Localization of Fluorescein-Labeled Antinucleoside Antibodies in Glomeruli of Patients with Active Systemic Lupus Erythematosus Nephritis

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ABSTRACT Renal tissues from two groups of patients were studied with fluorescein-labeled (Fl-) antibodies (Abs) to immunoglobulins, complement, and antibodies prepared in rabbits against BSA conjugate of 5-methyluridine (T) and cytidine (C), the latter two of which react specifically with denatured DNA. The first group consisted of 13 SLE patients, and the second consisted of 53 patients with non-SLE nephropathies. The data obtained from the two groups of patients were used for comparison, and they showed the following:

(*a*) Fl-Abs to immunoglobulins and complement were bound in the glomeruli of tissues from all patients with active SLE glomerulonephritis characterized by deposits of foreign material in glomerular capillary walls (GCW). The fluorescent pattern was granular, corresponding to the distribution of the glomerular deposits, as seen by electron microscopy. Fl-Abs reactive with thymine and cytosine were bound in the GCW of eight of the nine patients with active SLE glomerulonephritis and showed the same granular distribution. The capacity of these latter Fl-Abs to stain the GCW was removed by absorption with the homologous antigen or denatured DNA.

(*b*) Fl-Abs to immunoglobulins, complement, and pyrimidine bases of DNA did not react with the GCW of two SLE patients without clinical and histologic evidence of glomerulonephritis or with the sclerotic glomeruli of two uremic patients with chronic "burned out" lupus nephritis.

(*c*) The glomeruli of 47 of the 53 patients with other nephropathies bound Fl-Abs to immunoglobulins and complement to some extent, and in 26, the localization appeared as marked as in the patients with active SLE glomerulonephritis. Fl-Abs reactive with thymine and cytosine were bound in the GCW of only one of the renal tissues from the 53 non-SLE patients. In the remaining 52, no binding was seen.

(*d*) The findings are consistent with the hypothesis that antigen-antibody complexes, formed by denatured DNA, specific antibody, and complement, are present in the deposits of foreign material accumulated in the GCW of patients with active SLE glomerulonephritis, and that they may contribute to the pathogenesis of this renal disease.

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INTRODUCTION

Patients with systemic lupus erythematosus (SLE) may develop proliferative and membranous glomerulonephritis with deposits of foreign material along glomerular basement membranes (1–3). The presence of immunoglobulins and complement has been demonstrated in glomerular lesions by immunofluorescence techniques (4–7), and antinuclear antibodies have been found in eluates from the glomeruli (8–11). Fluorescein-labeled, highly purified antibody to DNA obtained from the serum of a lupus patient has localized in the glomeruli of two patients with SLE nephritis. Antibodies reacting with DNA, nucleoprotein, or neuclei were eluted from the glomeruli. These studies support the hypothesis that human lupus nephritis is provoked by circulating antigen-antibody complexes containing DNA and antibody to DNA (11).

This report describes the results of the studies of renal tissues from 13 SLE patients: nine with active glomerulonephritis, two with chronic glomerulonephritis,

and two without glomerulonephritis. The presence of immunoglobulins, complement, and denatured DNA in these tissues was determined by the immunofluorescence technique. Antibodies specific for the pyrimidine bases of DNA, thymine and cytosine, were used in searching for denatured DNA (12–14). Unlike the DNA-reactive antibodies in sera of some patients with SLE, the antibodies specific for the pyrimidines do not react with native DNA (13–15).

MATERIALS AND METHODS

Patient Material

Patients with SLE. 13 female patients, 18–47 yr old, had shown symptoms associated with SLE for 3 months to 15 yr. The more pertinent clinical, laboratory, and pathologic findings in these patients are summarized in Table I, where it is seen that eight patients were under the care of Dr. Andres in the Renal Unit at the II Clinica Medica, University of Rome, and that four were from the Columbia Presbyterian Medical Center (CPMC), where Dr. Christian had charge of them. We are indebted to Dr. D. Davids of St. Luke's Hospital for making available the tissue and the clinical and laboratory findings of patient BW.

