The delayed appearance of an antinuclear factor and the diagnosis of systemic lupus erythematosus in glomerulonephritis

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Summary

In 11 patients who presented with apparently idiopathic glomerular disease the antinuclear factor (ANF) was absent initially but was eventually detected during observation extending over 6 years. In 4 patients a diagnosis of systemic lupus erythematosus (SLE) has now been made and the disease treated. Of the remaining 7, 2 had conditions known to be associated with a positive ANF, and in 4, drug therapy induced the ANF. Clinical features, complement studies and measurement of anti-DNA antibody were of value in distinguishing those patients with SLE from the others.

Introduction

Prompt treatment may dramatically affect the outcome of lupus nephritis (Cathcart *et al.*, 1976; Levinsky, Cameron and Soothill, 1977; Dosa *et al.*, 1978). It is therefore crucial to distinguish it from idiopathic glomerulonephritis as early as possible but this may be difficult especially in the absence of extrarenal manifestations of systemic lupus erythematosus (SLE), since renal biopsy appearances are not diagnostic (Simenoff and Merrill, 1964). The detection of circulating antinuclear factor (ANF) is widely used as a screening test for SLE and while false positive results are common (Svec and Veit, 1967; Bartholomew, 1974; Burnham, 1972; Alarcon-Segovia, 1976), the test is so sensitive that some authorities suggest that a single negative ANF excludes the diagnosis (Hughes, 1974; Friou, 1972).

Eleven patients were seen who developed glomerulonephritis and in whom the ANF was

initially negative but subsequently became positive. In 4, a diagnosis of SLE has now been made.

The patients

Details of the 11 patients are given in Table 1. In the first 4 patients SLE has been diagnosed.

Case 1

A 19-year-old Chinese girl developed a nephrotic syndrome and acute renal failure in 1967. The LE cell test was negative. Her renal function improved with steroids and azathioprine but proteinuria persisted. In February 1972 the ANF was negative. The steroids were tailed off and stopped in November 1972 and, in May 1973, when she was asymptomatic, the ANF became positive. The total haemolytic complement (CH_{50}) and C3 were low. Anti-DNA binding was 22% (normal range 0-20%).

Case 2

A 16-year-old girl developed a nephrotic syndrome in June 1966. The ANF was negative. The proteinuria resolved with steroid therapy. The ANF became positive when the proteinuria recurred in October 1966. During a third relapse in August 1973 the anti-DNA antibody was elevated and CH_{50} low.

Case 3

A 49-year-old woman developed a nephrotic syndrome and microscopic haematuria in 1975. The ANF was negative and serum complement normal. Four months later when she was well the ANF

TABLE 1. Résumé of clinical and laboratory data on 11 patients: Cases 1-4: SLE diagnosed. Cases 5-6: Other conditions associated with a positive ANF. Cases 7-10: Positive ANF associated with drug therapy. Case 11: Unclassified

	Sex and age (in years) at onset of renal lesion	Interval: from onset to positive ANF	Pattern of ANF	Grade of ANF*	A.R.A. criteria fulfilled		Complement		Anti-DNA Antibody		Response of renal lesion to steroids
					At onset of illness	Subsequently	When ANF first positive	Subsequent	When ANF first positive	Subsequent	
1	F (19)	6 years	Nonspecific	+++	2	4	↓	↓	↑	↑↑	+
2	F (16)	5 months	Nonspecific	+++	2	4					+
3	F (49)	8 months	Nonspecific	+	2	4	N	N	N	↑↑	+
4	M (19)	3 months	Speckled	+++	1	4	↓	↓	↑	↑	+
5	M (38)	17 years	Speckled	+	1	2	N	N	N	N	†
6	M (59)	4 years	Nonspecific	++	2	2	N	↓	N	N	o
7	F (51)	3 years	Nonspecific	++	1	3	↓	↓	↑	↑	†
8	M (27)	4.5 years	Nucleolar/ Homogeneous	++	2	3	↓	↓			†
9	F (39)	3 years	Nonspecific	+++	2	2		N			o
10	M (35)	3 years	Speckled	+	2	2		N		N	†
11	F (23)	6.5 years	Nonspecific	+	2	2	↓	N	N	↑	+

*Visual assessment; N, normal; o, nil; †, steroids not given; A.R.A., American Rheumatism Association.

became positive but anti-DNA antibody and complement were normal. The anti-DNA antibody was subsequently elevated in June 1976 when she developed leucopenia and thrombocytopenia and a falling creatinine clearance.

