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REVIEW

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Autoantibodies to the ribosomal P proteins in systemic lupus erythematosus

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Abstract This paper describes the clinical significance of antibodies to the ribosomal P proteins in systemic lupus erythematosus. It appears that liver disease due to the lupus process and not attributable to viral infection, alcohol or drugs is associated with anti-ribosomal P. In addition, there is a strong relationship to central nervous system disease and nephritis of antibodies to ribosomal P proteins. The prevalence of the anti-P antibodies is strongly related to disease activity wherein disease remission is associated with disappearance of anti-P antibodies. These phenomena taken together suggest an immunopathogenic role for anti-P antibodies. This idea is strongly supported by the observation that immunoglobulin G containing anti-ribosomal P activity binds and penetrates living cells with profoundly inhibitory effects on protein synthesis. Finally, a new era of research has been uncovered by the observation that in 54 of 55 instances normal sera passed over a ribosome-sepharose column unmasks anti-P antibodies, which can be eluted from the ribosome column with 3.0 M magnesium chloride. This suggests that anti-idiotypes regulate the expression of anti-P antibodies in normal persons and in lupus patients this regulation is ineffective, with the development of free anti-P antibodies in a proportion of patients with active disease.

Key words Clinical significance · Anti-ribosomal P

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Introduction

Understanding the clinical and immunological findings in patients with systemic lupus erythematosus (SLE) has been characterised by the identification of auto-antigenic targets to which sufficient concentrations of autoantibodies appear that are associated with precipitin formation in agar gels. These antigens have been called extractable nuclear antigens (ENA), which means they are easily extractable from tissue homogenates with neutral buffers at isotonic salt concentrations. These include the following specificities: Sm, nRNP, Ro/SSA, La/SSB, and most recently the ribosomal P proteins.

It is this last group of proteins that are the subject of this article. The ribosomal P proteins were first described by Elkon et al. [1], who showed that antibodies to ribosomal P protein bound three proteins of molecular weight 38, 19 and 17 kD by Western blotting. These three proteins were associated with the large ribosomal subunit [1]. In a subsequent study from Elkon's group, it was shown that the three antigenic proteins: P_0, P_1 and P_2, shared an epitope expressed in the carboxyl terminal 22 amino acids, which was highly acidic [2]. Antibodies to this single epitope reacted with a linear antigenic determinant and it was shown that there were additional antibodies that recognised conformational determinants [2].

Materials and methods

Antibodies to ribosomal P proteins were measured as described in previous work [3, 4].

Results

Early on, after recognition of the ribosomal P proteins, the close association of antiribosomal P antibodies with psychosis was described [5, 6]. In addition, the paired fluctuations of anti-P protein levels and psychotic episodes were described [6]. The utility of the radioimmunoassay using the synthetic carboxyl terminal 22 amino acid peptide to measure quantitative changes in the antiribosomal P protein antibody was demonstrated [6]. While there has been some controversy about the relationship of antiribosomal P protein antibodies and psychosis, the weight of the evidence favours a real relationship as recently discussed [7]. Finally, one group has found a strong relationship between antiribosomal P antibodies and depression [8]. Recognition of the relationship with liver disease and nephritis with antiribosomal P protein antibodies has flowed from various sources. The first suggestion of a relationship of anti-P derived from the study of a single patient who developed what proved to be chronic active hepatitis in parallel with the development of anti-P protein antibodies [3]. Biochemical evidence of disease was present for four years before a biopsy was performed. Previously, the patient had been followed for five years for SLE with manifestations of a photosensitive malar rash, polyarthritis, oral ulcers, grand mal seizures, hyper- and hypopigmented skin changes, ANA at a titre of 1/4860, anti-dsDNA titre of 1/270 on the Crithidia assay and hypocomplementaemia. All of these clinical and serological findings so characteristic of SLE ultimately disappeared concomitant with the development of abnormalities in liver function and an anti-P precipitin. There was no evidence of hepatitis B or C infection and tests for smooth muscle antibodies and anti-mitochondrial antibodies were also negative.

The second piece of evidence testifying to a link between hepatitis and anti-P protein antibodies came from a retrospective chart review [4]. Of 131 patients with SLE, 4 (3%) had liver involvement that could only be ascribed to the disease itself and two additional cases from elsewhere were also studied. There was no serological evidence of hepatitis B or C infections and the clinical involvement resembled chronic active hepatitis. Only one patient had low titre anti-smooth muscle antibodies and none had anti-mitochondrial antibodies. Anti-ribosomal P antibodies were present in all six patients with lupus hepatitis compared to only 2 (10%) of 20 controls with lupus but no liver disease (P=0.0001, odds ratio 96).