Patients with nephropathies other than SLE nephritis. Renal tissues from 53 patients with various nephropathies summarized in Table III were selected for testing with fluorescein-labeled antibodies specific for thymine and cytosine, as well as with labeled antibodies to immunoglobulins and to complement. They were chosen to serve as controls because in most instances the amount of immunoglobulins and complement and the pattern of distribution in GCW resembled those found in active SLE nephritis. The age range was comparable with the lupus patients. One-third of the patients were women. This group of 53 patients, from whom biopsy or autopsy specimens were obtained, included 39 cared for at the Rome Clinic by Dr. Andres, 4 at the CPMC under the care of Dr. Christian, and 10 with renal allografts from Dr. T. E. Starzl, Colorado University Medical Center, Denver, Colo., or Dr. K. A. Porter, St. Mary's Hospital, London. Clinical, laboratory, and light and electron microscopic data have been used for the diagnosis, and most of the findings have been reported elsewhere (16–20).

Tissue processing

Renal biopsies were obtained with a Silverman needle with Franklin modification under local anesthesia or by "open biopsy." The tissues were divided into three parts; one part was fixed in formalin or Bouin's solution for light microscopy, the second was treated with osmium tetroxide in preparation for embedding in Araldite for electron microscopy, and the third part was quick-frozen in a bath of dry ice and alcohol and was sectioned in a cryostat at 4 μ thickness for immunofluorescent study.

Antisera: preparation and control

The following antisera were purchased, supplied by courtesy of various investigators, or prepared in our laboratories: anti-IgM, Hyland Div., Travenol Labs, Inc., Costa Mesa, Cal.; anti-IgA, Dr. R. D. Rosen (21); anti-lambda, Dr. E. R. Osserman (22); anti-kappa, Dr. E. R. Osserman (22); anti-β1C, Hyland Laboratories and Farbwerke Hoechst; anti-fibrinogen, Dr. F. Gorstein[1]; Anti-IgG, prepared in our laboratories or by Dr. A. J. L. Strauss (23); anti-BSA, prepared in our laboratories; anti-C'1q, Doctors J. H. Morse and C. L. Christian (24); and anti-T and anti-C, prepared by Doctors Beiser and Erlanger (12–13).

All antisera were tested by immunoelectrophoresis to establish their potency and specificity. Globulins were separated from the above antisera by sodium sulfate precipitation and were labeled with fluorescein using a technique already reported (25). Before any new fluorescein-conjugated antibody was used, its optimal dilution was determined by testing on a series of sections of tissue known to contain the specific antigen.

The preparation of the antisera reactive with thymine and cytosine has been described (12–13). Conjugates of bovine serum albumin (BSA) with 5-methyluridine (T) and with cytidine (C) were prepared, and rabbits were injected in the foot pads with the conjugates in complete Freund's adjuvant (26). Antibody for BSA, when present, was removed by absorption, and the antisera reacted only with homologous nucleoside-BSA conjugates and with denatured DNA. There was no crossing with heterologous nucleoside-BSA conjugates (15). The tests used were quantitative complement fixation (27) and quantitative precipitin reactions (28). Controls for the fluorescein staining experiments were prepared by absorbing aliquots of the fluorescein-labeled antisera with T, C, or with denatured DNA. Three successive absorptions were performed with T and C using 5 μg N antigen each time per 0.5 ml of serum and with denatured DNA using 40 μg of denatured human DNA each time per milliliter of serum. DNA was denatured by placing a solution (800 μg/ml) containing 1% formaldehyde in a boiling water bath for 10 min, followed by chilling quickly in an ice bath. After each antigen addition, the mixture was placed in a 37°C water bath for 1 hr and then was refrigerated overnight. After centrifugation, the procedure was repeated. The supernatant fluids after the third addition of antigen had been diluted less than 20%.