Case 4

A 20-year-old Indian man developed bilateral loin pain, a deep vein thrombosis and a nephrotic syndrome in December 1975. The ANF was negative. A venogram showed a partial right renal vein occlusion. In February 1976 when his symptoms had resolved but proteinuria persisted, the ANF became positive and the anti-DNA antibody was raised. CH₅₀ and C4 were low. The Coombs' test was positive, he had cold agglutinins and thrombocytopenia. There were granular casts in the urine. Renograms and scintiscanning demonstrated the resolution of the renal vein thrombosis following steroids and anticoagulants, but proteinuria persisted, the ANF remained positive and anti-DNA antibody, C1q and C3 abnormal.

Case 5

The ANF in this case was associated with another connective tissue disease.

A 54-year-old man with a 16-year history of rheumatoid arthritis treated with steroids was investigated in 1974 for proteinuria and haematuria. The ANF was negative at this time but positive one

year later. He was taking prednisolone 10 mg/day during this period.

Case 6

A paraprotein may have acted as an ANF in this patient.

A 59-year-old man developed a nephrotic syndrome in 1969. The ANF was negative. He suffered a retinal vein thrombosis in 1970 and a myocardial infarction in 1972. An IgG paraprotein was detected in his serum 9 months after the ANF first became positive in 1973. There was no evidence of malignant myeloma but there were K light chains in his urine. The anti-DNA antibody was elevated but serum complement was normal. Following cytotoxic therapy the proteinuria almost disappeared, the creatinine clearance rose, the ANF and paraprotein could no longer be detected and the anti-DNA antibody level returned to normal.

Drug therapy is implicated in the development of a positive ANF in 4 patients. In one, penicillamine induced a lupus syndrome.

Case 7

A 38-year-old woman developed polyarthritis in 1972. Rheumatoid factor and ANF were negative. She started penicillamine therapy in 1974 and 12 months later developed a nephrotic syndrome. The ANF was positive, anti-DNA antibody elevated and C1q low. The penicillamine was stopped and 9 months later the proteinuria had resolved. The ANF remains positive with a grossly elevated anti-DNA antibody.

Cases 8, 9, 10

In these 3 patients the ANF became positive after methyl dopa therapy. The Coombs' test was positive in Case 8. The development of a positive ANF was unrelated to the clinical condition of the patients and with the exception of an isolated low C3 in Case 8, was not accompanied by abnormality of anti-DNA antibody or serum complement. The ANF remains positive in Case 10 4.5 years after the methyl dopa was discontinued.

Case 11

The final patient apparently has idiopathic glomerulonephritis which has followed a relapsing course. The initial episode in 1968 responded to steroid therapy. CH₅₀ was low in 1973 and in 1974 the ANF, which had previously been negative, became positive while her disease was in remission and she was on no treatment. The anti-DNA antibody was normal. Complement levels have subsequently been normal and the anti-DNA antibody had been elevated on only one occasion and no other features of SLE have emerged.

