BANTAO Journal 2 (2): p 15; 2004

Correlation of Anti-dsDNA and Anti-Ribosomal P Antibodies with Glomerulonephritis in Patients with Systemic Lupus Erythematosus

Daniela Monova[1], T. Argirova[2], Simeon Monov[3]
Medical Institute – MIA[1], Medical University - Sofia, Clinic of Rheumatology[3], Sofia University, Department of Biochemistry, Faculty of Biology[2], Sofia

Introduction

Autoimmune diseases are characterized by the presence of autoantibodies targeted against cellular proteins and nucleic acids. They serve as useful markers for the clinician use. Some autoantibodies are disease specific and therefore they are valuable diagnostic tools for clinicians. In Systemic Lupus Erythematosus (SLE), antibodies to double stranded DNA and anti - Sm are widely used as diagnostic markers. Heterogenous antiribosomal antibodies are found in SLE, rheumatoid arthritis and other connective tissue diseases. Autoantibodies to ribosomal P protein have been recognized since 1985 [6]. Antibodies directed against the ribosomal P proteins (P0 with Mn of 38 kD, P1 of 19 kD and P2 of 17 kD) have been suggested to be specific markers of the neuropsychiatric manifestations of the disease [3]. Recently it has been shown that elevated antiribosomal P antibody levels were not specific for psychosis [5, 20]. In addition, antibodies to ribosomal P protein in SLE have also been associated with general disease activity [7].

The relationship of autoantibodies and lupus nephritis has been studied intensively for the past 35 years. One of the earliest specificities recognized to have a relationship with nephritis is the dsDNA-anti-dsDNA system [8,10]. Other specificities that have been studied by the criterion of enrichment in glomerular eluates include anti-Ro/SSA [11], antibodies to collagen-like region of C1q [12,16], anti-myeloperoxidase [13], and anti-ribosomal P antibodies and the presence of active nephritis [4,14,15]. Finally, although the experiments were not quantitative, early studies with glomerular evaluates showed that the most frequently found antibody was that directed to nucleoprotein [10]. It is, therefore, likely that anti-nucleoprotein antibodies form specific deposits which can participate in the pathogenesis of lupus nephritis.

Patients and Methods

We compare SLE patients with nephritis and precipitating anti-P to SLE patients without nephritis and anti-P precipitins with regard to the presence or the absence to other antibodies. We also compared a second group of SLE patients who had elevated levels of anti-P antibodies by ELISA to a group of SLE patients who were without anti-P antibodies by this method.

In this study we find strong correlation between the presence of both anti-ds DNA and anti-ribosomal P antibodies and, in the case of anti-ds DNA antibodies, titers are higher in nephritic than in nonnephritic patients.

We studied 74 patients (67 females, 7 males) with SLE (Table 1). Antiribosomal P antibodies were found in 38 of them. ELISA method for determination of antiribosomal antibodies were used [2].

There were no statistically significant differences in the frequency of antibodies to Ro, La, RNP or Sm between the two groups (anti-P positive group and anti-P negative group). A major serological difference between the groups was an increased prevalence of anti-double- stranded DNA in the anti-P positive group (30/38) versus the control group with patients without antiribosomal P antibodies (8/36).

Evidence for kidney disease was taken as persistent proteinuria >0,5 g/24 h or serum creatinine > 140 □mol/l. All patients with evidence for kidney disease had renal biopsy. In the anti-P positive group 30 patients had persistent proteinuria: 9 patients had membranous glomerulonephritis (MGN), 19 - diffuse proliferative glomerulonephritis (DPGN), 3 – mesangial proloferative lupus nephritis (MesPGN), 1 – focal lupus nephritis (FLGN). In the anti - P negative group 3 had DPGN. The difference in prevalence of renal disease between these two groups is statistically significant ($P<0,0001$). We observed 6 SLE patients whose nephrotic episodes were associated with the appearance of antibodies to native DNA.

In the group with anti-P antibodies, 9 patients (№1,8,10,11,15,20,27,29,31) were found to have had liver disease, compared with 4 (№4,10,30,35) in the control group. Evidence for liver disese was taken as simultaneous elevations of three or more liver enzymes (i.e., SGOT, SGPT, γGTP, alkaline phosphatase, total bilirubin).

