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Heterogeneity of immune complex-derived anti-DNA antibodies associated with lupus nephritis

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Heterogeneity of immune complex-derived anti-DNA antibodies associated with lupus nephritis. The mechanisms responsible for the tissue injuries associated with lupus nephritis have not yet been well explained. We have investigated the characteristics of anti-DNA antibodies in circulating immune complexes (CIC) and in the deposits of renal glomeruli in patients with active lupus nephritis. The CIC-derived antibodies expressed anti-DNA idiotypes (Id) designated as 0-81 Id and NE-1 Id, and bound mainly to single-stranded DNA but never to glomerular basement membrane (GBM) antigens. On the other hand, the immunoglobulins (Ig) eluted from renal glomeruli of lupus patients reacted not only with DNA but also with GBM, proteoglycan, and heparan sulfate. The binding of glomeruli-deposited Ig was markedly low when GBM antigens were used after treatment with heparitinase, suggesting that some anti-DNA antibodies may bind directly to GBM antigens associated with heparan sulfate, and form in situ IC in renal glomeruli. It was also revealed that the renal eluates obtained after passing through GBM antigen-coupled Sepharose lost the binding ability with GBM but still retained DNA-binding and 0-81 Id activity, showing the participation of circulating IC-derived anti-DNA antibodies in the glomerular deposits. Theoretically there may be two mechanisms in the pathogenesis of lupus nephritis through the deposition of circulating IC and through in situ formation of anti-DNA IC in renal glomeruli. The diversity of histological features in lupus kidneys may be attributed to the heterogeneity of the mechanisms.

Anti-DNA antibody activity is closely associated with the disease activity of systemic lupus erythematosus (SLE) and the autoantibodies have been assessed to play an important role in the pathogenesis of the disease, especially in the renal lesions. This was confirmed by evidence showing the deposition of anti-DNA antibodies and their idiotypes (Id) in the renal glomeruli [1–3]. Thus, SLE has been held to be typical of immune complex diseases. Several mechanisms have been proposed to account for the pathogenesis of the disease. One is the deposition of circulating anti-DNA immune complexes in the renal glomeruli or in the skin, resulting in tissue injuries through autoimmune or inflammatory processes. Definite evidence for the role of circulating anti-DNA IC, however, has been sparse [4, 5] because of the difficulty of exposing antigen-binding sites in the IC-forming antibodies [6, 7]. Another mechanism may be in situ immune complex formation of anti-DNA antibodies in the renal tissues. It has been suggested that the cationic anti-DNA antibodies interact with negatively-charged glomerular basement membrane (GBM) antigens in the renal glomeruli of lupus mice [8, 9]. Human anti-DNA antibodies also have the ability to bind with proteoglycan or heparan sulfate which are normal constituents of GBM [10]. Izui, Lambert and Miescher also indicated that DNA could bind to positively charged GBM components in mice and subsequently react with serum anti-DNA antibodies [11]. These findings, however, have been obtained from studies using monoclonal or serum anti-DNA autoantibodies, of which the role in renal injuries in vivo has not been evident. Thus, the exact mechanisms responsible for the disease have not been thoroughly elucidated [12, 13].

We recently identified specific clonotypes expressing anti-DNA Id (0-81 Id) in circulating IC of patients with active lupus nephritis [14]. Since the immunoglobulins eluted from renal glomeruli of lupus patients also included 0-81-Id positive antibodies, anti-DNA antibodies expressing 0-81-Id might be nephritogenic. We demonstrate here the binding specificity of the antibodies originating from circulating immune complexes and from their renal eluates.

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Methods

Samples

Plasma samples were obtained from 15 patients with active lupus nephritis and from 25 controls including other renal diseases. All SLE patients studied here fulfilled the 1982 criteria of the American Rheumatism Association (ARA) for the diagnosis of SLE. Disease activity was defined by clinical signs and symptoms (nephritis, arthritis, serositis, cerebral signs) according to the criteria of Budman, Lizzio and Reeves [15]. All of the patients with active lupus nephritis presented here showed abnormal urinalysis, a reduced total hemolytic complement level (CH_{50}) and active nephropathy as determined by renal biopsy [16, 17].

