CASE REPORTS

A prospective study on antiribosomal P proteins in two cases of familial lupus and recurrent psychosis

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Abstract

In two siblings with systemic lupus erythematosus (SLE), who experienced two episodes of psychosis each, a longitudinal study of autoantibodies, including antibodies to ribosomal P proteins, is described. In two of three evaluable periods of 15 weeks antedating psychosis a rise followed by a spontaneous drop in anti-P levels was recorded. In the third period antibodies to ribosomal protein P were absent. It is concluded that results with single samples are not informative, and that frequent measurement of antibodies to ribosomal protein P in patients with SLE may have limited predictive value for psychosis.

Neuropsychiatric manifestations occur in about half of all patients with systemic lupus erythematosus (SLE).¹ Immunologically mediated vascular injury and the action of autoantibodies reactive with neurones are thought to play a part in their pathogenesis, but the cause and effect of these abnormalities are still not understood.²

Recently, Bonfa *et al* described an association between psychosis in patients with SLE and the presence of autoantibodies to ribosomal P proteins,^{3,4} and found that serum levels of these antibodies increase before the onset of psychosis.⁴

We determined levels of autoantibodies, including antibodies to ribosomal protein P, in multiple serum samples from two carefully reported siblings with SLE who had two episodes of psychosis each. An increase, followed by a spontaneous fall in antiribosomal P protein levels was reported in two of three periods of 15 weeks antedating psychosis. In the third period autoantibodies to ribosomal P proteins were absent.

The data show that findings on anti-P in single samples are not informative and suggest that frequent measurement of antibodies to ribosomal protein P during the follow up of patients with SLE may have predictive value for psychosis.

Case reports**CASE 1**

An Indonesian woman, born in 1957, was diagnosed as having SLE in May 1982 when she had discoid skin lesions, butterfly rash, non-infectious fever, arthralgias, proteinuria (1.5 g/24 h), leucopenia ($3.4 \times 10^9/l$), and thrombocytopenia (platelets $69 \times 10^9/l$). Tests for anti-nuclear antibodies, autoantibodies to double stranded DNA (anti-dsDNA), and platelets were positive, and serum complement was low.

Antibodies to extractable nuclear antigens were absent. A kidney biopsy showed mesangial glomerulonephritis (WHO classification IIb). Serum creatinine was normal, the sediment unremarkable. She responded well to treatment with prednisone 40 mg/day (0.7 mg/kg). Because of platelet counts below $20 \times 10^9/l$ she was referred to our hospital in January 1983. The bone marrow biopsy specimen showed active thrombopoiesis. Treatment with high dose prednisone (>40 mg/day) was effective, but relapses occurred with lower doses. Therefore, splenectomy was performed in February 1984. For the next nine months there was a complete clinical remission. Then she was admitted because of fever, malaise, leucocytopenia (active granulopoiesis in the bone marrow), a vasculitic skin rash, and psychosis, while taking 12.5 mg prednisone/day. No infectious cause could be found. She responded well to treatment with prednisone (1 mg/kg) and clopenthixol chloride, but normalisation of her mental status occurred only over several months. In February 1985 azathioprine 150 mg/day was introduced and prednisone was tapered off.

In August 1987 she was admitted while taking 2.5 mg prednisone and 150 mg azathioprine daily for evaluation of complaints of diarrhoea. No cause was found. On admission she was apathetic, but over two weeks she developed a florid psychosis. The computed tomography brain scan and cerebrospinal fluid were normal. After one week's treatment with haloperidol her mental state had not improved, and a mucosal ulcer on the lower lip and leucopenia and thrombocytopenia were found. The daily dose of prednisone was increased to 60 mg. The mucosal and haematological abnormalities responded very rapidly; the psychiatric manifestations disappeared gradually over two months. The daily dose of prednisone was tapered slowly; the clinical remission persisted.

CASE 2

This patient, a younger sister of case 1, born in 1960, was diagnosed as having chronic discoid lupus erythematosus at the age of 25. Both her parents and eight other siblings were healthy without signs of an autoimmune disease. Treatment with topical corticosteroids and anti-malarial drugs was successful. In September 1987 she was admitted to another hospital because of malaise, myalgias, fever (39°C), and psychiatric abnormalities (bradyphrenia, blocking of thoughts, and paranoia). Oral ulceration and lymphadenopathy were found. Laboratory abnormalities included non-haemolytic anaemia

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(109 g/l), leucocytopenia ($3.0 \times 10^9/l$), raised creatine kinase and complement concentrations. Tests for antinuclear antibodies, anti-dsDNA, and antibodies to extractable nuclear antigens were negative. Systemic lupus erythematosus was diagnosed and treatment with 60 mg prednisone/day (1.2 mg/kg) was started the day after admission. After two days she left hospital against medical advice. Six days later she was seen at our outpatient department, still using 60 mg prednisone/day. The psychiatrist confirmed the presence of psychosis. There was diffuse loss of hair, proximal muscle weakness, (still) raised creatine kinase levels, and electromyographic findings compatible with myositis. Over the next month there was a complete recovery except for fatigue and alopecia. The daily dose of prednisone was gradually reduced.

