Autoantibodies to Ribosomal P Proteins in Systemic Lupus Erythematosus

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Systemic lupus erythematosus is considered the prototype of systemic autoimmune diseases in which autoantibody production is the prominent feature of the immune system dysregulation. These autoantibodies are directed against fundamental intracellular antigens, mainly of nuclear but also of cytoplasmic origin or localization in the cell. Among the anti-cytoplasmic antibodies, anti-ribosomal protein antibodies are the most predominant and the ribosomal P proteins the major targeted antigens [1,2]. The P proteins are three highly conserved phosphorylated proteins (P0, P1, P2) of 38, 19 and 17 kDa apparent molecular weight, respectively, located within the large 60S subunit of the eukaryotic ribosome. The anti-P immunodominant epitope has been identified as a single linear sequence within the 22 amino acid carboxy-terminal peptide (C-22) common to the three P proteins 3], even though the antibody binding seems to be mediated also by conformational determinants on P proteins [3].

The diagnostic significance of anti-P antibodies

During the last 15 years many studies on anti-ribosomal P antibodies have been published. Nevertheless, growing attention is still devoted to the clinical and pathogenetic significance of these antibodies. It has been largely ascertained that they occur almost exclusively in 15–20% of randomly selected Caucasian SLE patients [1,4,5] and are a specific serological marker of SLE and lupus-like disease [6]. Their prevalence is even higher in juvenile-onset SLE than in the adult-onset type [7], and antibody levels are strongly affected by disease activity or therapy [8–10], both in adult and pediatric SLE patients [7,11] [Figure 1].

An evaluation of the autoantibody frequencies in multiethnic populations of patients with SLE showed that anti-P antibodies are more frequently observed in Malaysian-Chinese patients (36–38%) [12,13] and less commonly in Bulgarian patients (6%) [13]. Although found in a relatively small proportion of patients, they tend to be associated with an overt and more generalized form of SLE [14] and with particular clinical features, such as diffuse neuropsychiatric abnormalities, primarily psychosis and/or severe depression, and hepatic and renal involvement [Table 1]. However, the clinical significance of anti-P antibodies is still the object of investigation due to contradictory findings, particularly concerning their role and diagnostic value in patients with neuropsychiatric dysfunction. More than 12 years ago, Bonfa et al. [15] first reported on a strong association between lupus psychosis and anti-P antibodies and also suggested that the antibody level selectively fluctuates during psychotic exacerbations. Since then, either confirmatory or conflicting findings have emerged [5,11,14,16–19]; thus the true predictive value of anti-P positivity for the presence of neuropsychiatric disease in SLE has not yet been defined. However, based on an indirect estimation, it seems to be rather low (approximately 0.30) [20,21]. Such discrepancies are due to methodological differences among the studies, one of the most important being the application of different non-established criteria in defining central nervous system involvement in patients with SLE. Moreover, the pathogenesis of

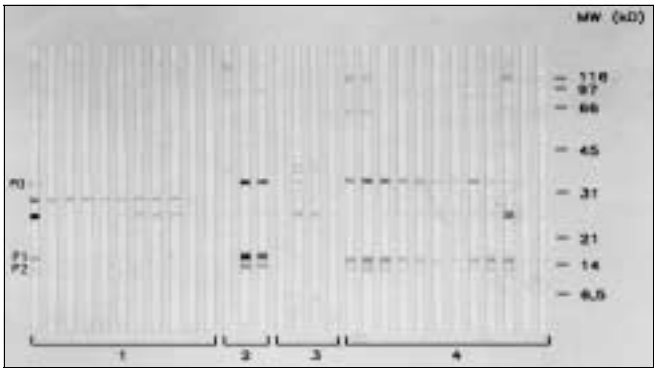

Figure 1. Western blot against 15% SDS-PAGE cytoplasmic Raji cell extract of serial serum samples from four SLE patients (1–4) over a mean follow-up period of 5 years (range 3–8 years). Molecular weight markers are indicated in kilodaltons on the right.

SLE = systemic lupus erythematosus

Table 1. Frequency (% values) and clinical associations of elevated serum levels of anti-P antibodies in different SLE patient cohorts

Studies	No. of patients	Mean age (yr)	Overall patients	Frequency (%) of anti-P antibody								
				Active disease	Inactive disease	Multisystem organ disease						
						Skin	Joint	Blood	Lung	Kidney	CNS	Psychosis/ depression
Bonfa [4]	50	22	34	–	–	36	36	30	30	25	41	100
Bonfa [15]	75	–	47	–	–	–	–	–	–	–	52	90
Schneebaum [16]	269	–	19	–	–	–	–	–	–	–	29	54
Sato [8]	138	33	30	42	8	–	–	–	–	–	–	73
Teh [5]	116	33	16	–	–	–	–	–	–	–	20	17
Nojima [17]	91	36	42	–	–	46	41	46	33	41	63	90
Yoshio [18]	70	32	41	41	–	–	–	–	–	–	58	30
Chindalore [10]	69	30	30	30	–	23	31	33	32	39	37	–
Tzioufas [19]	178	36	19	–	–	–	–	–	–	–	39	–

neuropsychiatric disease in SLE is multifactorial and could account for the difficulty in achieving a correct diagnosis and therapeutic strategy. More recently, it has been suggested that anti-P antibody positivity is more frequently found in patients with diffuse rather than focal manifestations of SLE-associated neuropsychiatric dysfunction [19], thus this serologic test should be performed in suspected cases [19,22].