Statistically significant differences in two groups suggest that antiribosomal P-antibodies identify a subset of SLE patients at higher risk for kidney involvement. Antibodies to ribosomal P proteins have been shown to be more prevalent in patients with juvenile - onset SLE than in adult SLE.

Correspondence to: Daniela Monova, 24 Veliko Turnovo st., Sofia-1504, Bulgaria, E-mail: dmonova@yahoo.com

Table 1. Autoantibody profile of anti-P SLE and control SLE*

Anti- P (+) group			Anti- P (-) group		
№, Sex, Age	**Kidney**	**Serology**	**№, Sex, Age**	**Kidney**	**Serology**
01. F., 19	**DPGN**	**ds, Ro, RNP**	***1. F., 26***		**Sm, RNP**
02. F., 21	**DPGN**	**ds**	***2. F., 27***		
03. F., 24	**MGN**		***3. F., 28***		**Ro, La**
04. F., 20	**DPGN**	**Ro, La, RNP**	***4. F., 35***		**Ro**
05. F., 18	**DPGN**	**Ro, Sm, RNP**	***5. F., 34***		**Ro**
06. M.,20	**MGN**	**ds**	***6. F., 35***		**Ro, La**
07. F., 19	**MGN**	**ds, La, RNP**	***7. M.,19***		**ds**
08. F., 32	**DPGN**	**RNP**	***8. F., 22***		**ds, La**
09. F., 26	**MGN**	**ds, Ro**	***9. F., 24***		**RNP**
10. F., 27	**DPGN**	**ds**	***10. M.,30***		**ds, RNP**
11. M.,23	**DPGN**	**ds**	***11. F., 34***		**Ro,RNP**
12. F., 22	**DPGN**	**ds**	***12. F., 37***		
13. F., 19	**DPGN**	**ds, La, RNP**	***13. F., 28***	**DPGN**	**Ro**
14. F., 33	**MGN**		***14. F., 33***		
15. F., 22	**MGN**	**ds, Ro, La, Sm RNP**	***15. F., 22***		**ds, Ro, RNP**
16. F., 30	**DPGN**		***16. F., 35***		
17. F., 27	**DPGN**	**ds, Ro**	***17. F., 34***		**RNP, Sm**
18. M., 24	**DPGN**	**ds,**	***18. F., 36***		**Sm**
19. F., 28	**MesPGN**	**ds,**	***19. F., 34***		
20. F., 25	**FLGN**	**ds, La, RNP**	***20. F., 33***		**Ro**
21. F., 30	**MGN**	**RNP**	***21. F., 36***		**ds**
22. F., 22	**DPGN**		***22. F., 29***		**RNP**
23. F., 20	**MesPGN**	**ds, Ro**	***23. F., 28***		**RNP**
24. F., 19	**DPGN**	**ds, Ro, Sm**	***24. F., 30***		
25. F., 34	**DPGN**	**ds, La**	***25. M., 27***	**DPGN**	**ds**
26. F., 29	**DPGN**		***26. F., 30***		
27. F., 28	**MGN**	**Ro, RNP**	***27. F., 24***	**MGN**	**Ro, RNP**
28. M., 21	**MGN**	**ds, RNP**	***28. F., 34***		**La**
29. F., 27	**MesPGN**	**ds, La, RNP**	***29. F., 30***		**Ro**
30. F., 21	**DPGN**	**ds, La**	***30. F., 31***		**ds, Sm**
31. F., 20	**DPGN**	**ds, RNP**	***31. F., 29***		**Ro**
32. F., 30	**DPGN**	**ds, Ro**	***32. F., 33***		
33. F., 25			***33. F., 32***		**Ro, La**
34. F., 29		**Ro**	***34. F., 28***		**Ro**
35. F., 32		**ds, Sm**	***35. F., 29***	**DPGN**	**ds, La**
36. F., 26		**Ro**	***36. F., 30***		**Ro**
37. F., 25		**RNP**			
38. F., 24		**La**			

* All patients except N14 in anti-P positive group and №22, №34 in anti-P negative group are ANA positive > 1:160

Results

Serological and clinical data were collected on 38 patients with high titers of anti-ribosomal P antibodies. Of these 38 patients, 32 had evidence of nephritis either at the time their serum was studied for anti-P antibodies or within the preceding 5 years. All of these patients satisfied the ACR criteria for the diagnosis of SLE [19]. The 6 patients without nephritis also satisfied ACR criteria for SLE and did not have any evidence of renal disease in the 2 years preceding the demonstration of precipitating anti-ribosomal P antibodies. The serological data comparing these two groups are listed in Table.2.