Renal biopsies

Renal biopsies have been performed on all 11 patients, repeatedly in 7, and a wide range of glomerular abnormalities found (Table 2). Initial biopsies from 3 patients (Cases 2, 6 and 7) showed no significant changes at the light microscope level but in all 3, either initially or subsequently, there was immunofluorescent and ultrastructural evidence of glomerular immune complex deposition, and one (Case 6) also showed typical Bence-Jones tubular casts ultrastructurally. Seven patients (Cases 1, 3, 5, 8, 9, 10 and 11) showed a variable diffuse mesangial proliferative glomerulonephritis in their first biopsies. Of these, 5 (Cases 1, 3, 8, 9 and 10) had repeat biopsies, all of which showed progression towards glomerulosclerosis and in 3 (Cases 1, 3 and 8) this had advanced to an 'end-stage kidney'. The remaining patient (Case 4) showed the classical appearances of established membranous glomerulopathy.

Discussion

A diagnosis of SLE has been made in 4 of 11 patients in whom the ANF was initially negative. This experience contradicts the view expressed that a single negative ANF is strong evidence against the diagnosis and the test need only be repeated when laboratory error or other cause of a negative ANF, e.g. immunosuppressive therapy, is suspected (Friou, 1972). A delay in the appearance of an ANF was due to steroids in only one of these patients (Case 1). Of the other 3, it became positive as the clinical illness remitted in 2 and only during a second exacerbation in one.

Case 5 has another connective tissue disease, a frequent cause of a positive ANF (Bartholomew, 1974). In this patient, the delayed appearance of the ANF may have been due to long-term steroid therapy. IgG paraprotein with specific anti-DNA antibody activity has been reported (Cryer and Kissane, 1976) and it is probable that the paraprotein detected in Case 6 was acting as an antinuclear factor. Penicillamine and methyl dopa are known to cause lupus syndromes (Elsas *et al.*, 1971; Feltkamp, Dourhout-Mees and Nieuwenhuis, 1970) and these drugs appear to be responsible for the late appearance of an ANF in 4 of the patients (Cases 7, 8, 9 and 10). The immunochemical disturbance may persist after withdrawal of the drug, as in Cases 7 and 10 (Alarcon-Segovia *et al.*, 1967). The pattern of staining of the ANF and titre of antibody are said to be helpful in distinguishing lupus and non-lupus patients (Husain *et al.*, 1974; Epstein, 1977); this was not the case in the present patients although the antibody was not titrated.

The renal biopsies of patients with renal manifestations of SLE show a wide variety of changes on light microscopy (Cameron *et al.*, 1977; Rothfield, 1977) but none of the present patients showed the abnormalities said to be particularly associated with the disease, haematoxyphil bodies, segmental fibrinoid necrosis, hyaline thrombi and 'wire loops' (Pollak, Pirani and Schwartz, 1964; Dujovne *et al.*, 1972; Pollak and Pirani, 1974; Meadows, 1978). The 4 patients in whom SLE was diagnosed illustrate the range of appearances possible in the disease. Renal immunofluorescence is reported to be invariably positive in SLE. IgG is the usual localizing immunoglobulin although both IgM and IgA are often found (Koffler *et al.*, 1969; Pollak and Pirani, 1974; Cavallo, Cameron and Lapenas, 1977; Cameron *et al.*, 1977) and no specific patterns of immunoglobulin deposition may be considered specific for the condition. Electron microscopy may also reveal a wide variety of changes and electron-dense deposits may be seen in any location (Comerford and Cohen, 1967; Dujovne *et al.*, 1972; Pollak and Pirani, 1974; Baldwin *et al.*, 1977). While subepithelial, intramembranous, subendothelial and mesangial electron-dense deposits were all present together in biopsies from 2 of the lupus patients (Cases 1 and 3), they were also present in a non-lupus patient (Case 9). The changes seen on electron microscopy in Case 8 were attributable to hypertension, and the Bence-Jones tubular casts in Case 6 accord with the finding of a monoclonal immunoglobulin in the patient's serum. Detailed examination of renal biopsy material and the study of the evolution of the renal lesion on repeated biopsies has not helped distinguish between the lupus and non-lupus patients.

