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Research Article

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Cross-reactivity of Human and Murine Anti-DNA Antibodies with Heparan Sulfate

The Major Glycosaminoglycan in Glomerular Basement Membranes

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Abstract

In 30 of 33 human systemic lupus erythematosus (SLE) sera and in 10 sera from MRL/l mice with spontaneous SLE, antibodies against heparan sulfate were detected. The anti-heparan sulfate titers showed a significant correlation with the anti-DNA antibody titers. By inhibition studies it was demonstrated that heparan sulfate could inhibit the binding of anti-DNA antibodies to DNA, whereas DNA could block the binding to heparan sulfate. That this reaction is due to crossreactivity of anti-DNA antibodies was further substantiated by the finding that two monoclonal anti-DNA antibodies also bound to heparan sulfate. Antibodies eluted from human and mouse kidneys with diffuse SLE glomerulonephritis showed a similar binding to DNA and heparan sulfate when these eluted antibodies were tested in vitro. Heparan sulfate is the major glycosaminoglycan constituent of the glomerular basement membrane. Our findings suggest that heparan sulfate might serve as a target antigen in vivo for crossreactive anti-DNA antibodies.

Introduction

Systemic lupus erythematosus (SLE)[1] is an autoimmune disease characterized by a great variety of apparently unrelated antibodies (1, 2), thought to be a consequence of polyclonal B cell activation. Recent observations mainly obtained with monoclonal anti-DNA antibodies, however, indicate that the B cell activation in SLE might be more restricted than assumed until now (3). It was shown that in addition to binding to DNA, human and murine monoclonal anti-DNA antibodies could also react with several crossreactive moieties like cardiolipin and other negatively charged phospholipids in micellar form (4, 5). The moieties recognized in these crossreactive molecules are thought to be the phosphodiester-linked phosphate groups, which show similarities to the sugar-phosphate backbone of DNA. However, these phosphodiester-linked phosphate groups are not the only crossreactive moieties recognized, since monoclonal anti-DNA antibodies can also bind to Raji-cells (6), B cells, T cells, erythrocytes (7), and the cytoskeletal protein vimentin (8). Moreover, we have recently reported that a monoclonal anti-DNA antibody can bind to hyaluronic acid and chondroitin sulfate (9). These molecules do not contain phosphodiester-linked phosphate groups but have other repeating negatively charged units. Apparently also these structures can serve as crossreactive moieties for anti-DNA antibodies. The observed crossreactivity is not a unique property of monoclonal anti-DNA antibodies, because also with polyclonal anti-DNA antibodies crossreactions were demonstrated with Raji-cells (10) and negatively charged molecules like cardiolipin (11), hyaluronic acid, and chondroitin sulfate (9). It seems that in SLE sera, a number of autoantibody specificities can be attributed to anti-DNA antibodies reacting with widely different molecules which all contain repeating negatively charged groups. We now present evidence that monoclonal and polyclonal anti-DNA antibodies can bind to heparan sulfate, the major glycosaminoglycan constituent of the glomerular basement membrane (12, 13).

Methods

Anti-dsDNA positive sera. Sera containing anti-DNA antibodies were obtained from SLE patients and from 24-wk-old MRL/Mp/lpr/lpr (MRL/l) mice (originally obtained from The Jackson Laboratory, Bar Harbor, Maine). These MRL/l mice develop spontaneously an autoimmune disease very similar to human SLE, characterized by formation of anti-DNA antibodies, and diffuse proliferative glomerulonephritis (14). After clotting for 3 h at room temperature and subsequent centrifugation for 5 min at 2,000 *g*, the sera were aliquoted and stored at −20°C. Test samples were thawed only once, and heat-inactivated for 30 min at 56°C before use. These sera were selected because they contained antibodies against dsDNA, as revealed by the dsDNA-Farr assay or *Crithidia luciliae* assay. Blood from healthy volunteers and BALB/c mice served as normal control sera. In addition sera from patients with biopsy-proven primary glomerulopathy (minimal lesions, focal glomerulosclerosis, membranous glomerulopathy and IgA nephropathy) and secondary glomerulopathy (Wegener's granulomatosis, Schönlein-Henoch purpura and periarteritis nodosa) were used for control experiments.

Monoclonal anti-DNA antibodies. Monoclonal anti-DNA antibodies were kindly provided by Drs. T. Westgeest and R. Smeenk (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and produced as described (9).