Finally, a case control study found a relationship between both hepatitis and nephritis and the presence of antiribosomal P protein antibodies [9]. The relationship of anti-P protein antibodies to nephritis received additional support from a retrospective study of SLE patients as they entered periods of active nephritis and remission. Four such patients had measurably elevated antibodies to ribosomal P protein during periods of active nephritis and a return to normal levels during periods of remission of their nephritic episodes [10].

A retrospective study of 69 SLE patients was carried out to study the association of antiribosomal P antibodies and active nephritis in SLE [11]. Antiribosomal P antibodies were positive in 21/69 patients with active disease with an overall prevalence of 30.4%. Prevalence of anti-P antibodies in patients with active nephritis was 75.0% (15/20), P value by Fishers' exact test of 8.39×10^{-7}. In 12 of 13 patients (92.3%) anti-P disappeared during periods of disease remission, P=0.0002. In nine of twelve patients (75%) titres of anti-dsDNA correlated with anti-P during disease exacerbation and remissions, P=0.004. These data support a possible role for anti-P in immunopathogenesis and a strong association between anti-P antibodies and anti-dsDNA antibodies.

A recent retrospective analysis of the presence or absence of nephritis and the relationship to serological findings has been published [12]. The specificity with the tightest relationship to nephritis was anti-LPL (lipoprotein lipase) with an odds ratio of 5.28 and a P value of 0.0019 while anti-P antibodies had an odds ratio of 3.47. There was synergy between anti-P and anti-LPL with 20 of 22 patients having nephritis for an odds ratio of 17.11 and a P value of 2.33×10^{-5}. Thus, virtually all patients with these two antibodies had nephritis. The figures are impressive and are the most impressive serological correlations made thus far in lupus nephritis.

Finally, a kidney became available for study from a patient who expired in 1979 and whose kidney eluate was studied previously for enrichment of anti-Ro antibodies [13]. This eluate was now studied for enrichment of anti-P in the eluate compared to the serum level. Indeed the eluate was enriched thirty-fold for anti-P activity compared to the serum providing evidence for the participation of anti-P in the lupus nephritis process [14].

Discussion

What then do these correlations mean? In these cases the implication is that antibodies to ribosomal P proteins are the pathogenic agents: i.e., the anti-P antibodies directly injure tissues of the liver, kidney and brain not unlike the tissue damage mediated by antibodies to dsDNA in lupus patients. Firstly, antibodies to ribosomal P proteins have

been shown to bind a membrane form of the P_0 38-kDa ribosomal phosphoprotein [15]. In addition, it has been shown that affinity-purified antibodies to the ribosomal P proteins bind living HepG2 cells and subsequently penetrate these live cells and cause cellular dysfunction [16]. Binding and penetration of anti-P antibodies is the property of $F(ab^1)_2$ fragments as well as whole IgG molecules, showing that neither binding nor penetration depends on Fc fragments or their cognate receptors. Confocal microscopy shows that internalised antibody concentrates in perinuclear vesicles (presumably lysosomes), but substantial quantities of antibody are also found in the cytosol. This intracellular antibody adversely affects the synthesis of apolipoprotein B resulting in a three-fold increase in cellular cholesterol with lipid droplet accumulation as seen in some chronic liver diseases. It also has a profound inhibitory effect on global protein synthesis as measured by ^{35}S methionine incorporation. Indeed, anti-P IgG at 10.0 mg/ml when incubated with live $HepG_2$ cells inhibits ^{35}S methionine incorporation 99.83%, while normal IgG at 10.0 mg/ml inhibits ^{35}S methionine incorporation 17.99%. Incubation beyond 24 h results in morphological changes in the cells incubated with anti-P IgG with the eventual admission to the cells of ethidium homodimer. These studies describe a model of cellular injury affected by specific antibody to ribosomal P protein that may underlie the liver disease occurring in lupus patients that is not due to alcohol, drugs or viral infection [16].

The other kind of data that suggests an immunopathogenic role for antiribosomal P antibodies is the clinical data that anti-P antibodies share uniquely with anti-dsDNA antibodies. These include the findings that both anti-dsDNA and anti-P are disease specific, vary with disease activity, bind and penetrate cells in culture, are not found in the sera of normals or first degree relatives, and deposit in tissues at the site of cellular injury such as the kidney.