Staining of tissues

Staining of sections with the fluorescein-labeled globulins was carried out according to a technique already described (25). In an attempt to sharpen the staining with anti-T and anti-C, in six instances (EC [second biopsy], MTN [post-mortem biopsy], VC, ID, LS, and DW), additional sections were first treated with 0.02 M citrate buffer, pH 3.2, for 15–30 min, or with 0.2 M NaCl for 1–2 hr at room temperature (11). They were then washed with phosphate buffer at pH 7.2 before staining with Fl-anti-T and with Fl-anti-C. Some sections were also eluted with physiological saline for the same interval of time. Unlabeled antibody was applied first to some tissue sections in order to test for blocking of the subsequent reaction with the labeled antibody.

RESULTS

Histologic studies (Table I). (*a*) In the tissues obtained on initial renal biopsies from the first nine patients (CM, EC, MTN, VC, AC, LS, ID, DW, and BW), glomerular lesions characteristic of, or compatible with, those seen in patients with diagnosis of active SLE ne-

[1]Gorstein, F., and E. Puszkin. Immuno-electronmicroscopic appearance of fibrin. Data in preparation.

TABLE I

Summary of Clinical and Laboratory Data for 13 SLE Patients

Patient	Age	Prior to biopsy/autopsy: Duration symptoms	Prior to biopsy/autopsy: Course of disease, treatment	At biopsy/autopsy and after biopsy: LE cells	Anti-nu.Ab	BUN	Creatinine clearance	Urinary findings	Histologic, clinical evaluation, course
	yr					*mg/100 ml*	*ml/min*		
CM, Rome Clinic 9/65	19	10 mo	Rash of face, chest, migratory arthralgia, fever, proteinuria, pleural effusion. R_x antibiotics, insufficient steroids. On admission also myo- and pericarditis.	pos	pos	100	40	protein 6–8 g casts rbc	Severe membranous, proliferative nephritis with hematoxylin bodies and wire loops. No improvement with immunosuppressive treatment. Left hospital in extremus.
EC, Rome Clinic 6/67	40	6 yr	Initially migratory arthralgia, fever. R_x salicylates. 5½ yr postsurgery, proteinuria, edema. Admission diagnosis, glomerulonephritis, nephrotic syndrome.	neg	neg	15	89	protein 10–16 g casts rbc	Severe proliferative and membranous GN with wire loops. EM shows subendothelial and subepithelial deposits, also present between mesangial cells. R_x 60 mg prednisone/day.
9/67			Readmitted for biopsy.	neg					EM shows some slight improvement of renal lesion.
3/68			Readmitted, in fairly good condition.	pos	pos	15	94	protein 3 g	Steroids decreased, azathioprine started. 4/68 biopsy: decreased proliferative changes, persist deposits. Clinical condition improving.
2/70			Readmitted for observation. Good condition on prednisone 30 mg, azathioprine 50 mg/day.	neg	neg	25	—	protein 2–3 g	
MTN, Rome Clinic 7/67	23	3 yr	Migratory arthralgia, fever, edema, proteinuria. R_x antibiotics. 1 yr pericardial effusion, LE+, proteinuria, casts.	pos	pos	60	52	protein 10–15 g macroscopic blood	Severe membranous, proliferative GN with hematoxylin bodies and wire loops. R_x steroids and azathioprine. Remarkable clinical improvement.
11/67			Puncture to remove a persistent pericardial effusion during which patient died.						Needle biopsy taken. PM showed a decrease in amounts of deposits in GCW.

