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Relationships between antibodies to native DNA and glomerulonephritis in systemic lupus erythematosus

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SUMMARY

Anti-native DNA antibodies have been evaluated in forty-six lupus patients' sera for antigen-binding capacity, affinity and precipitating activity. Diffuse proliferative glomerulonephritis was significantly correlated with the presence of high serum level of free anti-native DNA antibodies. Weak affinities were more often found in patients with than without glomerular changes but several patients had high-affinity anti-DNA antibodies and severe glomerulonephritis. No correlation was found between anti-DNA antibody-precipitating activity and renal lesions.

INTRODUCTION

It is generally accepted that anti-native DNA antibodies are found at high level almost exclusively in systemic lupus erythematosus (SLE) and thus constitute an excellent diagnostic test (Pincus *et al.*, 1969; Edmonds *et al.*, 1975; Hughes, 1971). A fairly good correlation is observed between serum levels and disease activity (Schur & Sandson, 1968). In addition, as shown by elution studies (Koffler, Schur & Kunkel, 1967) they play a major role in determining renal lesions by the mediation of DNA–anti-DNA complexes. However, detection of anti-DNA antibodies has been difficult because of lack of sensivity and specificity of the tests formerly used. The radioimmunological Farr test which uses immunoglobulin (Ig) precipitation by ammonium sulfate has facilitated their detection (Pincus *et al.*, 1969; Hughes, 1971). This technique is a sensitive detector of all antibodies capable of binding to DNA, independent of their biological properties (Farr, 1958). Results of the test are positive in 75–80% of lupus patients (Pincus *et al.*, 1969; Hughes, 1971). It remains to be determined, however, whether quantitative or qualitative differences in anti-DNA antibodies could account for differences in physico-chemical properties of immune complexes and thus possibly explain the degree of renal damage. We have approached this problem by comparing renal histological data with quantitative and qualitative analysis of anti-DNA antibodies (i.e. measurement of their affinity and precipitating nature).

MATERIAL AND METHODS

(1) *Patient selection.* Sera from forty-six patients, selected by the A.R.A. criteria (Cohen *et al.* 1971) were examined. Patients had clinical or biological renal manifestations assessed by clinical examination (blood pressure, oedema . . .) urinary tests (proteinuria, haematuria, leucocyturia) or renal function measurement (creatininaemia). None of the patients considered had yet received significant doses of steroids or immunosuppressive drugs. Transcutaneous renal biopsies were obtained in thirty-seven of these patients and detailed results are published elsewhere (Hill, Hinglais, Tron *et al.*, 1976). In order to simplify the analysis, patients were placed in five groups: (1) diffuse proliferative glomerulonephritis (GN), twenty patients; (2) segmentary and focal GN, three patients; (3) extra-membranous GN, three patients; (4) isolated granular deposits of immunoglobulins (visible in immunofluorescence) eleven patients; (5) patients not having had a renal biopsy because they did not present any urinary abnormality (neither proteinuria, haematuria, renal failure, nor hypertension) as opposed to all patients, from the four other groups, except one patient from the fourth group, nine patients.

All patients from group 1, 2 and 3 had urinary abnormalities, only ten out of twenty-six showed increased creatininaemia. Patients from group 4 never showed renal failure but ten out of eleven had proteinuria and/or haematuria.

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(2) *DNA-binding assay.* ^{14}C-labelled DNA, 0·30 ml (*E. coli*, Amersham) (10 μg/ml) was diluted in 1·05 ml borate buffer in 0·15 ml normal human serum to obtain 1·50 ml of solution at 2 μg/ml DNA; 0·1 ml of the test serum diluted in pure borate buffer was added to 0·1 ml of the DNA solution described above and incubated 30 min at 37°C and then 18 hr at 4 °C. At the end of this incubation period at 4 °C, Ig was precipitated by adding 1·8 ml of 55% ammonium sulfate. The tubes were incubated for 30 min at 4 °C and then centrifuged at 1000 *g* for 30 min at 4 °C. The supernatant was eliminated and the precipitate thus obtained was diluted in 1 ml HCl 0·1 N and transferred into counting flasks. Radioactivity was evaluated after addition of 10 ml Instagel and related to the total amount of radioactivity initially introduced.

(3) *Affinity evaluation.* Two different techniques were used.

(a) *Displacement technique.* ^{14}C-labelled DNA, 0·05 μg was incubated with 0·015 ml of the test serum, diluted so as to produce a binding of about 40%. Cold native *E. coli* DNA (Worthington) was then added to a concentration 200 times higher than that of hot DNA. Immunoglobulins were then precipitated by 50% ammonium sulphate after 1, 5, 20 and 60 min incubation. The Farr technique was then followed as described above. DNA displacement was evaluated in function of incubation time (Fig. 2).