Anti-DNA antibody assays. Detection of anti-DNA activity was performed with the *Crithidia luciliae* and Farr assays as described by Aarden and co-workers (15, 16). Furthermore, an enzyme-linked immunosorbent assay (ELISA) was used. Polystyrene microtiter plates (Costar, Cambridge, MA) were precoated with 150 μl of 0.5 mg/ml protamine chloride (Kabi AB, Stockholm, Sweden) per well during a 2-h incubation and washed subsequently with phosphate-buffered saline (PBS). The DNA used for

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1. *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; GBM, glomerular basement membrane; SLE, systemic lupus erythematosus.

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the ELISA was commercially obtained calf thymus DNA (Sigma Chemical Co., St. Louis, MO). This DNA reacted in the ELISA only with a reference anti-DNA antiserum (ANA human reference serum 1, lot 82-0007; AF-CDC Reference Lab, Centers for Disease Control, Atlanta, GA) and not with other reference sera directed against non-DNA nuclear antigens (anti SS-B: ANA human reference serum 2, lot 82-0008; anti-RNP: ANA human reference serum 4, lot 82-0010; anti-Sm: ANA human reference serum 5, lot 82-0011). This DNA was coated in a concentration to ensure maximal binding (100 μl, 50 μg/ml, per well) overnight at room temperature. The plates were washed five times with PBS containing 0.05% vol/vol Tween 20 (wash buffer). To avoid nonspecific binding, the plates were subsequently coated for 2 h with 1% wt/vol gelatin dissolved in PBS and then washed five times with wash buffer. The sera were diluted in PBS, containing 1% wt/vol gelatin, in twofold dilution steps (final vol 100 μl). After 1 h of incubation the plates were washed five times with wash buffer. Then, 100 μl of peroxidase-conjugated rabbit anti-human light and heavy chain or rabbit anti-mouse Ig (Miles Laboratories, Elkhart, IN) (diluted 1:500 in PBS containing 1% wt/vol gelatin) were added and incubated for 1 h. After washing five times a second peroxidase-labeled antibody (goat anti-rabbit IgG, Miles) was added to intensify the signal. The plates were washed again five times and 100 μl freshly prepared substrate solution, 0.8 mg/ml 5'aminosalicylic acid dissolved in 50 mM phosphate buffer, pH 6.0, containing 0.8 μl/ml 30% vol/vol H_2O_2, was added to each well. After 30 min the signal was measured at 450 nm in a Titertek multiskan (Flow Laboratories, McLean, VA). To determine blank values, wells were treated in the same way as mentioned above, but instead of serum, 1% wt/vol gelatin dissolved in PBS was used.

ELISA for detection of antibody activity against heparan sulfate. Heparan sulfate was purchased from Seikaguku Kogyo Ltd. (Tokyo, Japan). Electrophoresis on cellulose acetate showed that this preparation contained only heparan sulfate and was not contaminated with other glycosaminoglycans, like chondroitin sulfate and hyaluronic acid. Polystyrene microtiter plates (Costar), precoated with 150 μl of protamine chloride (0.5 mg/ml) were coated overnight at room temperature with heparan sulfate (100 μl, 2.5 μg per well). Coating of serial dilutions of heparan sulfate revealed that 0.4 μg per well was the minimal amount still giving a maximal ELISA signal. To ensure saturated binding an excess of this amount (2.5 μg per well) was subsequently used in all determinations. In control experiments heparan sulfate was digested enzymatically with heparitinase (Seikaguku Kogyo Ltd.) (17). After cleavage the reaction mixture was dialyzed overnight against PBS to remove cleaved oligosaccharides. Although the heparan sulfate preparation showed no optical density at 260 nm, in a number of experiments heparan sulfate was treated with DNAse I (Boehringer, Mannheim, Federal Republic of Germany) to exclude any possible contamination with DNA. DNAse treatment was performed by adding an equal volume of 1.0 mg/ml DNAse I to a solution of 50 μg/ml heparan sulfate in PBS in the presence of 5 mM $MgCl_2$. This mixture was incubated during 60 min at 37°C, and subsequently dialyzed against PBS. In all experiments sera were also tested on wells only coated with gelatin (1% wt/vol). In none of those, binding to gelatin was observed. If bovine serum albumin (BSA, Sigma) was used as coated antigen in the ELISA, microtiter plates were coated with 150 μl BSA (100 μg/ml). The ELISA was performed similarly as described for heparan sulfate.