TABLE I—(*Continued*)

Patient	Age	Prior to biopsy/autopsy: Duration symptoms	Prior to biopsy/autopsy: Course of disease, treatment	At biopsy/autopsy and after biopsy: LE cells	Anti-nu.Ab	BUN	Creatinine clearance	Urinary findings	Histologic, clinical evaluation, course
	yr					*mg/100 ml*	*ml/min*		
VC, Rome Clinic 12/67	18	14 mo	Migratory arthritis, fever, muscle pain, proteinuria, hematuria, edema, pleural effusion. R_x antibiotics.	pos	pos	90	45	protein 10–12 g	Membranous GN with wire loop changes, mesangial cell proliferation. EM subepithelial and subendothelial deposits. Improvement on azathioprine and prednisone. Condition remains satisfactory.
AC, Rome Clinic 2/68	29	13 mo	Migratory arthralgia. 8 months later edema, hematuria. Diagnosed GN, R_x antibiotics. After development of peritoneal, pericardial, pleural effusion.	neg	pos	80	90	protein 8–10 g	Severe proliferative GN, hematoxylin bodies, wire loops. R_x azathioprine and prednisone. After 5 months improved.
9/69			Readmitted for biopsy. Edema and effusion gone, condition good.	neg	neg	35	—	protein 2–4 g	Proliferative and membranous changes decreased. R_x prednisone and azathioprine continued.
2/70			Readmitted for routine check up. Condition remains satisfactory.	neg	neg	30	95	protein 1–3 g	
LS, CPMC 7/60	25	1 yr	Joint pains, fever, lymph nodes tender, enlarged. Murmurs, in systole, diastole. Proteinuria, hematuria.	pos	pos	45	—	protein 4 g casts rbc	Biopsy 10/68. Severe diffuse, proliferative and membranous nephritis with wire loops. Prednisone, azathioprine started.
ID, CPMC '59	47	10 yr	Original diagnosis mild rheumatoid arthritis. 1/66 developed nephrotic syndrome. LE cells neg. 2/66 renal biopsy showed membranous nephritis. LE cells positive. Final admission 4/69 for cerebral thrombosis, renal failure.	pos	pos	117	—	protein 4–6 g	4/69 autopsy. Membranous nephritis. Thickened GBM resembling wire loops.

TABLE I—(*Continued*)

Patient	Age	Prior to biopsy/autopsy: Duration symptoms	Prior to biopsy/autopsy: Course of disease, treatment	At biopsy/autopsy and after biopsy: LE cells	Anti-nu.Ab	BUN	Creatinine clearance	Urinary findings	Histologic, clinical evaluation, course
	yr					*mg/100 ml*	*ml/min*		
DW, CPMC	31	15 yr	1945 skin rash. 2 similar episodes in next 9 yr. 1954, polyarthritis, rash. 1956, LE cells neg. 1957, muscle biopsy, vasculitis, episcleritis, '60, arthralgias, pneum. pneumonia. On 2nd admission, viridans sepsis, latex 1:2000, LE cells pos. Pleurisy, gastric washings pos. for AFB. 1963 fever, adenopathy, proteinuria ++++ BUN 30. 1968, BUN 162. 5/69 admitted with azotemia BUN 100, started on hemodialysis.	pos	pos	100	—	protein 4–6 g	10/69 renal transplant. Own kidney, most glomeruli with sclerosis. In others, active inflammatory lesions with diffuse subepithelial and subendothelial deposits.
BW, St. Luke's 10/69	41	3 mo	Skin rash, fatigue, loss of appetite and weight. Multiple enlarged lymph nodes. Hypergamma-globulinemia. Improved on prednisone.	pos	pos	85	—	protein 3–4 g	10/69 renal biopsy. Light micros. variable glomerular damage. EM shows subendothelial and mesangial deposits.
CC, Rome Clinic 2/5/70	18	3 yr	Fever, arthralgia, rash, purpura. After 3 months pleural and pericardial effusions, glomerulonephritis. LE cells+; Anti-nu.Abs+; proteinuria, 8–10 g; casts, rbc, BUN 70 mg/100 ml. R_x antibiotics, steroids azathioprine with only short intervals, for 2 yr. At admission hypertension and renal failure.	pos	pos	120	15	protein 10 g casts rbc	Severe chronic glomerulonephritis with diffuse sclerosis.