Monoclonal antibodies

Human monoclonal anti-DNA antibodies (0-81 and NE-1) were obtained from EBV-transformed B cell clones originated from SLE with active lupus nephritis [18]. 0-81 Antibody binds preferentially to single-stranded (ss)-homopolymer with pyrim-

Table 1. Specificity of monoclonal antibodies

mAb	Class	Specificity
0-81	human IgM	ssDNA
NE-1	human IgM	ss/dsDNA
7F4	human IgM	ss/dsDNA
D1E2	mouse IgG_1	idiotypes of 0-81
1F5	mouse IgG_1	idiotypes of NE-1

idine bases and NE-1 reacts not only with ssDNA but also with double-stranded (ds)DNA, dsRNA, zDNA and cardiolipin [17, 19]. 7F4 was from human-human hybridoma originated from a normal subject and reacted with ss/dsDNA, cardiolipin and vimetin. Two types of monoclonal anti-idiotypic antibody (D1E2 and 1F5) to human monoclonal anti-DNA antibodies (0-81 and NE-1) were obtained from mouse hybridoma using cells immunized with purified 0-81 or NE-1. D1E2 binds to idiotypes (Id) which have the antigen-binding site of 0-81 and inhibits the binding of 0-81 to ssDNA but does not react with NE-1. 1F5 specifically blocks the interaction between NE-1 and dsDNA but never DNA-binding of 0-81 or 7F4 (Table 1) [20].

Reagents

Calf thymus DNA was obtained from Worthington Biochemicals. The DNA was further purified into double-stranded (ds) and single-stranded (ss) DNA as described before [21]. Cardiolipin and histones were purchased from Sigma Chemical Company (St. Louis, Missouri, USA) and haparan sulfate from bovine kidney, hyaluronic acid from hog skin, chondroitin sulfate from whale cartilage, chondroitin sulfate B from shark cartilage, and chondroitin sulfate C from hog skin were obtained from Seikagaku Koygo Ltd. (Tokyo, Japan). Heparitinase from Favobacterium heparinum, trypsin and nuclease S_1 were also purchased from Seikagaku Kogyo, and DNAse I from Sigma Laboratory.

Preparation of glomerular basement membrane (GBM) antigens and proteoglycan

Glomeruli were isolated from fresh swine kidneys at 4°C by the sieving method [22]. The efficiency of the glomerular isolation was monitored by checking the suspension under a dissecting microscope. The glomeruli thus obtained were then sonicated for six minutes at 200 W and centrifuged at 3000 rpm in 1 M NaCl solution. After being dissolved in distilled water, the pellets were washed five times with centrifugation at 3000 rpm for 10 minutes. The GBM fractions were dissolved with 0.15 M Tris-HCl buffer, pH 2.3 at 1% final concentration, digested with collagenase and then with deoxyribonuclease at 37°C for two hours. The obtained supernatant was concentrated and dialyzed against distilled water for five days and finally lyophyilized.

The GBM fraction was chromatographed in a DEAE-cellulose column equilibrated with 50 mM sodium phosphate (pH 7.0) containing 7 M urea. The column was washed with the same buffer and linear gradient elution was carried out with NaCl in the buffer. After pooled and submitted to a second DEAE-cellulose chromatography, the major proteoglycan fraction was further filtrated on a sepharose CL-6B column equilibrated and eluted with 4 M guanidine HCl, 0.05 M sodium phosphate, pH 7.0. GBM was treated with heparitinase, at 37°C for 30 minutes, dialyzed against 0.15 M PBS, pH 7.2 for overnight and then used as antigens in some experiments.

Isolation of immune complexes from plasma

Circulating immune complexes (IC) were isolated from the plasma sample using anti-C_3 affinity columns. In order to prepare the column, highly purified human C_3 was first obtained [23, 24] and immunized to rabbits. The immunized sera were purified by affinity chromatography using human C_3-Sepharose CL-6B [25]. Then the obtained anti-C_3-rabbit IgG was coupled to Sepharose and used for the isolation of the circulating IC. The plasma sample was first precipitated by 3.5% polyethylenglycol at final concentrations. The precipitates were dissolved in PBS, pH 7.2 and passed through an affinity-purified anti-C_3 IgG-coupled Sepharose. After washing with PBS, the immune complexes bound to anti-C_3 Sepharose were eluted with 0.15 M glycine-HCl buffer, pH 2.3, neutralized with 3 M Tris-HCl, pH 10.0 and then stored at −70°C until use.

Elution of immunoglobulins from kidneys

The renal tissues were obtained from three autopsy cases with lupus nephritis (T.M, S.C, and N.T), from one case with chronic glomerulonephritis (N.Y.), and from three control subjects with esophageal cancer, gastric cancer or Behget's disease, who had not manifested the glomerular injuries. The renal histological findings in T.M, S.C, and N.T showed diffuse proliferative glomerulonephritis classified as IV by WHO criteria [7]. Each sample was minced into small pieces, suspended in 0.15 M PBS, pH 7.2, and then homogenized in a chilled Waring Blender. Then the renal glomeruli were obtained by sieving methods as described for the preparation of GBM antigen. The obtained glomeruli were washed repeatedly with PBS by centrifugation at 2000 × g until the optical density of the supernatant read less than 0.05 at 280 nm, then suspended in citrate buffer, pH 3.2 and incubated at 37°C for one hour with continuous shaking. After being centrifugated at 2000 × g 4°C for 15 minutes, the eluates were pooled and dialyzed against water for two hours, then against 0.02 M PBS, pH 7.2 for 24 hours. The eluates were concentrated approximately 25-fold with an Amicon filter and stored at −70°C until use.