Table 2. Autoantibody profiles in SLE patients with anti-P precipitins with and without nephritis

№ of patients	SLE patients with nephritis		SLE patients without nephritis	
№ with anti-dsDNA	**22**	**68,75 %**	**1**	**16, 67 %**
№ with anti-Ro	**10**	**31,25 %**	**2**	**33,33 %**
№ with anti-La	**8**	**25 %**	**1**	**16, 67 %**
№ with anti-Sm	**3**	**9,38 %**	**1**	**16, 67 %**
№ with anti-RNP	**13**	**40,62 %**	**1**	**16, 67 %**
№ with 0 precipitins	**5**	**15,62 %**	**1**	**16, 67 %**

There is a highly significant difference in the prevalence of positive anti-dsDNA antibodies. As seen, 22 of 38 (57,89%) of the nephritic group had positive tests, while only 1 of 6 or 16,67 %, had positive tests in the no nephritis group. There were no statistically significant differences noted for any of the other serological tests which included precipitins for anti-Ro, anti-RNP or anti-Sm. There is an obvious difference not only in the qualitative presence of anti-dsDNA antibodies, but also a sizable quantitative difference, with the maximum titers in the nephritis group being substantially higher than those in the no nephritis group. The titer in the nephritis group was about 14 times higher than in the no nephritis group.

We also studied serum samples from 36 SLE patients without anti-ribosomal P precipitins and those data are listed in table 3. Of these 36 patients, 4 had nephritis, while 32 did not. Of these 36 patients 8 patients had anti-dsDNA antibodies. As with the patients with precipitating anti-P, no other autoantibody specificity had a statistically significant higher prevalence in one group or the other.

Table 3. Autoantibody profiles in SLE patients with no anti-P precipitins with and without nephritis

№ of patients	SLE patients with nephritis		SLE patients without nephritis	
№ with anti-dsDNA	**2**	**50 %**	**6**	**18,75 %**
№ with anti-Ro	**2**	**50 %**	**12**	**37,5 %**
№ with anti-La	**1**	**25 %**	**5**	**15,62 %**
№ with anti-Sm	**-**	**-**	**4**	**12,5 %**
№ with anti-RNP	**1**	**25 %**	**8**	**25 %**
№ with 0 precipitins	**-**	**-**	**8**	**25 %**

Thus, the simultaneous presence of anti-dsDNA and anti-P over a wide range of antibody concentrations appears to be strongly correlated with the presence of nephritis.

Discussion

In this study we find a strong relationship between the presence of both anti-ds DNA and anti-ribosomal P protein antibodies and nephritis in SLE (Table 1). First, it suggests that the presence of two "pathogenic" autoantibodies is more nephritogenic than either autoantibody when it occurs alone. Yet, these relationships are not perfect, as SLE patients with only one of the two "pathogenic" antibodies occasionally develop nephritis, and also uncommonly SLE patients with both antibodies apparently escape renal injury, at least during the duration of follow-up utilized in this study.

It is, of course, likely that even those antibodies such as anti-dsDNA and anti-ribosomal P that are closely associated with nephritis are themselves heterogeneous with respect to"pathogenicity". Indeed, the variable behavior of monoclonal anti-dsDNA antibodies with cells suggests a mechanism for variable pathogenicity. As described in a recent review, some anti-dsDNA antibodies bind but do not penetrate cells in culture, some do penetrate and home to the nucleus, while a third variety penetrates the cellular membrane and resides in the cytoplasm even after 24 h [13]. Interestingly, anti-ribosomal P antibodies consistently bind and penetrate cells and are capable of inhibiting protein synthesis [9,18]. Anti-dsDNA antibodies are also very effective inhibitors of in vitro translation [1,21]. These jointly held properties of anti-dsDNA and anti-ribosomal P may account in part for their pathogenicity in vivo. In addition, a variety of membrane receptors for anti-dsDNA on cells in culture and even whole animals have been described [17]. Little wonder that with such heterogeneity of the behavior and biological effects on cells and tissues that heterogeneity in "pathogenicity" might be expected. So far, there are no data that might link these cellular and molecular heterogeneous effects to pathogenicity. It may also be true that there are other "pathogenic" autoantibodies whose presence or absence, in addition to anti-dsDNA and anti-P antibodies, might make a decisive contribution to the renal outcome in individual patients with SLE. These might include antibodies to the collagen-like regions on C1q [12,16], anti-

myeloperoxidase [13] and perhaps other as yet unidentified autoantibodies.

