Pathogenesis of membranous nephropathy in systemic lupus erythematosus: possible role of nonprecipitating DNA antibody

The biological importance of qualitatively different subpopulations of antibody in determining the inflammatory consequence of circulating immune complexes in animal models of chronic serum sickness and human systemic lupus erythematosus (SLE) is well recognised. The precise role of antibody precipitability, however, is unclear since numerous investigations have shown seemingly conflicting results. We undertook a study to evaluate the amount and character of antibody to native DNA in untreated patients with SLE alone or SLE with membranous nephropathy or diffuse proliferative glomerulonephritis.

Patients, methods, and results

Native DNA was purified by ribonuclease treatment and deproteinisation with choloroform-isoamyl alcohol and then labelled *in vitro* with radioactive ^{125}I.[1] Before the assay was performed ^{125}I-DNA antigen stock solution was appropriately diluted and aspirated through a nitrocellulose filter to remove denatured or single-stranded determinants. All lupus sera were heat deactivated at 56 C for 30 minutes and assayed in triplicate. Total DNA antibody was measured by a modification of the Farr ammonium sulphate technique.[2] Spontaneously precipitating antibody to native DNA was determined essentially by the same technique except that the incubation period of test serum with ligand was prolonged to one week and immune complexes were harvested by centrifugation without the addition of ammonium sulphate. Subtracting the results from those obtained with the identical test serum in the ammonium sulphate assay yielded a measure of non-precipitating antibody to DNA.

A sensitive independent qualitative measure of precipitating antibody to DNA was obtained by a discontinuous modification of a described counter-immunoelectrophoretic technique, using multiple concentrations of DNA (20, 5, and 1·25 mg/l) and simultaneously run deoxyribonuclease-treated controls to ensure reaction specificity.[3]

Percutaneous needle biopsy specimens of the kidney and serum samples were obtained from patients with SLE who had had neither corticosteroids nor immunosuppressants. Renal biopsy specimens were analysed by electron, fluorescent, and light microscopy. At least two whole glomeruli were examined by electron microscopy in each specimen. We used strict criteria for diagnosing membranous nephropathy: there had to be (*a*) diffuse capillary wall thickening with no or only minimal local increase in mesangial matrix and no proliferation of mesangial or endothelial cells on light microscopy; (*b*) characteristic interrupted, granular, monotonous membrane-oriented immunoglobulins and complement components on immunofluorescent microscopy; and (*c*) epimembranous and intramembranous deposits plus very occasional mesangial dense deposits with the complete absence of subendothelial deposits on electron microscopy. Patients were selected for study if they could provide enough pretreatment serum and a "classical" biopsy specimen.

The percentage DNA binding reported for the ammonium sulphate and spontaneous precipitation assays were those obtained with a 1/4 dilution of test serum in borate buffer and represented, respectively, measures of total and precipitating antibody to native DNA (see table).

Comment

In SLE and animal models of chronic serum sickness conflicting evidence exists about the role of antibody precipitability in the pathogenesis of renal disease. Using two-stage electroimmunodiffusion to examine SLE sera, others showed a high degree of correlation between renal involvement and the absence of precipitating antibody to DNA, 64-75% of patients with lupus nephritis having no demonstrable electroimmunoprecipitins.[4] The measurement of spontaneously precipitating and total antibody to radiolabelled DNA, however, has led to the conflicting observation that all patients with SLE and clinically determined mild or severe nephritis had precipitating antibody to DNA.[5]

The results of our investigation show that patients with diffuse proliferative glomerulonephritis and those with active SLE without renal disease have a vigorous native DNA antibody response that is significantly precipitating in character. In contrast, patients with membranous nephropathy are unique in synthesising distinctively less total native DNA antibody that is almost exclusively non-precipitating. The formation of complexes with native DNA and non-precipitating DNA antibody either in the circulation or in situ may be essential to the development of membranous lupus nephropathy.

Native DNA antibody profile of patients with diffuse proliferative nephritis, active SLE without renal disease, and membranous nephropathy

Case No	Counter-immuno-electro-phoresis*	Ammonium sulphate (%)†	Spontaneously precipitating (%)†	Ratio‡	Total antigen binding capacity (µg/l)
		Diffuse proliferative nephritis (A)			
1	+	99·5	58·6	0·59	>2537
2	+	101·4	97·2	0·96	6592
3	+	82·4	60·5	0·73	25395
4	+	101·5	35·3	0·35	26624
Mean		96·2	62·9	0·66	
		Active lupus without renal disease (B)			
5	+	91·4	78·6	0·86	13107
6	+	25·7	5·8	0·23	>3174
7	+	73·7	56·9	0·77	>2355
8	+	88·9	68·3	0·77	6592
Mean		69·9	52·4	0·66	
		Membranous nephropathy (C)			
9	−	22·5	0	0	256
10	−	36·6	1·2	0·03	243
11	−	6·2	0·6	0·10	49
12	−	31·9	1·9	0·06	627
13	trace +	35·9	0	0	262
Mean		26·6	<1	0·04	

Analysis of variance for differences between the means of the three groups:
C *v* A $P < 0{\cdot}001$; $P < 0{\cdot}001$.
C *v* B $P < 0{\cdot}01$; $P < 0{\cdot}005$.
A *v* B NS; NS.

* + = Precipitin reaction of neat test serum with 20, 5, or 1·25 mg DNA/l.
† Ammonium sulphate and spontaneously precipitating results expressed as percentage of ^{125}I-DNA bound by antibody.
‡ Ratio = Spontaneously precipitating : ammonium sulphate.
Case 8 did not undergo renal biopsy.

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