Measurement of antibody activity

Each sample obtained from circulating IC, the glomerular eluates or monoclonal antibody was fractionated in 4.5 M urea-containing PBS (0.15 M) with gel tiltration using a Sephacryl S-300 column. The resulting fractions were dialyzed against 0.15 M PBS, pH 7.2 and then tested for the antibody activity.

Antibody activity was measured mainly with modified solid phase radioimmunoassay [19]. SsDNA (2 μg/ml), dsDNA (2 μg/ml), GBM, proteoglycan, heparan sulphate, hyaluronic acid or chondroitin sulphate A, B, C at 2.5 μg/ml were incubated in 96-well polystyrene microplates (Coster 2985) precoated with 100 μl of protamine (1 mg/ml) overnight at room temperature. Excess antigens were removed and 200 μl of 3% fetal bovine serum (FCS) were added to each well. After incubation for 60 minutes, followed by extensive washing with PBS containing 0.1% bovine serum albumin (BSA) and 0.05% Tween-20, serially diluted samples were allowed to react with antigen in the wells for 90 minutes at 37°C. After the wells were washed with

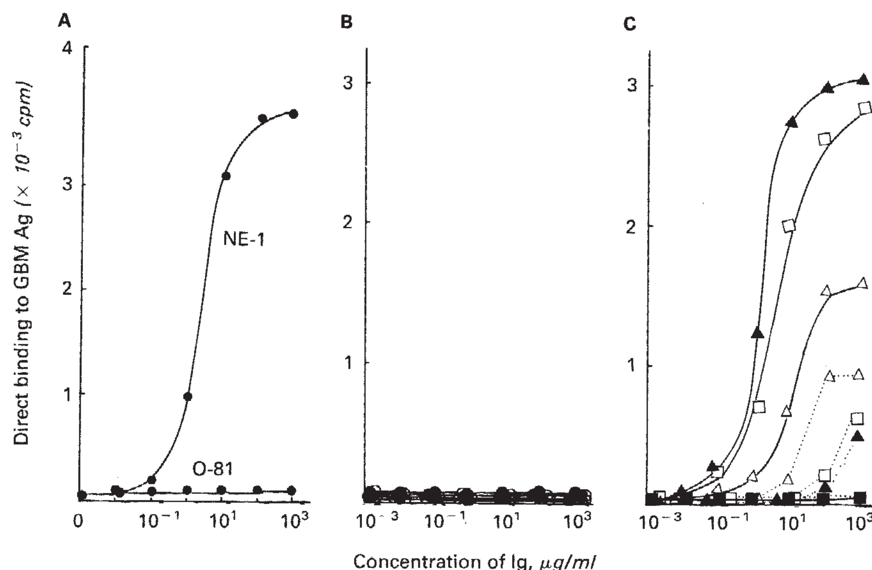

Fig. 1. *Direct binding to GBM antigen.* Each sample was tested for the binding ability to GBM antigen (-) or to heparitinase-treated GBM antigens (. . .) as described at **Methods**. **A.** Samples from human monoclonal antibodies NE-1 or 0-81. **B.** Samples from Immunoglobulins isolated from circulating immune complexes of SLE (●) or other kidney diseases (○). **C.** Samples from glomerular IgG of SLE case T.M (▲), S.C (□) and N.T (△) or of kidney disease case, N.Y (■).

washing buffer, affinity-purified anti-human γ $F(ab')_2$ or anti-human μ $F(ab')_2$ labeled with ^{125}I was added. The mixture was incubated for two hours at room temperature, and the radioactivity bound to each well was determined by a gamma counter.

Competitive inhibition RIA

A solid-phase competitive inhibition RIA was performed by an inhibition to DNA-binding of anti-DNA antibodies. Prior to inhibition, each sample was diluted to the middle point of the maximum binding to DNA. A mixture of the test sample and 50 μl of inhibitors at varying dilutions were incubated for 90 minutes in the well coated with ssDNA at 37°C. The results of inhibition were expressed as percentage inhibition of anti-DNA antibody-binding activity. Competitive inhibition assays were also performed by a modified Farr assay. A 10 μl sample of the purified antibodies from immune complexes was mixed with 100 μl (3×10^4 cpm) of ^{125}I-labeled *E. coli* plasmid DNA (1000 bp, Nippon DPC Corporation, Chiba, Japan) and 10 μl of unlabeled inhibitor at varying dilutions. After they had been incubated for 90 minutes at room temperature, an equal volume of saturated ammonium sulphate was added to the mixtures. The immune complexes were precipitated by centrifugation (2000 × g) at 4°C for 15 minutes and the radioactivity was determined on a gamma counter.