Inhibition studies. To demonstrate the specificity of the crossreactivity of anti-DNA antibodies with heparan sulfate, inhibition experiments were performed with DNA and heparan sulfate. SLE sera ($n = 6$) diluted to a concentration giving 75% of the maximal signal in the ELISA, were preincubated with an equal volume of 100 μl of DNA or heparan sulfate at concentrations varying from 0.25 to 100 μg/ml for 1 h at 37°C and subsequently for 1 h at 0°C. DNA used for these inhibition studies was commercially obtained calf thymus DNA (Sigma). To exclude the possibility that the observed inhibition is due to non-DNA nuclear constituents eventually present in this DNA preparation, we performed inhibition studies with DNA after digestion with DNAse I. The sera preincubated with heparan sulfate or DNA were tested in the ELISA with DNA or heparan sulfate as coated antigen.

Inhibition studies on anti-DNA binding were also performed with Cibacron Blue F3GA sepharose 6B (Pharmacia, Uppsala, Sweden), a sulphonated polyaromatic, polyanionic dye. SLE sera ($n = 3$) diluted to a concentration giving 75% of the maximal ELISA signal, were preincubated with an equal volume (100 μl) of PBS containing varying amounts (0.002–1.2 mM) of Cibacron Blue. The experiments were further carried out as described for the DNA and heparan sulfate inhibition studies.

To exclude that DNA, heparan sulfate or Cibacron Blue inhibited anti-DNA binding nonspecifically, they were tested for their ability to inhibit an unrelated antigen-antibody interaction, using BSA (Sigma) and rabbit anti-BSA (Dako, Copenhagen, Denmark). The anti-BSA antiserum was diluted to a concentration giving 75% of the maximal ELISA signal, with BSA as coated antigen. Diluted anti-BSA antiserum (100 μl) was incubated with 100 μl solution containing the same range of concentrations of DNA, heparan sulfate, or Cibacron Blue as used in the DNA, heparan sulfate, or Cibacron Blue inhibition experiments of the SLE sera. The experiments were further carried out as described above, and the supernatants were tested in the ELISA with BSA as coated antigen.

ELISA with different salt concentrations. To test the influence of ionic strength on antibody binding, ELISAs with DNA or heparan sulfate were performed with different NaCl concentrations. After coating of the appropriate antigen under standard conditions, serum dilutions of three different SLE sera were applied in the presence of increasing NaCl concentrations (range, 0.15–3 M NaCl). After the first incubation step, the ELISA was further carried out according to the standard procedure. The NaCl concentrations used did not induce a dissociation of the coated antigens from the microtiter plates, since preincubation for 1 h at room temperature of the coated antigens with the used salt concentrations did not alter the subsequent binding of a known SLE serum.

Elution of antibodies from kidneys with diffuse SLE nephritis. Kidneys from 22–26-wk-old MRL/l mice, BALB/c mice, a part of a kidney from a patient with SLE glomerulonephritis, and a part of a normal human kidney were used for elution studies. These MRL/l mice had all high anti-dsDNA antibody titers and diffuse proliferative glomerulonephritis with glomerular IgG deposits on immunofluorescence. Also the patient with a classic SLE and a biopsy-proven diffuse proliferative glomerulonephritis had high anti-dsDNA antibody titers. The kidneys used for control experiments showed no abnormalities on light microscopy and immunofluorescence. The kidney tissue was homogenized and washed extensively in 0.01 M PBS until no absorbance at 280 nm was found in the wash solution. The tissue homogenate was resuspended in 0.1 M sodium acetate buffer (pH 7.0) containing 10 mM calcium chloride and 5 mM magnesium chloride. To 15 g of tissue homogenate, 1 mg DNAse I and 10 U heparitinase were added. This mixture was incubated for 4 h at 37°C and 16 h at 4°C under constant stirring. The tissue homogenate was spun down in a SW34 rotor at 6,000 rpm for 15 min at 4°C. The pellet was resuspended in 30 ml 0.02 M citrate buffer pH 2.85, and under constant stirring incubated for 2 h at room temperature. After centrifugation (15 min, at 4°C in a SW34 rotor, 6,000 rpm) the elution procedure was repeated once. The supernatant was brought back immediately to pH 7 with Tris buffer. The precipitate formed during pH titration was removed by centrifugation. The eluate was dialyzed overnight against PBS, and subsequently concentrated with an Amicon YM 50 filter to a volume of 5 ml (Amicon Corp., Danvers, MA). The IgG content was determined nephelometrically. The control eluates of the BALB/c kidneys and the normal human kidney were concentrated to the same IgG concentrations as the MRL/l eluate and the human SLE eluate, respectively. Additionally, an eluate of rat kidneys with experimentally induced Heymann's nephritis was used for control experiments. This eluate (IgG concentration 160 μg/ml) was a generous gift from Dr. E. de Heer (Department of Nephrology, Leyden State University, Leyden, The Netherlands) and obtained as published before (18).