TABLE I—(*Continued*)

Patient	Age	Prior to biopsy/autopsy: Duration symptoms	Prior to biopsy/autopsy: Course of disease, treatment	At biopsy/autopsy and after biopsy: LE cells	Anti-nu.Ab	BUN	Creatinine clearance	Urinary findings	Histologic, clinical evaluation, course
	yr					*mg/100 ml*	*ml/min*		
PP, CPMC 7/60	22	3 mo	Rash, fever, poliarthralgia. LE cells positive. Pleural effusion, proteinuria, hematuria, maintained on prednisone. 9/67, renal insufficiency, LE cells negative. 10/68, final admission.	neg	neg	195	—	protein 4.3 g	10/68 autopsy. Hypercellularity. Thickened basement membranes, extensive glomerulocapsular adhesions, fibrin deposits. Architecture of glomeruli lost due to severe, diffuse sclerosis.
SA, Rome Clinic 6/13/67	29	3 mo	Rash (face, chest), migratory arthritis, fever, pleural effusion. Salicylates.	pos	pos	15	110	normal	Normal kidney, both by light and EM. Steroids and azathioprine.
5/10/68			Readmitted for routine check up.	neg	neg	20	95	normal	Purpura. Skin vasculitis. Steroids and azathioprine.
3/2/69			Readmitted for 3 months, pregnancy. Spontaneous abortion.	neg	neg	18	116	normal	Satisfactory condition. Rash, face. Steroids and azathioprine.
2/28/70			Readmitted for observation.	neg	neg	20	100	normal	Muscle pain migratory arthralgia. Steroids and azathioprine.
PC, Rome Clinic 8/5/67	25	1 yr	Original diagnosis mild migratory arthritis. 4/67 developed skin rash, muscle pain, pleural and pericardial effusions.	pos	neg	20	98	normal	Normal kidney both by light and EM. Steroids and azathioprine.
5/3/68			Readmitted for routine check up.	neg	neg	15	—	normal	Muscle pain. Satisfactory condition.

phritis, were found by light and electron microscopy (1–3). Patients AC, MTN, EC, LS, DW, and BW had a variety of proliferative and membranous changes, the pattern and the severity of which varied from glomerulus to glomerulus. Patients VC and ID had a diffuse membranous glomerulonephritis of moderate severity with slight evidence of proliferation. Patient CM had a severe acute proliferative and membranous glomerulonephritis with polymorphonuclear leukocytes and hematoxylin bodies. In all these patients, the electron microscopic studies showed the presence of aggregates of foreign electron-opaque material between proliferating cells and on both sides of the glomerular basement membrane. In the second and third biopsy of patient EC and in the second biopsy of patient MTN, the glomerular changes appeared less severe than when first seen. By electron microscopy there were fewer electron-opaque foreign depositis seen within the GCW of the second and third biopsies than were found in the first biopsy. In patient EC, lesions of "membranous transformation" (29) began to be evident in the third biopsy. In patients CC and PP, lesions of severe chronic nephritis, with diffuse glomerulovascular sclerosis, were seen, whereas the light and electron microscopic studies of tissues obtained from patients SA and PC showed only normal renal structures.

(*b*) All the renal tissues obtained from the third group of patients with glomerular diseases other than SLE glomerulonephritis have been examined by light and electron microscopy. All of theme were studied with the immunofluorescence technique, and some were also studied with the immunoferritin technique. The histologic and immunologic findings have been reported elsewhere (16–20).