(b) *Scatchard's technique.* Evaluation of the association constant (Soothill & Steward, 1971).

The percentage of bound hot DNA was determined at different test serum dilutions: 1/10, 1/40, 1/160, 1/640. The following equation (Soothill & Steward, 1971) was then applied:

$$\frac{1}{\mathrm{AgAb}} = \frac{1}{\mathrm{K}} \times \frac{1}{\mathrm{Ag}} \times \frac{1}{\mathrm{Abt}} + \frac{1}{\mathrm{Abt}},$$

where Ag is the free-antigen concentration, AgAb the bound-antigen concentration, Abt the number of sites per antibody molecule and K the equilibration constant. The curve 1/Ag Ab function of 1/Ag was established. Its slope is (1/K) (1/Abt) and its intersection with the abcissa line gives Abt. This method is not, in theory, directly applicable to lupus sera, which contain heterogeneous antibodies, and DNA which is a multivalent antigen. Association constants may be calculated by this technique exclusively with homogeneous antibodies directed against monovalent antigens but one should mention, however, that this technique has been used with reasonable accuracy by Steward & Petty (1972) for the evaluation of anti-BSA antibody affinity.

(4) *Counter-Immunoelectrophoresis* (CIE). CIE technique has been performed as described elsewhere (Schuller *et al.*, 1976). Native DNA was put in the presence of variable dilutions of test sera. The titre of anti-DNA antibodies was defined as the maximum serum dilution giving a visible precipitate.

RESULTS

DNA binding (Fig. 1)

Sera of the forty-six patients showed a binding between 17 and 95% for a serum dilution of 1/10. Results are given in Fig. 1. The twenty patients with diffuse GN and the three patients with segmentary and focal GN showed a binding higher than or equal to 50% in twenty-two out of twenty-three cases. Two out of three patients with membranous GN showed a binding lower than 50%. The third had a binding higher than 90% but showed necrotic lesions. The eleven patients with isolated Ig deposits and the nine patients without urinary symptoms (not biopsed) showed very heterogeneous binding values (between 22 and 86%) but, on an average, significantly lower than the other patients studied.

Evaluation of anti-DNA antibody affinity

(1) *Displacement technique.* Twenty-three patients were studied by the displacement technique: ten with diffuse GN, five with membranous or focal GN and eight with normal kidneys (with isolated Ig deposits). Three types of curves were obtained (Fig. 2). Flat curves were defined by a decrease in DNA binding induced by cold DNA of less than 25% after 60 min. They prove the absence of displacement and correlate with a high affinity (+ + +). Hyperbolic curves were associated with a decrease in DNA binding greater than 50% in less than 10 min. They indicate a low affinity (+). The intermediate curves were considered to correlate with a middle affinity (+ +). The results presented in Table 1 show that high affinity antibodies were more often found in patients with a normal kidney, but this was not absolute: several patients with kidney disease showed antibodies with high affinity. Because of the small number of cases studied, the differences observed are not statistically significant. No correlation was observed between antibody affinity and creatininaemia or proteinuria (Fig. 3).

(2) *Scatchard's technique* (Table 2). Twenty-eight patients were studied by the Scatchard's technique:

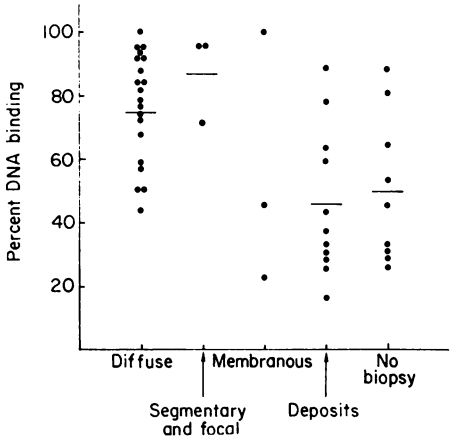

FIG. 1. Correlation between DNA binding and renal histology.

eleven with diffuse GN, five with other nephropathies, six with isolated Ig deposits and six with normal kidneys. Results are reported in Table 2 and Fig. 4.

The lowest affinities were observed in the group with diffuse GN, whereas subjects with normal kidneys had affinities higher than 4×10^{11}, but here again the differences are not significant. Results produced by both techniques were compared in fourteen patients. A good correlation was found in twelve of these cases (see Table 2).

Study of the precipitating nature of anti-DNA antibodies by counter-immunoelectrophoresis (CIE)

(a) In a first study we compared the percentage of binding obtained with the Farr technique and the titre of anti-DNA antibodies evaluated by CIE. No significant correlation was found. This conclusion was confirmed in a larger series (Fig. 5) (Schuller *et al.*, 1976).