In March 1988, while receiving 20 mg prednisone/day, there was recurrence of myositis, leucocytopenia, a butterfly rash, and vasculitis on the palms of the hands. Prednisone was increased to 50 mg/day. Within 24 hours she became overtly psychotic, and was admitted to a psychiatric hospital after several attempts to commit suicide. Prednisone was continued and combined with haloperidol, promethazine chloride, and diazepam. At discharge, two months later, she was still depressed, anxious, and bradyphrenic. These symptoms gradually disappeared as the daily dose of prednisone was tapered. In October 1988, while receiving 22.5 mg prednisone/day, azathioprine (100 mg/day) was introduced and neuroleptic drugs stopped.

Materials and methods

From both patients serum samples were obtained during follow up and stored at -80°C until use. Samples were tested without knowledge of the clinical data.

Antibodies to ribosomal phosphoproteins were determined by immunoblotting and measured by an enzyme linked immunosorbent assay (ELISA). We used an internal reference serum, which reacted in a similar fashion to a reference serum, in both the immunoblotting assays and ELISAs.

In the immunoblotting assay⁵ a rat liver ribosomal extract, essentially prepared as described by Fairhurst,⁶ was used and serum samples were tested in a 1:25 dilution.

For the ELISA a synthetic peptide containing 22 amino acids as described by Elkon *et al*⁷ was synthesised by solid phase methods. It was found to be 50% pure by high performance liquid chromatography. The peptide was coated overnight onto an ELISA plate (Dynatech, Plochingen, FRG) at a concentration of 5 µg/ml. Serum samples were diluted 1:50 in phosphate buffered saline (10 mM NaH_2PO_4 , pH 7.4, 150 mM NaCl) containing 0.02% Tween-20 (Sigma) and 0.2% gelatin (Merck) and incubated for two hours with the plates. Mouse monoclonal antihuman IgG (CLB-MH-16-E), coupled to horseradish peroxidase was used as a conjugate in a 1:1000 dilution. Plates were developed with 4-chloro-1-naphthol (Sigma) and read in a Titertek Multiscan reader. We tested 200 healthy blood bank donors (150 male, 50 female,

median age 33 years) and 48 patients positive for anti-dsDNA (five men, 43 women, median age 43 years) in this ELISA. The mean extinction with the normal donors was 0.11 and the highest value was 0.25. The extinctions with the samples positive for anti-dsDNA also did not exceed 0.25. For quantitation, titration curves of the test samples were made, using twofold serial dilutions. On each plate a titration curve of the reference serum, arbitrarily defined as having 100 U antibodies to P proteins, was included. The titre of a serum was defined as the reciprocal dilution at which the extinction was 1.0. In order to obtain units, the ratio between the titre of the test serum and the reference serum was calculated and multiplied by 100. Values above 10 units were considered positive.

Antibodies against RNP, Sm, La/SS-B, and Ro/SS-A were determined by counterimmunoelectrophoresis according to the method of Kurata and Tan.⁸

Serum samples were screened for anti-dsDNA by indirect immunofluorescence on *Criethidia luciliae*⁹; positive sera were tested in a modified Farr assay.¹⁰ Values above 10 U/ml (using 50 µl serum corresponding with more than 15% binding of 100 ng ³H labelled circular bacteriophage (PM₂) DNA) are positive. Selected sera were tested in the polyethylene glycol assay.⁹

Antibodies to cardiolipin (ACA) and tetanus toxoid were determined with ELISA techniques.^{11 12}

Concentrations of the complement components C3 and C4 were measured by laser nephelometry (Hyland, Costa Mesa, California) and of C1q by radial immunodiffusion.

Results

In patient 1 the anti-P value increased from 12 U (two weeks after splenectomy) to 23 U over a period of seven months. Simultaneously, anti-dsDNA increased from 11 to 470 U/ml. Five weeks later, at the time of psychosis, antibodies to P proteins were absent, and anti-dsDNA 1100 U/ml. Since then, antibodies to P proteins have remained undetectable for at least four years. This period included a second exacerbation with psychosis (unrelated to infection, metabolic disturbances, or drugs), which was again preceded by a rise in anti-dsDNA from negative (week 197) to 644 U/ml (week 244). Except for samples drawn between weeks 197 and 244, which were low positive (<80 U) for ACA-IgG, all samples were negative for both ACA-IgG and ACA-IgM. The titre of antibodies to tetanus toxoid showed only minor unrelated fluctuations. Concentrations of C3 and C4 were low in all samples. From week 187 onwards C1q concentrations being low before, reached normal.