TABLE 2. Summaries of pathological appearances

Case number	Biopsy number	Date	Light microscopy	Immunofluorescence	Electron microscopy
1	1st	June 1973	MPGN→sclerosis	+ IgA, IgM and C3	As LM. Mesangial, sub-endothelial and occasional intramembranous EDD
	2nd	Sept. 1976	End-stage kidney	+ + + IgM, Clq and C4; + + IgG and C3	As LM. Mesangial, sub-endothelial, intramembranous and subepithelial EDD
2	1st	July 1966	NLMC	Not done	Not done
	2nd	Sept. 1973	NLMC	All negative	No glomeruli
	3rd	Feb. 1977	NLMC	+ + IgM and C4; + IgA, IgG, C3 and C1q	Occasional mesangial and paramesangial EDD
3	1st	Oct. 1975	Variable MPGN	No glomeruli	As LM. Focal sub-endothelial EDD. Occasional platelet aggregates.
	2nd	March 1976	Variable MPGN→sclerosis	Focal and segmental + IgG and C3	No glomeruli As LM
	3rd	Dec. 1978	End-stage kidney	+ IgM, C3 and C4	As LM
4	1st	Sept. 1976	Membranous	+ + + IgG, Clq, C4 and Properdin; + + IgA	Predominantly sub-epithelial and occasional intramembranous EDD
5	1st	March 1975	MPGN	+ IgA	As LM. Occasional paramesangial EDD
6	1st	Jan. 1970	NLCM	Not done	Marginal increase in mesangium; no EDD identified
	2nd	Nov. 1973	Mild MPGN→sclerosis	Focal and segmental + IgG and IgM	As LM. Occasional glomerular collagen. Bence-Jones tubular casts
7	1st	Feb. 1976	NLMC	+ + IgG and IgM	Mild mesangial increase; occasional subepithelial EDD
8	1st	June 1970	Mild MPGN	Not done	As LM. Wrinkling and focal thickening of membranes; occasional intraluminal fibrin
	2nd	Dec. 1971	End-stage kidney	Not done	No glomeruli
	3rd	Oct. 1973	End-stage kidney	+ + IgM and C3	As first biopsy. No EDD seen
9	1st	Sept. 1969	MPGN	Not done	Not done
	2nd	Oct. 1973	Variable MPGN→sclerosis	+ + IgA, IgM and C3	As LM. Subendothelial, intramembranous and subepithelial EDD. Numerous intramembranous extensions of mesangial cell cytoplasm
10	1st	July 1971	MPGN	Segmental + + IgM and C3	As LM. Occasional mesangial and paramesangial EDD
	2nd	March 1974	MPGN→sclerosis	Focal and segmental + + C3 and + IgM	As first biopsy, but with increase in mesangial matrix
11	1st	Feb. 1973	Variable MPGN	+ IgM and C3	As LM. No definite EDD seen

MPGN = diffuse mesangial proliferative glomerulonephritis.

NLMC = no significant light microscope changes.

LM = Light microscopy.

EDD = electron dense deposits.

The ANF is fallible as a screening test for SLE. While false positives are frequent, more significantly, the test may initially be negative. Linstedt et al. (1977) have reported 7 patients with lupus nephritis in whom a persistently negative ANF was associated with positive anti-DNA antibodies. In common with others, the present authors have found anti-DNA antibody and serum complement levels valuable in making a diagnosis (Davis *et al.*, 1973; Cameron *et al.*, 1976). While isolated abnormal levels occurred in the non-lupus patients, the association of a positive ANF, high anti-DNA antibody, and low complement levels have usually occurred in those in whom lupus has been diagnosed. The late appearance of a positive ANF in the absence of other clinical or laboratory evidence of disease activity should suggest a cause other than SLE.

Cohen and Canosa (1972) have commented that lupus patients frequently fail to fulfill the American Rheumatism Association criteria for the diagnosis at first presentation and this has been the experience of the present authors. A single negative ANF must not be accepted as excluding SLE. Repeated estimations are required and, when accompanied by other immunological measurements, may make earlier diagnosis possible. As therapy for severe renal lupus improves, this becomes increasingly desirable.

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