(b) In a second study we related the titre of precipitating antibodies to the existence of a nephropathy. For this study, we have divided the patients in two groups, those with diffuse, segmentary and focal or membranous GN, and those having only isolated Ig deposits or a normal kidney in the absence of urinary abnormalities. No significant difference was found between titres of precipitating antibodies in the two groups, although the patients of the first group tended to show lower titres (Fig. 5).

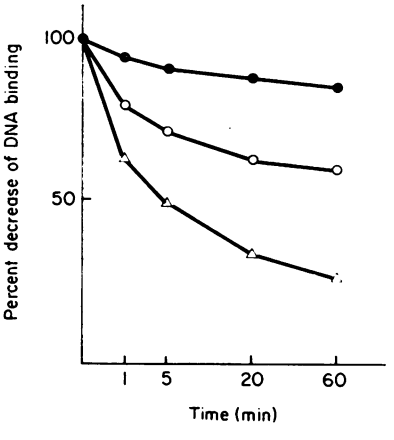

FIG. 2. Evaluation of anti-DNA antibody affinity by the displacement technique. (●) High affinity; (○) intermediate affinity; (△) low affinity.

TABLE 1. Correlation between affinity (evaluated by the displacement technique) and renal lesions

	Membrano-proliferative GN	Other GN	Normal kidney	
Low affinity	5	3	3	11
Intermediate affinity	2	1		3
High affinity	3	1	5	9
Total	10	5	8	23

TABLE 2. Correlation between the displacement technique and the Scatchard's analysis for the evaluation of anti-DNA antibody affinity

Patient	Histological pattern	Displacement technique	Scatchard's analysis	Correlation
GAU	D	+	4×10^{11}	Yes
BOU	D	++	$2{\cdot}3\times10^{12}$	Yes
BEU	NK	+	$4{\cdot}7\times10^{11}$	Yes
GAR	D	+	7×10^{11}	Yes
VEN	D	+	$1{\cdot}6\times10^{11}$	Yes
GOH	SF	+++	8×10^{12}	Yes
DOUGUE	NK	+++	$6{\cdot}3\times10^{12}$	Yes
LARD	D	++	3×10^{12}	Yes
THEB	D	+++	$4{\cdot}6\times10^{10}$	No
BLE	SF	+	$2{\cdot}5\times10^{11}$	Yes
HEN	D	++	$7{\cdot}5\times10^{11}$	Yes
DRIAN	D	++	$8{\cdot}5\times10^{11}$	Yes
DUBON	Deposits	+++	4×10^{11}	No
MAOU	D	+	$2{\cdot}6\times10^{11}$	Yes

Displacement technique: see the Materials and Methods section for +, ++ and +++ Scatchard's analysis definition. Data given are association constants. It should be realized, however, that in the experimental conditions used, functional rather than intrinsic affinities are measured. D = Diffuse glomerulonephritis: SF = segmentary and focal glomerulonephritis; NK = normal kidney; deposits = isolated Ig deposits.

DISCUSSION

Glomerulonephritis is a frequent and severe consequence of SLE. Factors explaining the occurrence of GN in a given patient are unknown. The subject however has elicited much interest since its elucidation could cast some light on the general problem of why certain immune complexes are nephrotoxic while others are not.

Our results indicate above all, that the existence of a renal lesion in SLE is very significantly correlated with the presence of high serum titres of free anti-DNA antibodies. Immunological correlations were essentially studied with histologic lesions since creatininaemia or proteinuria were found to provide much less consistent and meaningful data. In that respect, it is significant that all patients with diffuse GN in our study have a binding level higher than 50%; these results have been confirmed in a larger series (Tron & Bach, 1976). This data may be taken at first approximation in contradiction with the hypothesis that nephrotoxic immune complexes are in antigen excess (Dixon, 1968) if DNA is accepted as a relevant antigen in SLE pathogenesis, as suggested by elution studies (Koffler *et al.*, 1967; Winfield,

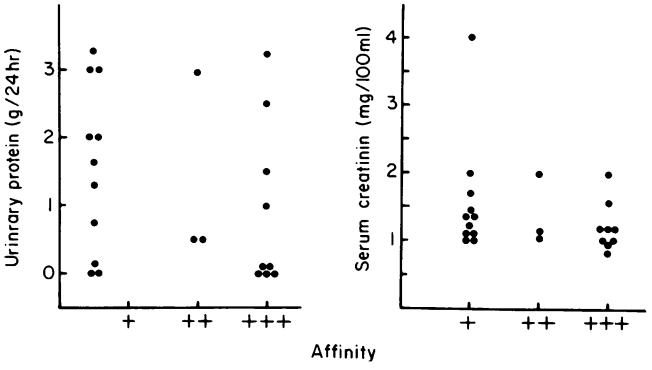

FIG. 3. Absence of correlation between affinity of serum anti-DNA antibodies (displacement technique) and proteinuria (left) or creatininaemia (right).