Results

Reactivity of human and murine SLE sera with heparan sulfate. Of 33 human SLE sera (positive in the Farr assay, the *Crithidia*

luciliae test and DNA-ELISA), 30 showed a positive binding with heparan sulfate as tested in the ELISA. A representative serum is shown in Fig. 1. As can be seen from Fig. 2, we found a clear correlation (r = 0.73) between the anti-DNA titer and the anti-heparan sulfate titer in these 33 SLE sera. The mean anti-DNA titer of these sera was approximately three times higher (mean log 2 titer±SD; 8.7±1.9) than the mean anti-heparan sulfate titer (7.2±2.5). Of 33 SLE sera, 3 showed no binding activity for heparan sulfate although an evident binding to DNA was present. A significant correlation (r = 0.77) was also found between the anti-DNA titer and anti-heparan sulfate titer in 10 MRL/l mouse sera, which were all positive in the Farr assay and *Crithidia luciliae* test (data not shown). The binding activities of a serum pool from 40 healthy volunteers and of 8 sera from patients with rheumatoid arthritis were negative in the DNA as well as in the heparan sulfate ELISA. Also in sera from normal BALB/c mice (n = 40) anti-DNA as well as antiheparan sulfate activity was never detected. The observed binding of SLE sera to heparan sulfate can not be attributed to the higher IgG concentration of these sera since the mean IgG concentration of these SLE sera (22±9 mg/ml; range, 7.0–41 mg/ml) was only two to four times higher than the control sera (10 mg/ml) while the average binding to heparan sulfate of these SLE sera was ~100 times higher than of the control sera. Furthermore, there was no correlation between the anti-heparan sulfate titer and the IgG concentration. Sera from patients with primary glomerulopathy [minimal lesions (n = 3), membranous glomerulopathy (n = 4), focal glomerulosclerosis (n = 2), and IgA nephropathy (n = 3)] and patients with glomerulopathy secondary to systemic diseases other than SLE (Wegener's granulomatosis (n = 12), Schönlein-Henoch purpura (n = 5), and periarteritis nodosa (n = 4)) were also tested for their reactivity in the DNA and heparan sulfate ELISA. None of these sera showed a positive binding to either of these antigens. To exclude any DNA contamination of the heparan sulfate preparation, 10 human SLE sera were tested with heparan sulfate pretreated with DNAse I. We observed no difference in binding activity of these sera with heparan sulfate whether it was pretreated with DNAse I or not. However, treatment of heparan sulfate with heparitinase abolished the binding activity of these SLE sera (n = 10) (data not shown).

Inhibition studies. To demonstrate that the binding to heparan sulfate was due to crossreactivity of anti-DNA antibodies,

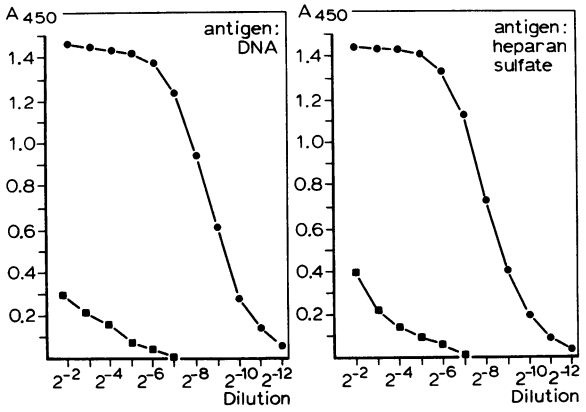

Figure 1. Binding activity in ELISA to DNA and heparan sulfate of a human SLE serum (● — ●) and a pool of normal human serum (■ — ■). The results are expressed as the absorption at 450 nm (A450).