TABLE II

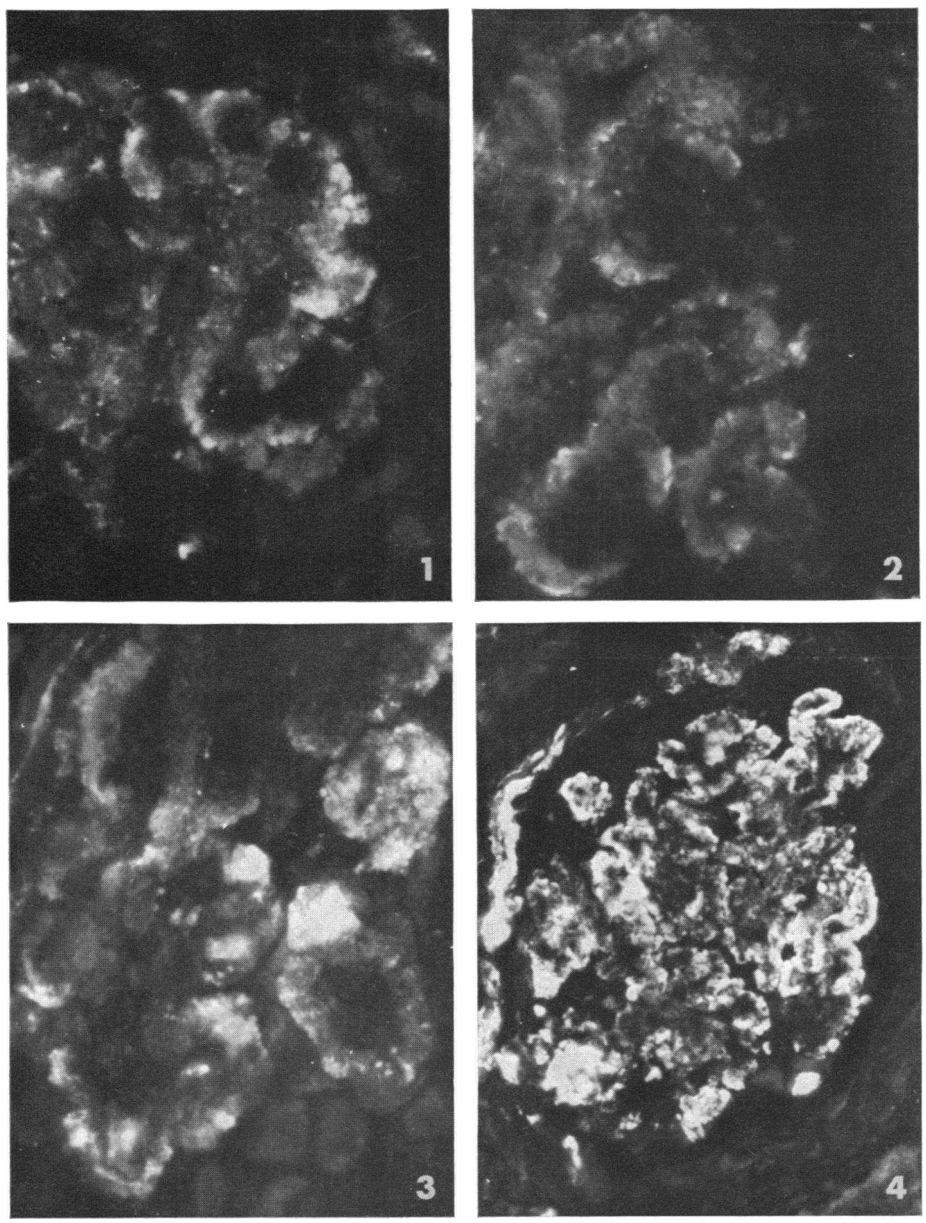
Localization of Fl-Antibodies to Immunoglobulins, Complement, and Nucleosides in Glomeruli of Renal Tissues from 13 SLE Patients