Taken together, these data argue powerfully for an immunopathogenic role for antibodies to the ribosomal P proteins in lupus patients.

A new era was opened in the understanding of the regulation of autoimmune responses in lupus patients by the observation that anti-P antibodies were present in virtually all normal persons, but were masked by normal IgG presumably containing an anti-idiotype to anti-P antibodies [17]. Sera from healthy adults were applied to affinity columns coated with ribosomes, and the affinity purified fractions were analysed for anti-P antibodies by ELISA and immunoblot. Anti-P antibodies were detectable in serum only after affinity chromatography were predominantly of the IgG isotype, stained Hep-2 cells in the characteristic anti-P pattern and demonstrated specificity for all three ribosomal phosphoproteins, P_0, P_1 and P_2. Using batch affinity chromatography, anti-P autoantibodies were identified in the affinity-purified serum fractions of 54 of 55 healthy individuals. Anti-P antibodies from healthy adults generally bound less ribosomal P antigen per mg/ml IgG than anti-P from patients. Inhibition studies revealed that autologous serum contained an IgG inhibitor of anti-P antibody. These data are interpreted to mean that masked anti-P antibodies are present in the healthy normal population and presumably means that the detection of these antibodies in lupus patients represents disruption of these described regulatory networks operative in the normal population.

It is predictable that more will be learned about the regulation of the anti-P antibodies in healthy individuals and the factors affecting this regulation will be identified so as to bring greater understanding to this autoimmune response.

References

1. Elkon KB, Parnassa AP, Foster CL (1985) Lupus autoantibodies target ribosomal P proteins. J Exp Med 162:459–471
2. Elkon KB, Skelly S, Parnassa AP, Moller W, Danko W, Weissbach H, Brot N (1986) Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. Proc Natl Acad Sci USA 83:7419–7423
3. Koren E, Schnitz W, Reichlin M (1993) Concomitant development of chronic active hepatitis and antibodies to ribosomal P proteins in a patient with systemic lupus erythematosus. Arthritis Rheum 36:1325–1328
4. Arnett FC, Reichlin M (1995) Lupus hepatitis: an under-recognized disease feature associated with autoantibodies to ribosomal P. Am J Med 99:465–472
5. Bonfa E, Elkon KB (1986) Clinical and serological associations of the anti-ribosomal P protein antibody. Arthritis Rheum 29:981–985
6. Bonfa E, Golombek SJ, Kaufman LD, Skelley S, Weissbach H, Brot N, Elkon KB (1987) Association between lupus psychosis and anti-ribosomal P protein antibodies. N Engl J Med 317:265–271
7. Reichlin M (2003) Ribosomal P antibodies and CNS Lupus. Lupus 12:916–918
8. Schneebaum AB, Singleton JD, West SG, Blodgett JK, Allen LG, Cheronis JC, Kotzin BL (1991) Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. Am J Med 90:54–62
9. Hulsey M, Goldstein R, Scully L, Surbeck W, Reichlin M (1995) Anti-ribosomal P antibodies in systemic lupus erythematosus: a case-control study correlating hepatic and renal disease. Clin Immunol Immunopathol 74:252–256
10. Martin AL, Reichlin M (1996) Fluctuations of antibody to ribosomal P proteins correlate with appearance and remission of nephritis in SLE. Lupus 5:22–29
11. Chindalore V, Neas B, Reichlin M (1998) The association between anti-ribosomal P antibodies and active nephritis in systemic lupus erythematosus. Clin Immunol Immunopathol 87:292–296

12. Reichlin M (2005) Serological correlations with nephritis in systemic lupus erythematosus. Clin Immunol 117:12–14
13. Maddison PJ, Reichlin M (1979) Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. Arthritis Rheum 22:858–863
14. Reichlin M, Wolfson-Reichlin M (1999) Evidence for the participation of anti-ribosomal P antibodies in lupus nephritis. Arthritis Rheum 42:2728–2729
15. Koren E, Reichlin MW, Koscec M, Fugate RD, Reichlin M (1992) Autoantibodies to the ribosomal P proteins react with a plasma membrane-related target on human cells. J Clin Invest 89:1236–1241
16. Koscec M, Koren E, Wolfson-Reichlin M, Fugate RD, Trieu E, Targoff IN, Reichlin M (1997) Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. J Immunol 159:2033–2041
17. Stafford HA, Anderson CJ, Reichlin M (1995) Unmasking of anti-ribosomal P autoantibodies in healthy individuals. J Immunol 155:2754–2761