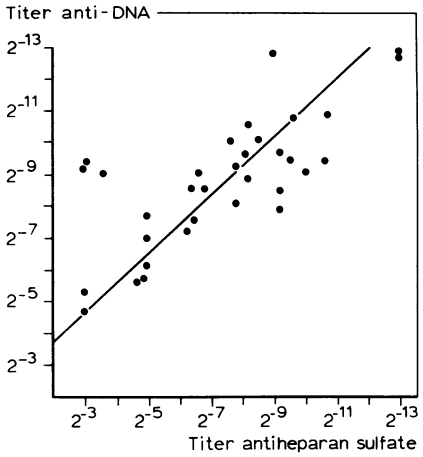

Figure 2. Correlation between the anti-DNA titer and the anti-heparan sulfate titer of 33 human SLE sera (r = 0.73; $P < 0.001$). The antibody titer for DNA or heparan sulfate is expressed as the dilution which gives a A450 signal of 0.5 in the ELISA.

blocking experiments with DNA and heparan sulfate were performed. Inhibition studies were done with five human SLE sera and with a serum pool of MRL/l mice (n = 40). These sera had a comparable binding activity to DNA as to heparan sulfate. The maximal inhibition observed with DNA or heparan sulfate for the different sera on subsequent binding to DNA is shown in Table I. As can be seen the range of inhibition of DNA to subsequent binding to DNA varied from 28 to 98% for the various sera. Heparan sulfate did also inhibit binding to DNA, although for some sera (3 and 5) the inhibition was lower than with DNA. The maximal inhibition obtained with DNA on subsequent heparan sulfate binding varied from 60 to 100% and was comparable to the inhibition of heparan sulfate on subsequent heparan sulfate binding (Table I). When in inhibition experiments DNA was used after digestion with DNAse I we found a complete loss of the inhibitory effect indicating that non-DNA nuclear antigens were not responsible for the inhibition. If heparan sulfate was digested with heparitinase the inhibitory effect of heparan sulfate was completely abolished in all four SLE sera tested. Fig. 3 depicts in detail the inhibition curve observed for serum 1, showing that heparan sulfate and DNA inhibit in a similar, dose-dependent way the binding of DNA (Fig. 3 *A*) or

Table I. Percentage of Maximal Inhibition by DNA or Heparan Sulfate on Subsequent DNA and Heparan Sulfate Binding (of a Serum Dilution Giving 75% of the Maximal ELISA Signal)

Coated antigen:	DNA		Heparan sulfate	
Fluid phase inhibitor:	DNA	Heparan sulfate	DNA	Heparan sulfate
HS 1	98	90	88	76
HS 2	92	94	99	95
HS 3	80	36	70	82
HS 4	28	23	60	80
HS 5	59	45	71	86
MRL/l serum pool	80	80	90	74

Amount of inhibitor per well, 10 μg. HS, human SLE serum.

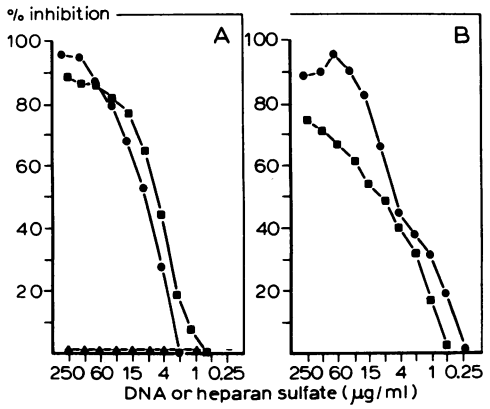

Figure 3. Inhibition of the binding of a human SLE serum preincubated with DNA (● — ●) or heparan sulfate (■ — ■) or heparitinase-treated heparan sulfate (▲ — ▲) on subsequent binding to DNA (*A*) or heparan sulfate (*B*). The results are expressed as percent inhibition calculated as: 1 − (A450 in the presence of inhibitor/A450 in the absence of inhibitor) × 100.

heparan sulfate (Fig. 3 *B*). Furthermore, it shows (Fig. 3 *A*) that digestion of heparan sulfate with heparitinase leads to a complete loss of the inhibitory effect of heparan sulfate.

The binding of anti-DNA antibodies to DNA could not only be inhibited by heparan sulfate, but also by another, unrelated, highly polyanionic structure: Cibacron Blue. In the three SLE sera tested we found that Cibacron Blue exerted the same inhibitory effect on DNA binding as heparan sulfate. Fig. 4 depicts the dose-dependent inhibition with Cibacron Blue for one of the sera analyzed.

To show that DNA, heparan sulfate, and Cibacron Blue did not inhibit nonspecifically the binding of anti-DNA to DNA, we performed similar inhibition experiments with these reagents in an unrelated antigen-antibody system, e.g., BSA-anti-BSA. Preincubation of anti-BSA antiserum with DNA, heparan sulfate or Cibacron Blue under the same conditions as used in the anti-DNA inhibition studies, did not inhibit at any concentration the subsequent binding of anti-BSA to BSA in the ELISA (data not shown).

