involvement performed at any time point during the course of SLE. The specificity for the diagnosis of CNS-SLE was 80% and the sensitivity only 26–42%, with a higher sensitivity for psychosis and mood disorder [22]. The third study was performed by Abdel-Nasser et al. [23] who evaluated 32 SLE patients for a battery of neuropsychiatric tests and concomitant levels of anti-Rib-P Abs. At least one neuropsychiatric manifestation was observed in 81% of patients, of which depression was the most common. Anti-ribosomal P antibodies were detected in 7/32 patients (22%); all 7 patients (100%) had clinical depression, suggesting a strong correlation (P < 0.01) between the two. In addition, the titers of anti-Rib-P Abs were relatively higher in all patients with neuropsychiatric SLE (P = 0.05).

Viewing the above findings as a whole suggests the notion that anti-Rib-P Abs need to be evaluated in the context of “active serology.” In other words, antibodies should be determined at the same time of symptoms assessment.

CONCLUSIONS

The lack of consistency regarding anti-Rib-P Abs prevalence and clinical correlations as observed in this study and in others limit their utilization as a diagnostic criterion for SLE disease. It seems that inconsistency between the presence of clinical manifestations and the time of blood sampling is one obstacle that can be overcome in the future by differentiating symptoms, especially CNS symptoms, according to their acuteness. Another drawback could be the different methods for antibodies detection currently used. An international standardization of tests for anti-Rib-P Abs is necessary. This will enable the use of one method or a combination of methods to determine the specificity and sensitivity of anti-Rib-P Abs as well as their “true” role in SLE.

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References

**Capsule**

**CD+4 cells control chronic viral infections by IL-21**

Chronic viral infections such as HIV and hepatitis B and C viruses are major public health concerns. T cell-mediated immune responses are critical for controlling viral infections. In contrast to acute infections, chronic viral infections are characterized by "exhausted" cytotoxic CD8+ T cells, cells that exhibit reduced proliferative capacity, cytokine secretion, and cytotoxicity. Treatments that reverse exhaustion result in increased viral control. Despite their exhaustion, these CD8+ T cells eventually help to control chronic infections by killing virally infected cells, and require CD4+ T cell help to do so. Three papers published in *Science* show that the cytokine, interleukin-21 (IL-21), known to be critical for the differentiation of certain CD4+ T cell effector subsets, is an essential factor produced by CD4+ T cells that helps CD8+ T cells to control chronic lymphocytic choriomeningitis virus infection in mice. Acute and chronic infections resulted in differing amounts of IL-21 production by virus-specific CD4+ T cells. CD8+ T cells required IL-21 directly, and when CD8+ T cells were unable to signal through IL-21 or IL-21 was not available, they were reduced in number, exhibited a more exhausted phenotype, and were not able to control the virus. In contrast, the absence of IL-21-dependent signaling did not affect primary CD8+ T cell responses to acute infection or responses to a viral rechallenge, suggesting that differentiation of memory CD8+ T cells is independent of IL-21.

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"A belief which leaves no place for doubt is not a belief; it is a superstition"

Jose Bergamin (1895-1983), Spanish writer, essayist, poet and playwright. Bergamin, whose father served as president of the canton of Málaga and his mother was a devout Catholic, was greatly influenced by both politics and religion and would attempt to reconcile Communism and Catholicism throughout his life, remarking “I would die supporting the Communists, but no further than that.”