Absorption of antibody activity in the samples by a DNA or an GBM antibody column

DNA or GBM antigens were coupled with CNBr-activated Sepharose 4B as described before [26]. The samples were passed through a DNA- or a GBM-coupled Sepharose 4B column and then tested to determine anti-DNA antibody activity.

Results

Direct binding of the glomerular deposited and circulating immune complex-derived antibodies

We isolated the immune complexes (IC) from plasma and the eluates from the renal glomeruli. Each sample was further fractionated as IgG or IgM with gel filtration in 4.5 M urea-containing PBS (0.15 M), and then tested for binding ability to DNA or GBM antigens by using a solid phase RIA. The results including human monoclonal anti-DNA antibodies (0-81, NE-1) are shown in Figures 1 and 2. IgM activity was found neither in the preparations from circulating IC nor in those from the glomerular eluates (data not shown). All samples from SLE bound to DNA. However, the reactivity to swine GBM which showed cross-reactivity with human GBM [27] was variable. The glomerular IgG from three cases with lupus nephritis strongly reacted with GBM antigens as well as with DNA. These samples also showed binding to proteoglycan, heparan sulfate and chondroitin A. On the other hand, circulating IC-derived antibodies from 15 SLE cases bound to DNA, but failed to react with GBM-associated antigens. The binding of the glomerular IgG from SLE was markedly low to heparitinase-treated or to pepsin-treated GBM antigens (Fig. 1, Table 2). All samples of the renal eluates and of circulating IC from 20 control cases bound neither to DNA nor to GBM antigens.

Competitive inhibition RIA

These results prompted us to further study the binding specificity of IC-derived antibodies by a competitive inhibition RIA.

DNA-binding of circulating IC-derived antibodies from SLE was blocked with the coexistence of free DNA but not with that of GBM antigens. Similar results were obtained with 0-81 (data not shown). On the other hand, the renal eluates decreased DNA-binding ability when free DNA or GBM antigens were added (Fig. 3). In some experiments, the inhibitor at the same concentrations were first added for 90 minutes in wells coated with ssDNA and washed. Then the test samples were again tested for the inhibitory ability. The results were the same as those in Figure 3 (data not shown).

We next sought to determine whether the glomerular eluates still retained DNA-binding ability after an absorption of GBM antigen-reactive antibodies. The glomerular eluates preab-