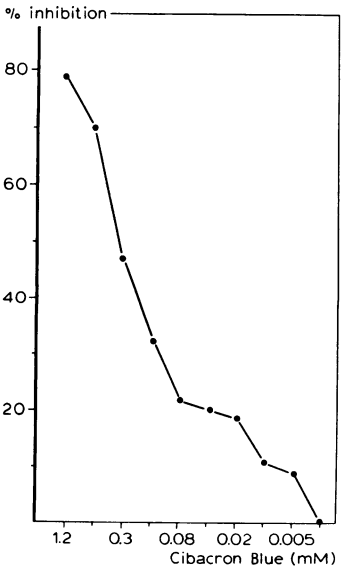

Figure 4. Representative experiment on inhibition with Cibacron Blue on anti-DNA binding of a human SLE serum. Inhibition was calculated as in Fig. 3.

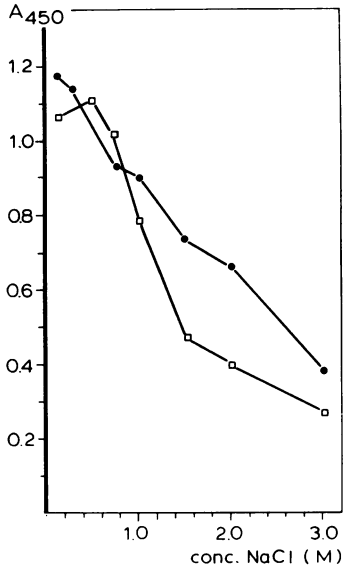

Figure 5. Influence of increasing ionic strength on the binding of a human SLE serum to DNA (● — ●) and to heparan sulfate (□ — □). The results are expressed as the absorption at 450 nm (A450).

Influence of the ionic strength on the binding of anti-DNA to DNA and heparan sulfate. To test the influence of ionic strength on the binding of anti-DNA to DNA and heparan sulfate ELISAs were performed in the presence of increasing concentrations of NaCl. Fig. 5 depicts results obtained with one of the tested sera, and shows that increase of the ionic strength resulted in a similar dose-dependent decrease of the binding to both DNA and heparan sulfate. The binding to heparan sulfate and DNA in two other sera tested was also equally sensitive to increasing salt concentrations although there were differences in sensitivity between the sera. As already mentioned this decrease in binding is not due to an enhanced dissociation of the coated antigens under the higher salt conditions used in the tests.

Binding of monoclonal anti-DNA antibodies to heparan sulfate. To substantiate further that antibodies with an anti-DNA specificity can bind to heparan sulfate, two monospecific murine monoclonal anti-DNA antibodies were tested for their binding activity to heparan sulfate. Both monoclonal antibodies showed a similar reaction pattern in the ELISA with either DNA or heparan sulfate. The binding activity of one monoclonal anti-DNA antibody is shown in Fig. 6.

Binding to heparan sulfate of antibodies eluted from SLE kidneys. To investigate whether anti-DNA antibodies deposited in vivo in SLE-glomerulonephritis could bind to heparan sulfate,

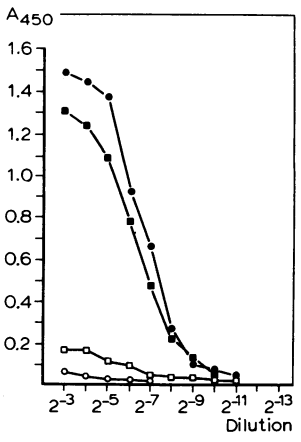

Figure 6. Reaction pattern of a mouse monoclonal antibody with DNA (● — ●) or heparan sulfate (■ — ■). In control experiments a mouse anti-human T cell monoclonal with the same subclass was tested for its reactivity to DNA (○ — ○) or heparan sulfate (□ — □). The results are expressed as the absorption at 450 nm (A450).