When patient 2 presented with psychosis in October 1987 (week 5) the anti-P level was 134 U. During treatment with prednisone there was a decrease (102 U; week 8), followed over the next 15 weeks by an increase to 632 U. Five weeks later, at the time of psychosis, the anti-P level was 274 U. During treatment anti-P levels reached values just above 40 U for at least 32

Figure 1 Results with anti-double-stranded DNA (anti-dsDNA) assays (Crithidia luciliae, polyethylene glycol (PEG) assay, and Farr assay), levels of antibodies to ribosomal P proteins (anti-P), antibodies to cardiolipin (ACA), antibodies to tetanus (anti-tet), and percentages of complement components C1q, C4, and C3 in case 1 (left panel) from January 1983 to December 1988, and in case 2 (right panel) from September 1987 to December 1988. For case 2 levels of creatinine kinase (CPK) are also given. The normal ranges for the complement components are indicated by vertical lines. S, Ps, and aza indicate splenectomy, psychosis, and azathioprine respectively. Note the logarithmic scale for anti-dsDNA levels measured by the Farr assay in case 1.

Figure 2 Immunoblot reactivity of a negative control serum (lane 1), a reference anti-P serum (lane 2), sera from patient 1 taken at weeks 66, 216, and 242 (lanes 3, 4, and 5 respectively), and sera from patient 2 taken at week 30 (lane 6) and week 52 (lane 7).

weeks. All samples were negative for anti-dsDNA in all three assays used. Except for three samples drawn shortly before the second exacerbation (weeks 23 to 28), which were low positive for ACA-IgG, all samples were negative for ACA. There was only minor fluctuation in the level of antibodies to tetanus toxoid. The concentration of C1q was normal in all samples. Concentrations of C4 and C3 were low in all samples taken before the second exacerbation but normalised during treatment.

Discussion

This study was prompted by the observation by Bonfa *et al* in two patients with SLE that anti-P levels increase before the onset of psychosis.⁴ Both our patients had serum samples positive for anti-P with both immunoblotting (fig 2) and ELISA (figs 1 and 3) techniques. Interestingly, the only detectable autoantibody in case 2 was anti-P. The sisters described in this study were discordant for antinuclear antibodies, anti-dsDNA, the concentrations of complement components over time, and clinical manifestations like nephritis and myositis. This finding is not surprising as a large study on familial lupus has shown that clinical and laboratory disease manifestations in pairs of siblings with SLE differ as often as those of control pairs.¹³ We found significant shifts in anti-P levels during two of three periods antedating psychosis. Because of the high number of samples we could restrict the most relevant period to 15 weeks. Within this period there was an increase followed by a spontaneous fall in anti-P levels

Figure 3 Anti-P levels in a 15 week period before and after onset of psychosis in patients 1 and 2. First and second episodes of patient 1 are indicated by 1-I and 1-II and for patient 2 by 2-I and 2-II. Week 0 corresponds with onset of psychosis. Note the logarithmic scale on the vertical axis.

before the first psychotic episode in case 1 and before the second episode in case 2 (figs 1 and 3). A similar pattern has been described previously for anti-dsDNA levels during exacerbations of lupus nephritis.¹⁴ It is tempting to interpret this pattern as an indication for complexation of antibodies to specific antigens and thus suggestive of a pathogenetic role for autoantibodies. The apparent autonomy of anti-P formation, which is manifested by the independence of anti-P levels from the level of other autoantibodies (anti-dsDNA, ACA) or polyclonal activation (antibodies to tetanus toxoid), supports this suggestion. The absence of anti-P before, during, and after the second episode of psychosis in case 1, however, argues against a causal relation between antibodies to P proteins and psychosis, and reduces the significance of the anti-P assay as a prognostic tool. The latter the more so as both episodes of psychosis in case 1 were clinically identical and no other cause than lupus could be found.

In conclusion, our data indicate that the levels of antibodies to P proteins in single samples are not informative and that frequent measurement of anti-P levels in order to find an increase followed by a spontaneous fall may

have predictive value for psychosis in patients with SLE. Clearly, more patients should be evaluated to determine whether the discordancy between anti-P and psychosis we noted in one out of three prepsychotic periods forms a rare exception or a regularly occurring phenomenon.

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