Even the relationship between anti-P antibodies and SLE-related nephritis or hepatitis is still a matter of controversy. At present, data on the true specificity of anti-P antibody in renal or hepatic involvement of SLE are uncertain even if both case-control and longitudinal studies assess a correlation between these features [9,10,23]. Diagnostic significance and putative nephritogenic or hepatogenic potential of anti-P are undoubtedly reinforced by anti-dsDNA antibody [7] with which, in many cases, anti-P coexist and simultaneously fluctuate [7,9,10]. Recently, Reichlin and Wolfson-Reichlin [24] reported fascinating evidence for the specific enrichment of anti-P activity in the renal eluate from a patient with SLE and rapid glomerulonephritis, thus supporting the possible involvement of anti-ribosomal P autoimmune response in immune-mediated renal damage. Liver dysfunction, correlated to anti-P in SLE, has biochemical and histologic characteristics resembling chronic active hepatitis not explained by viral infections, drugs or alcohol abuse. Patients with non-SLE-related autoimmune chronic active hepatitis did not have anti-P autoantibodies [23].

Development of the anti-P autoantibody response

The anti-P autoantibody elicitation pathway strikingly resembles that of an antigen-driven response with a characteristic immunoglobulin M to G isotype switching during time, either in humans or "P-peptide"-immunized animals [25]. At present, the mechanisms evoking the autoantibody response in SLE as well as in other systemic autoimmune diseases are still unknown. Recent developments over the last decade suggest that exposure to the immune system of modified intracellular molecules during apoptosis could be the triggering event leading to autoimmunity. It has recently been shown that the P proteins are also post-translationally modified (dephosphorylated) during the Fas ligand-induced apoptosis [26]. The P1 and P2 proteins are completely dephosphorylated whereas P0 is only partially. Since the epitope recognized by the antibodies on P proteins is normally phosphorylated, the apoptotic modification might be a crucial event for the antigenicity of P proteins. The correlation between the apoptosis-induced modifications of P proteins and the development of the autoimmune response is still under investigation.

Such findings do not seem to be consistent with the identification of "occult" IgG anti-P autoantibodies in virtually all healthy adults and children after serum affinity chromatography on ribosome columns [27,28]. Such natural autoantibodies are masked in untreated normal sera by a natural inhibitor, an IgG anti-idiotype to anti-P antibodies [29]. It has been hypothesized that a deficiency of the natural anti-P inhibitor with a disruption of the immune regulatory network occurs early in SLE patients.

Pathogenic potential of anti-P antibodies

There is evidence that these antibodies have a direct pathogenic role in SLE, even if the mechanisms are still unclear. A membrane form of ribosomal P0 protein is expressed on the surface of different human cells including hepatic, neuronal, fibroblast, endothelial and lymphoid cells, and functions as the cellular receptor that mediates the P0 antibody binding and penetration into living cells [30–33]. In addition, *in vitro* studies have demonstrated that anti-P antibodies act as potent inhibitors of protein synthesis and cellular function in living cell cultures in a dose and time-dependent manner [33,34], thus supporting their potential to induce cell or tissue immune-mediated dysfunction.

Laboratory determination of anti-P antibodies

Anti-P antibody detection is reliably performed by using either Western blot on ribosomal or whole cytoplasmic extract [Figure

Ig = immunoglobulin

1] or enzyme-linked immunosorbent assay on affinity-purified P proteins, recombinant P antigens or synthetic peptides. [6,19,35–37]. Good agreement and correlation in the results is generally obtained, most of all within highly positive sera.

Ribosomal proteins are known to strongly mediate antibody cross-reactivity. In fact they can be recognized by other antibodies such as anti-dsDNA or anti-Sm [38–40], but such cross-reaction phenomena do not reduce the value of anti-P binding identification or the characterization of antibody polyreactivity, being confined within human lupus sera or lupus-prone murine sera.

Conclusions

Sensitive serological testing of anti-P antibodies is of high diagnostic value in suspected SLE, particularly in pediatric-onset cases due to its high diagnostic specificity, even as a unique antibody species. Moreover, testing for this autoantibody may be clinically useful, primarily in lupus patients with active neuropsychiatric, hepatic or renal dysfunction, as a valuable predictor of disease course or response to therapy. However, at present the antibody predictive value for these clinical complications is still uncertain, since the majority of anti-P positives have neither anamnestic nor clinical evidence of the above cited manifestations. In order to progress in this setting of conflicting hypotheses, we suggest that efforts continue in both the clinical and biological characterization of the anti-P immune response – one of the most promising antibody systems involved in the etiopathogenesis of tissue damage in SLE.

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