Koffler & Kunkel, 1975). One cannot, however, extrapolate from the existence of serum free antibody to the composition of complexes deposited in the glomeruli. The possibility that renal lesions depend not only on the quantity of anti-DNA antibodies but also on their physico-chemical properties has been stressed by several authors. Soothill & Steward (1971) in particular suggested that SLE renal lesions could be associated with a lower affinity of anti-DNA serum antibodies than forms without renal lesions. This hypothesis was suggested by their demonstration that mouse strains which are the most suceptible to GN development after viral infections generally produce low-affinity antibodies (Soothill & Steward, 1971). Our results are not incompatible with this hypothesis since weak affinities are more often found in patients with GN. Antibody affinity, however, does not seem to be an absolute factor since we have observed numerous cases with high-affinity antibodies and renal lesions, and cases without GN and anti-

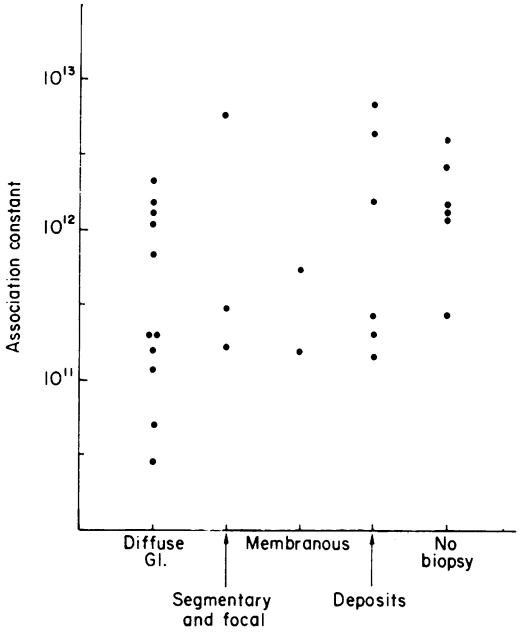

FIG. 4. Correlation between association constant (Scatchard's analysis) and renal histology.

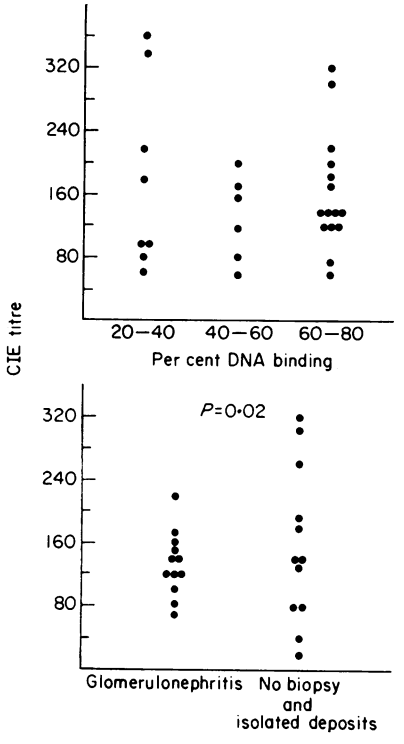

FIG. 5. Correlation between counter-immunoelectrophoresis and DNA binding (top) and renal histology (bottom).

DNA antibodies of low affinity. In addition we have not confirmed Steward's (Steward, 1976) data showing correlation between anti-DNA antibody affinity and proteinuria. However the validity and precision of the techniques used for the determination of affinity (even when considering functional affinity) are limited since the patients' sera contain heterogeneous mixtures of antibodies of various specificities and since DNA itself is a multivalent and not well-defined antigen. One should lastly mention that, at variance with our results and those of Steward (1976), Gershwin has reported the finding of generally high-affinity antibodies in cases with renal lesions (Gershwin & Steinberg, 1974).

Johnson *et al.* (1973) have reported that precipitating antibodies could more often be found in subjects without renal lesions than in patients with GN. Our results, like those of Gershwin & Steinberg (1974) are not in agreement with this hypothesis. Finally, the only factor which has been found to correlate consistently with the existence of renal lesions in SLE is the amount of anti-native DNA antibodies. Physicochemical properties and biological activities of antibodies, as tested by affinity measurement and precipitation, may play a role, but if so, present techniques do not allow its conclusive demonstration. Similarly, it should also be noted that no satisfactory correlation has been found between the class and sub-class of anti-nuclear antibodies and the existence of renal lesions, with the exception of Talal's recent report showing a greater severity of SLE in patients with IgG anti-DNA antibodies (Talal *et al.*, 1976). A direct study of the physico-chemical characteristics of complexes would seem to be necessary for obtaining more precise information on the possible differences between nephrotoxic and non-nephrotoxic complexes. It is also possible that the onset of renal lesions involves other factors, in particular at the level of complex deposition and phlogogenic action.

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