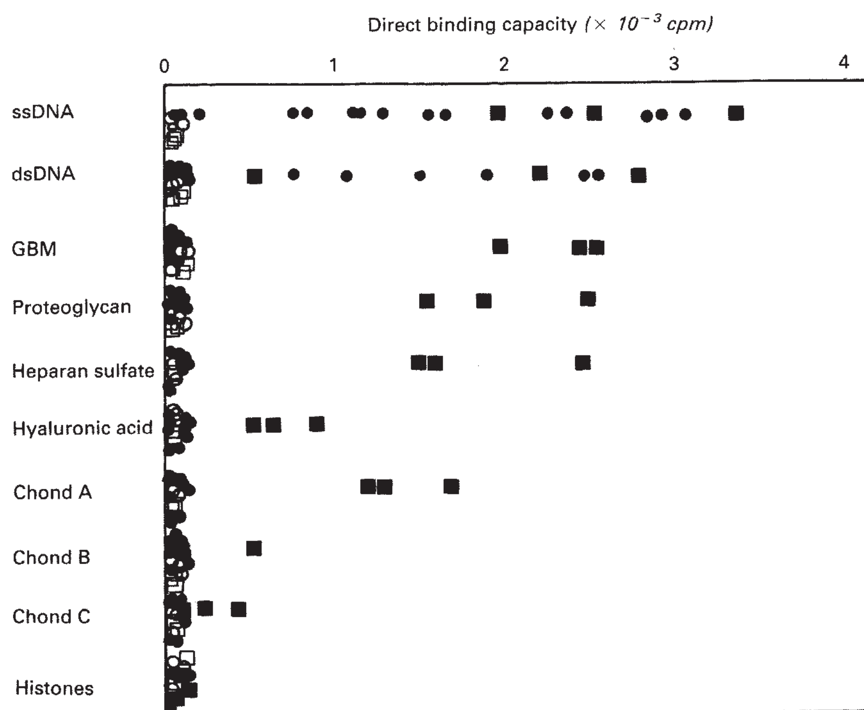

Fig. 2. *Direct binding to DNA or to GBM-associated antigens. Twenty μg/ml of Ig purified from circulating IC or from glomerular eluates were incubated with the indicated antigens precoated in the wells.* Then the direct binding ability was determined by a solid phase RIA. Symbols are: (●) samples from circulating IC of active lupus nephritis; (○) samples from circulating IC of other diseases; (■) samples from glomerular eluates of lupus nephritis; (□) samples from glomerular eluates of other diseases.

sorbed with an ssDNA or GBM-coupled column were tested for binding ability to DNA. The ssDNA column completely absorbed both DNA- and GBM-binding ability in the eluates (Table 3). The eluates after being preabsorbed with GBM also failed to bind with GBM but to some extent, retained the binding ability to ssDNA. DNA-binding capacity was inhibited when GBM-absorbed samples were tested with the coexistence of free ssDNA but not when tested with that of GBM antigens (data not shown).

Then we tested for the expression of anti-DNA idiotypes (Id) in IC-derived antibodies and in the glomerular eluates by a competitive inhibition RIA using human monoclonal anti-DNA antibodies (0-81, NE-1, and 7F4) or of murine monoclonal anti-Id antibodies (D1E2 and 1F5) as an inhibitor. As shown in Figure 4, DNA binding of the samples from the glomerular eluates of lupus patients and from circulating IC was strongly inhibited by 0-81 and D1E2 (anti-Id to 0-81) and to a lesser degree by NE-1 and 1F5 (anti-Id to NE-1). The pooled IgM, 7F4 and mouse IgG never blocked DNA-binding of the samples.

Discussion

This paper has described the properties of IC-derived anti-DNA antibodies. The reasons why we studied IC-derived antibodies in association with the pathogenesis of lupus nephritis are as follows. Circulating anti-DNA antibodies expressing 0-81- or NE-1-Id occurred in 73% of SLE patients with active lupus nephritis, but never in those lacking renal lesions [14]. Furthermore, 0-81 Id was proved to be deposited in lupus kidneys (Suzuki, manuscript submitted for publication) and 0-81- or NE-1 Id-positive anti-DNA antibodies could be eluted from the necropsy cases with active lupus nephritis [14]. These data indicate that circulating IC-derived anti-DNA antibodies expressing 0-81 Id may well be nephritogenic.

The samples from plasma or from the renal glomeruli might still be in a immune complex form, where antigen-binding sites of the antibodies were masked with IC-forming antigens, resulting in difficulty in analyzing the binding specificity of IC-derived antibodies. In order to overcome this problem, we separated the complex-derived antibodies from the bound antigens by using 6 M urea, followed by the gel filtration. The possibility remains that the renal eluates might include Ig originating in blood or in urine contaminants arising in the preparation. This is unlikely because we obtained renal glomeruli by a sieving method. The samples were more than 95% renal glomeruli (data not shown). Other evidence is that the Ig class of the renal eluates was exclusively IgG, whereas plasma or urine in the patients included both IgG and IgM. In addition, the spectrotypic profiles of Ig from the renal eluates were apparently different from those in plasma or urine [14]. These data suggest that the renal eluates presented here originated from IC in the glomeruli.

We should also discuss DNA contamination in GBM or proteoglycan antigens used in this experiments. Circulating IC-derived anti-DNA antibodies reacted with DNA, but failed to bind to GBM antigens, indicating that GBM or proteoglycan antigens at the concentration presented here did not contain immunologically active DNA. Another evidence is the failure of detecting DNA in GBM or proteoglycan using electrophoresis (data not shown), where 0.05 μg of DNA were detectable in the presence of ethidium bromide [28]. In addition, the glomerular eluates from SLE cases reacted with DNAse- or nuclease S_1 treated GBM as well as with the nontreated, but showed markedly decreased binding to heparitinase-treated GBM. The

Table 2. Effect of treatment with the enzymes on Ig-binding to GBM antigens

Treatment		Duration *min*	Binding to G1-Ig (T.M.)	G1-Ig (S.C.)	G1-Ig (H.K.)	CIC-NT (N.T.)
None		—	4712 ± 102	3954 ± 241	102 ± 11	123 ± 13
Heparitinase	0.1 U/ml	30	1031 ± 101	1599 ± 114		
	0.5 U/ml	30	830 ± 59	1054 ± 93	102 ± 10	119 ± 23
Trypsin	5 μg/ml	1	312 ± 25	213 ± 18		
	10 μg/ml	1	202 ± 34	159 ± 17	107 ± 9	115 ± 16
DNAse	100 U/ml	30	4599 ± 319	3990 ± 295	111 ± 14	
Nuclease S_1	100 U/ml	30	4752 ± 146	3888 ± 311	134 ± 8	

GBM antigen as treated with the indicated enzymes at 37°C for 1 or 30 min. The direct binding assay to the treated GBM was carried out as described in **Methods**, and total binding to the treated GBM-coated well minus the binding to BSA-coated well was described as the direct binding. Each assay was done in triplicate. Data are mean ± SE.