Clearly, there is much yet to learn about the immunological determinants of lupus nephritis and can expect more clarity of these phenomena in the future as these studies progress.

References

1. Alberdi, F. et al. Cross-reaction of lupus anti-dsDNA antibodies with protein translation factor EF-2. Clin. Immunol., 2001., 98, 293-300.
2. Argirova, T. et al. Detection of antiribosomal antibodies in human sera. Balk. J. Clin. Lab., 1997, 3-4, 29-31.
3. Bonfa, E. et al. Association between lupus psychosis and antiribosomal P protein antibodies. N. Engl. J. Med., 1987, 1, 265-271.
4. Chindalore, V., B. Neas et M. Reichlin. The association between anti-ribosomal P antibodies and active nephritis in systemic lupus erythematosus. Clin. Immunol. Immunopathol., 1998, 87, 292-296.
5. Derksen, R.H.W. M. et al. A prospective study of antiribosomal P proteins in two cases of familial lupus and recurrent psychosis. Ann. Rheum. Dis., 1990, 4, 779-782.
6. Elkon, K., A. Parnassa et C. Foster. Lupus autoantibodies target ribosomal P proteins. J. Exp. Med., 1985, 2, 459-471.
7. Hulsey, M. et al. Anti-ribosomal P antibodies in systemic lupus erythematosus. - Clin. Immunol. Immunopathol., 1995, 3, 252-256.
8. Koffler, D., P.H. Schur and H.G.Kunkel. Immunological studies concerning the nephritis of systemic lupus erythematosus. J. Exp. Med., 1967, 126, 607-624.
9. Koscec, M. et al. Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. J. Immunol., 1997, 159, 2033-2041.
10. Krishnan, C. et M.H. Kaplan. Immunopathologic studies of systemic lupus erythematosus. J. Clin. Invest, 1967, 46, 569-579.
11. Maddison, P.J. et M. Reichlin. Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. Arthritis Rheum., 1979, 22, 858-863.
12. Mannik, M, et M.H. Wener. Deposition of antibodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. Arthritis Rheum., 1997, 40, 1504-1511.
13. Mannik, M,, C.E. Merril et M.H. Wener. Antibodies to human myeloperoxidase in glomerular immune deposits of systemic lupus erythematosus. Lupus, 2000, 9, 607-613.
14. Martin, A. et M. Reichlin. Fluctuations of antibody to ribosomal P proteins correlate with appearance and remission of nephritis in SLE. Lupus, 1996, 5, 22-29.
15. Monova, D., T. Argirova and S. Monov. Antiribosomal P antibodies in patients with lupus glomerulonephritis. Nephrology, Hemodialysis and Transplantation, 1999, 1, 30-32.
16. Monova, D. et al. Autoantibodies against C1q: view on association between systemic lupus erythematosus disease manifestation and C1q-autoantibodies. Ann. Rheum. Dis., 2002, 6, 563-564.
17. Reichlin, M. Autoantibodies to ubiquitous intracellular antigens interact with living cells. Immunologist, 1998, 6, 76-78.
18. Stacey, D. W. et al. The inhibition of protein synthesis by IgG containing anti-ribosome P autoantibodies from systemic lupus erythematosus patients. - Arch., Biochem. Biophys., 267, 1988, 398-403.
19. Tan, E. M. et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum., 1982, 25, 1271-1277.
20. Teh, L. et al. Antibodies to protein P in systemic lulus erythematosus. Ann. Rheum. Dis.. 1992, 2, 489-494.
21. Tsuzaka, K. et al. Autoantibodies to double-stranded DNA immunoprecipitate 18S ribosomal RNA by virtue of their interaction with ribosomal protein S1 and suppress in vitro protein synthesis. Clin. Exp. Immunol., 1996, 106, 504-508.