antibodies were eluted from MRL/l kidneys and from a kidney of an SLE patient with a diffuse proliferative glomerulonephritis. For control experiments kidneys from normal BALB/c mice and a part of a normal human kidney were eluted. The MRL/l and human SLE eluate contained 128 and 263 μg IgG/ml, respectively, and were positive in the *Crithidia luciliae* test, in contrast with the eluate of normal BALB/c kidneys (IgG content: 4 μg/ml) or normal human kidney (IgG content 5 μg/ml). To exclude that the higher IgG content of the SLE eluates caused a nonspecific binding, both control (human and murine) eluates were concentrated to the same IgG content as the SLE eluates. As shown in Fig. 7 the MRL/l and human SLE kidney eluate showed a comparable binding in the DNA and heparan sulfate-ELISA, while the concentrated BALB/c and normal human eluates were negative for both antigens. The binding activity of IgG in the human and murine SLE eluates to heparan sulfate could be inhibited by DNA, indicating that both antigens were recognized by the same antibody specificity. Although we cannot exclude unequivocally that the entrapment of serum IgG in the kidney eluates contributed to the anti-DNA and anti-heparan sulfate binding activity of the eluates, this seems very unlikely since the specific anti-DNA activity per microgram IgG eluted from the human and murine SLE kidneys was, respectively, four and seven times higher than the anti-DNA activity per microgram serum IgG.

Since elution studies of normal kidneys cannot be regarded as optimal control experiments we analyzed in addition the anti-DNA and heparan sulfate binding activity of a kidney eluate of an experimentally induced Heymann's-glomerulonephritis in the rat. This eluate (160 μg IgG/ml) did not show any binding activity in the anti-DNA and anti-heparan sulfate ELISA.

Discussion

This study shows that in 90% of human SLE sera (selected for the presence of anti-dsDNA autoantibodies) and in all the murine SLE sera antibodies are present directed toward heparan sulfate. In these sera the anti-heparan sulfate titer was positively correlated with the anti-DNA titer (r = 0.73). Binding to heparan sulfate was not found with sera from healthy volunteers, patients with rheumatoid arthritis, or patients with primary or secondary glomerulopathy due to systemic diseases other than SLE.

Inhibition studies revealed that heparan sulfate could block the binding to DNA and that DNA could inhibit the binding to heparan sulfate, indicating crossreactive recognition of heparan sulfate by anti-DNA antibodies. The specificity of the anti-DNA binding to heparan sulfate was demonstrated in several ways. Firstly, we could exclude DNA contamination of the heparan sulfate preparation, since the optical density of the heparan sulfate preparation at 260 nm was zero and DNAse I treatment of heparan sulfate did not influence the subsequent binding of anti-DNA antibodies. Secondly, treatment of heparan sulfate with heparitinase not only abolished the subsequent binding of anti-DNA antibodies to digested heparan sulfate, but also the inhibitory effect of heparan sulfate on subsequent binding to DNA. Thirdly, heparan sulfate (as well as DNA or Cibacron Blue) did not inhibit antibody binding in an unrelated antigen-antibody system (BSA-anti BSA).

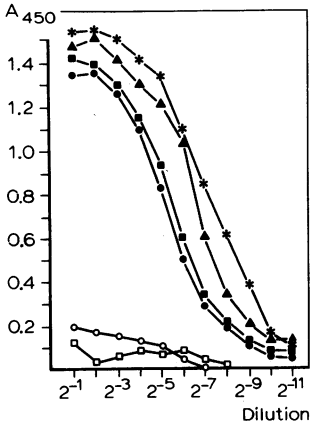

Figure 7. Reaction pattern of IgG eluted from a human SLE kidney with DNA (• — •) and heparan sulfate (▲ — ▲) and IgG eluted from MRL/l kidneys with DNA (● — ●) and heparan sulfate (■ — ■). Control experiments were performed with IgG eluted from BALB/c kidneys, normal human kidney, and rat kidneys with experimental Heymann's nephritis on DNA (○ — ○) and heparan sulfate (□ — □). The results are expressed as the absorption at 450 nm (A450).

That the binding of anti-DNA to heparan sulfate is due to a nonspecific charge interaction seems unlikely for different reasons. We did not observe any binding of non-SLE sera to heparan sulfate. If serum IgG would bind by a nonspecific charge interaction to heparan sulfate, one would expect that also some of these sera would show binding to heparan sulfate and that there existed a correlation between serum IgG concentration and heparan sulfate titer. Finally, the strongest argument can be derived from the studies showing the influence of ionic strength on antibody binding to DNA and heparan sulfate. Previous studies from Aarden and co-workers have demonstrated that nonspecific binding to DNA does not occur if the ionic strength exceeds 0.2 M NaCl, and that the binding of anti-DNA to DNA was of electrostatic nature (16, 21), since anti-DNA binding to DNA decreased with increasing ionic strength. We observed that the binding to heparan sulfate was equally influenced by increasing ionic strength as the binding to DNA, in each serum tested, although there existed differences in sensitivity for ionic strength among the different sera. Therefore, the binding of anti-DNA to heparan sulfate seems to be governed by the same electrostatic forces as the antigen-antibody interaction of DNA and anti-DNA.