G1-Ig (T.M.) or G1-Ig (S.C) was glomerular Ig from SLE patient, T.M. or S.C., and G1-Ig (H.K.) from renal disease, HK. CIC-NT was circulating IC-derived Ig from SLE patient, N.T.

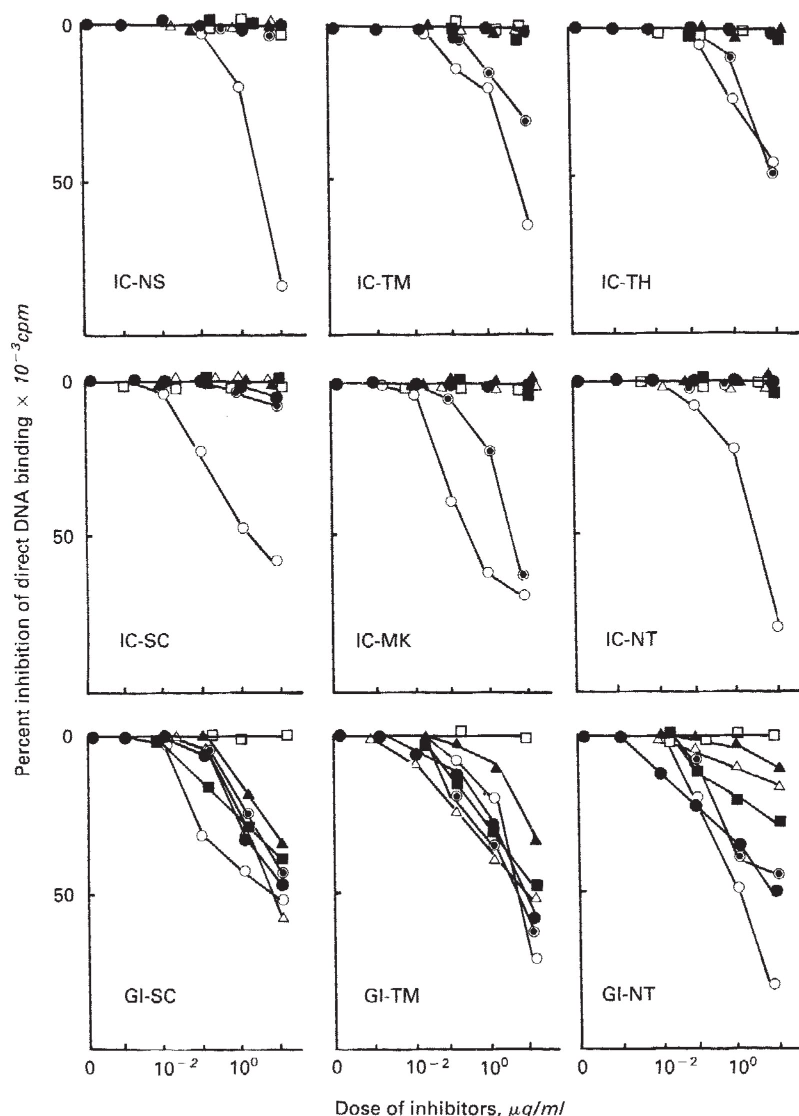

Fig. 3. *Competitive inhibition RIA using GBM-associated antigens.* Twenty μl/ml of samples from circulating IC (IC-NS, IC-TM, IC-TH, IC-SC, IC-MK, IC-NT) or from glomerular eluates (G1-SC, G1-TM, G1-NT) of SLE cases were incubated with a equal volume of each antigen at the indicated concentrations in wells precoated with ssDNA and then assayed for anti-DNA activity. The data were expressed as percent inhibition of the uninhibited DNA binding (1,500 to 5,700 cpm). Symbols for inhibitors are: (○) ssDNA; (◉) dsDNA; (●) GBM antigens; (△) chondroitin A; (▲) chondroitin B; (□) chondroitin C;(■) heparan sulphate.

above mentioned data indicate that glycosaminoglycan antigens may be responsible for the reaction of the glomeruli-deposited antibodies. Another problem is a possible involvement of histones in the GBM preparation. Histones have a very high affinity to the negatively charged GBM [29] and the possibility remains that GBM included histones in the preparation or that

Table 3. Absorption tests of glomerular Ig by an affinity column

Glomerular eluates from	Absorbent[a]	Direct binding[b] to				
		ssDNA	GBM	D1E2	1F5	mouse Ig
T.M.	BSA	5115 ± 112	4094 ± 153	3153 ± 54	2950 ± 212	211 ± 35
T.M.	ssDNA	311 ± 19	415 ± 111	294 ± 19	333 ± 39	192 ± 19
T.M.	GBM	2002 ± 101	302 ± 100	3095 ± 199	1219 ± 256	232 ± 18
T.M.	D1E2	2514 ± 112	3517 ± 299	199 ± 12	2755 ± 154	186 ± 19
T.M.	1F5	4080 ± 155	2958 ± 184	2998 ± 58	302 ± 50	180 ± 9
T.M.	mouse Ig	5508 ± 259	4155 ± 153	3004 ± 156	2859 ± 130	271 ± 20
C.S.	BSA	4008 ± 251	2859 ± 310	3513 ± 114	1912 ± 94	118 ± 19
C.S.	ssDNA	294 ± 18	639 ± 48	404 ± 14	724 ± 19	209 ± 13
C.S.	GBM	2043 ± 57	305 ± 100	3430 ± 195	897 ± 45	192 ± 8
C.S.	D1E2	2158 ± 154	3055 ± 195	293 ± 8	1738 ± 53	200 ± 11
C.S.	1F5	3078 ± 94	2440 ± 102	3315 ± 17	401 ± 18	198 ± 11
C.S.	mouse Ig	4199 ± 158	2958 ± 113	3499 ± 28	2000 ± 12	113 ± 18
N.Y.	BSA	214 ± 19	300 ± 7	295 ± 9	ND	ND
N.Y.	ssDNA	205 ± 15	271 ± 11	231 ± 14	ND	ND
N.Y.	GBM	211 ± 13	315 ± 29	219 ± 19	ND	ND
N.Y.	D1E2	198 ± 7	287 ± 18	242 ± 23	ND	ND

ND, not done

[a] Each sample was absorbed by an equal volume of the indicated antigen-coupled Sepharose and then tested for antigen-binding ability by ELISA

[b] cpm

[c] T.M. and C.S. were patients with active lupus nephritis and N.Y. was patient with chronic glomerulonephritis

part of the isolated anti-DNA antibodies are still complexed to nucleosomes [30]. The glomerular eluates, however, did not react with histones and we used IgG fraction obtained by Sephacryl S 300 gel filtration, indicating that the prepared glomerular IgG were not in a immune complex form.

Our data demonstrated that all samples from lupus glomeruli preferentially bound to GBM and its associated antigens. This findings is consistent with the previous observation that the antibodies eluated from MRL/lpr/lpr mice kidney reacted with heparan sulphate, the major glycosaminoglycan constituent of GBM [31]. This raises the possibility that anti-DNA antibodies deposited in the renal glomeruli might have originated from free anti-DNA antibodies in circulation, and that serum anti-DNA antibodies bound directly to structures within renal glomerular tissues which share the same epitopes recognized by the antibodies [32]. This is supported by in vivo studies, where exogeneously administered anti-DNA antibodies caused renal dysfunction in mice [33] or in the rat whose kidney was perfused [34]. Thus, certain antibodies which bind to GBM antigens may preferentially form in situ immune complexes at renal glomeruli in vivo.

Another important finding is that the glomerular eluates still retained anti-DNA activity even after a complete absorption of GBM-binding antibodies, indicating another mechanism for immune deposits in the renal lesions. DNA has the ability to bind to GBM in vitro through charge-charge interaction [11] and to localize within the glomeruli in vivo [35], so that circulating DNA could be planted in the glomeruli. Then the tissue-bound antigens might react with free anti-DNA antibodies in the circulation. Experiments suggesting this mechanism have recently been employed in vivo [36]. Our data may support this mechanism. We, however, prefer to speculate that circulating IC might be responsible for the immune deposition in renal tissues, as demonstrated by Dixon, Feldman and Vazquez [37]. This mechanism has been challenged by data showing unsuccessful trials for the presence of DNA antigens as well as antibodies in circulating IC [6, 7, 12, 13]. In our experiments, GBM-absorbed glomerular Ig reacted specifically with DNA and expressed anti-DNA antibody-associated 0-81 Id and NE-1 Id. We previously demonstrated that some populations of anti-DNA antibodies deposited in glomeruli were identical with those of circulating IC-forming anti-DNA antibodies expressing 0-81 or NE-1 Id when analyzed by an isoelectrofocusing study [14]. These data suggest that some types of antibodies deposited in renal glomeruli may originate from circulating IC. Thus, our data suggest that there might be at least two mechanisms for the immune deposits in renal glomeruli of lupus patients. One is associated with tissue deposition from circulating anti-DNA IC and the other with in situ IC formation at renal glomeruli by serum anti-DNA antibodies, which have a cationic charge [14].