Although our inhibition studies have revealed the crossreactive recognition of heparan sulfate by anti-DNA antibodies, they also showed that in certain sera, the inhibition of DNA binding obtained with heparan sulfate was less than that found with DNA, while the inhibition of DNA and heparan sulfate on heparan sulfate were comparable. This indicates that these sera contain additional noncrossreactive anti-DNA antibodies. Besides the amount of crossreactive anti-DNA antibodies, as revealed by heparan sulfate inhibition of anti-DNA binding, also the avidity of the anti-DNA antibodies seems to be important since in certain sera, DNA in the fluid phase was not able to prevent completely subsequent binding to solid phase DNA.

However, in certain sera the concentrations necessary for 50% inhibition of binding to both antigens were similar, indicating that dependent on the amount and avidity of the crossreactive anti-DNA antibodies heparan sulfate can serve under these circumstances equally well as antigenic target for anti-DNA antibodies as DNA. That anti-DNA antibodies could bind to heparan sulfate was unequivocally confirmed with the two monoclonal anti-DNA antibodies, since these monospecific antibodies bound equally well to heparan sulfate as to DNA.

The crossreactive recognition of anti-DNA antibodies is not restricted to heparan sulfate. We have previously reported that other polyanionic molecules such as chondroitine sulfate and hyaluronic acid, can also bind to anti-DNA antibodies (9). In addition, we have shown in this study that another unrelated highly anionic molecule, Cibacron Blue, could inhibit anti-DNA

binding to DNA to the same extent as heparan sulfate. This observation is in line with earlier reports in which Cibacron Blue was used to purify anti-DNA antibodies (19), or to inhibit anti-DNA binding to DNA in the Farr assay (20).

Primarily based on studies with monoclonal antibodies (22, 23) anti-DNA antibodies can be divided in antibodies reactive with DNA nucleotides or nucleotide sequences and those reactive with the sugar phosphate backbone of DNA. The latter are thought to be responsible for crossreactive binding of anti-DNA antibodies to several molecules (3). There still exists, however, some controversy about the composition and characteristics of the crossreactive targets. Stollar, Schwartz, and co-workers (4, 5) have proposed that the crossreactive moieties are phosphodiester-linked phosphate groups. We propose, however, based on our previous experiments with hyaluronic acid and chondroitin sulfate (9), the reported reactivity of anti-DNA antibodies with Cibacron Blue (19, 20) and the present results with heparan sulfate and Cibacron Blue that molecules with repeating anionic sites also can serve as crossreactive antigenic targets for anti-DNA antibodies.

The crossreactivity of anti-DNA antibodies with the glycosaminoglycan heparan sulfate may play an important role in the immunopathogenesis of SLE glomerulonephritis. Heparan sulfate is the major glycosaminoglycan constituent of the glomerular basement membrane (GBM), and has a major role in the maintenance of the charge selective barrier of the GBM (24). Furthermore, recent experiments have indicated that heparan sulfate may also have an important function in the integrity of the size-selective barrier of the GBM (25, 26). Reduction or neutralization of heparan sulfate in the GBM leads to proteinuria in experimental animals (24, 27). Clinically, a reduction of heparan sulfate-associated polyanionic sites has been held responsible for proteinuria in congenital nephrotic syndrome (28). In addition to their function in glomerular permeability, there is now also evidence that anionic sites within the glomerulus may determine the intraglomerular handling of circulating antigens, antibodies, and immune complexes (29). Therefore binding of anti-DNA antibodies to the heparan sulfate associated polyanionic sites in the GBM might be an important event in SLE nephritis either by interference with physiological functions and/or by triggering inflammatory reactions locally. This hypothesis implies that glomerulonephritis in SLE patients might occur by a direct binding of anti-DNA antibodies to heparan sulfate. Our finding that anti-DNA antibodies eluted from kidneys with diffuse SLE glomerulonephritis can bind to heparan sulfate in vitro supports this hypothesis. Studies are now in progress to see whether anti-heparan sulfate activity in the serum of SLE patients is correlated with the presence of glomerulonephritis.

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