It is very interesting to speculate that our data might be associated with histological or immunological features in renal glomeruli. Experimentally, the circulating IC brought about deposition on the mesangeal and the endothelial areas, whereas the subepithelial deposits, which can be observed in idiopathic membranoglomerulonephritis in humans, may arise by in situ IC formation associated with cationic charged antigens or antibodies [38, 39]. In lupus nephritis, immune deposits in subepithelial areas are often accompanied with mesangeal and subendothelial immune deposits, and their histological changes are diverse [16, 17], indicating that the complexity of renal lesions in SLE may be attributed to the heterogeneity of the mechanisms presented here. Thus, analysis of circulating nephritogenic antibodies in association with histological changes will provide us with important information concerning their clinical significance as well as their role in the pathogenesis of lupus nephritis.

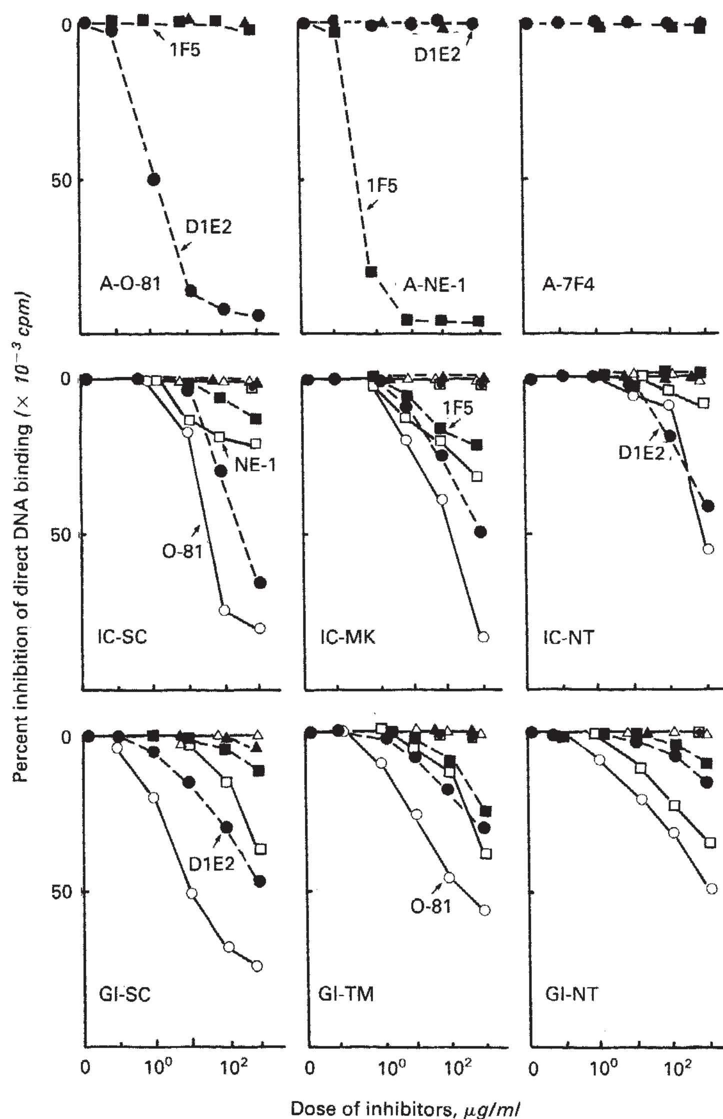

Fig. 4. *Competitive inhibition RIA using Id or anti-Id antibodies.* Twenty µl/ml of human monoclonal anti-DNA antibody (0-81, NE-1, 7F4), circulating IC-derived Ig (IC-SC, IC-MK, IC-NT) or glomerular eluates-derived Ig (G1-SC, G1-TM, G1-NT) were incubated with a equal volume of the indicated inhibitor in wells precoated with ssDNA and then assayed for anti-DNA activity. The uninhibited DNA-binding in each sample was 1,500 to 5,700 cpm. Symbols for inhibitors are: (○) 0-81; (□) NE-1; (▣) 7F4; (△) IgM; (●) D1E2; (■) 1F5; (▲) mouse Ig.

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