Patient and date of biopsy	Antisera to										
	IgG	IgM	IgA	Lam	Kap	C'1q	βIC	Fngn	T	C	BSA
CM											
10/65	−	−	−	−	−	−	+	++	+++	−	−
EC											
6/67	++	++++	−	−	−	++++	+	++	+++	+++	±
9/67	+++	++	−	−	−	++++	++	0/+	++++	++++	0
4/68	++	0	−	++	−	++	+	0/++	++	+	±
MTN											
7/67	++++	+++	0	+	++/+++	++++	++	+	+++	+++	±
11/67 (PM)	++	+++	−	−	−	++++	+	±	++	++	0
VC											
2/68	++++	±	±	±	0	++++	+++	0	++	±	0
AC											
3/68	++++	+	0	+/+++	++/+++	++++	+++	+	++	++	0
9/69	++++	++++	−	−	−	−	++++	−	+++	++	−
LS											
10/68	++	+++	−	+++	+++	++++	++++	+	+++	+++	+
ID*											
4/69	++++	+++	0/+	+++	±/+	±/+	+++	++	+++	++/+++	0
DW											
10/69	+++	+++	−	−	−	−	±/+	±	+/+++	++/+++	0
BW											
10/69	+++	+++	−	−	−	−	++++	−	0	0	0
CC											
4/70	±	±	−	−	−	±	±	0	0	0	0
PP*											
10/68	0	0	0	0	0	0	0	0	0	0	0
SA											
7/67	0	0	−	−	−	0	0	0	0	0	0
PC											
11/67	0	0	−	−	−	0	0	0	0	0	0

* Autopsy specimen.

Abbreviations: Fl-, fluorescein-labeled; SLE, systemic lupus erythematosus; lam, lambda light chain; kap, kappa light chain; Fngn, fibrinogen; PM, postmortem; T, 5-methyluridine-BSA; C, cytidine-BSA; −, test not done or no glomeruli in sections; 0, negative; ±, minimal in amount; +, slight in amount; ++, moderate in amount; +++, marked in amount; ++++, very marked in amount and extent.

Immunofluorescence studies. (*a*) The results of studies in which frozen sections of kidney from 13 SLE patients were tested with Fl-Abs specific for IgG, IgM, IgA, kappa and lambda light chains, β1C, C'1q, fibrinogen, T, C, and BSA are summarized in Table II. Only a few sections containing glomeruli were obtained from the biopsy of patient CM, and they were only tested with Fl-anti-T, Fl-anti-β1C and Fl-anti-fibrinogen. Antibodies to IgG, IgM, β1C, and C'1q were localized in a granular pattern in the GCW of biopsy specimens from eight



Figs. 1–14 are photomicrographs which show the results of staining with different fluorescein-labeled (Fl-) antibodies on sections of renal tissues of three SLE patients with active glomerulonephritis.

FIGURE 1 Patient MTN. Fl-anti-T localized in a granular manner in GCW. × 350.
FIGURE 2 Same patient. Fl-anti-C similarly localized in GCW. × 350.
FIGURE 3 Same patient. Granular localization in GCW of Fl-anti-IgM. × 450.
FIGURE 4 Same patient. Localization of Fl-anti-C'1q in a glomerulus and in a part of Bowman's capsule. × 300.

patients listed in the table. Labeled anti-IgA antibody did not yield significant reaction with any of the tissues studied. Light chain globulin fractions were present in variable degrees in GCW of patients EC, MTN, AC, LS, and ID. Anti-fibrinogen was mainly localized in the glomeruli of patients CM, EC, and ID. The fluorescent staining obtained with anti-T and anti-C in patients CM, EC, MTN, VC, AC, LS, ID, and DW was distributed in GCW in a granular manner (Figs. 1, 2, 5, 9, and 12) similar to that obtained with antibodies to immunoglobulins (Figs. 3 and 7) and complement (Figs. 4 and 8) and similar to the irregular distribution of the deposits of foreign material in GCW, as seen by electron microscopy. Renal tissues from patient BW did not bind the anti-T and anti-C sera. Anti-BSA was employed as control since BSA was part of the conjugate used for

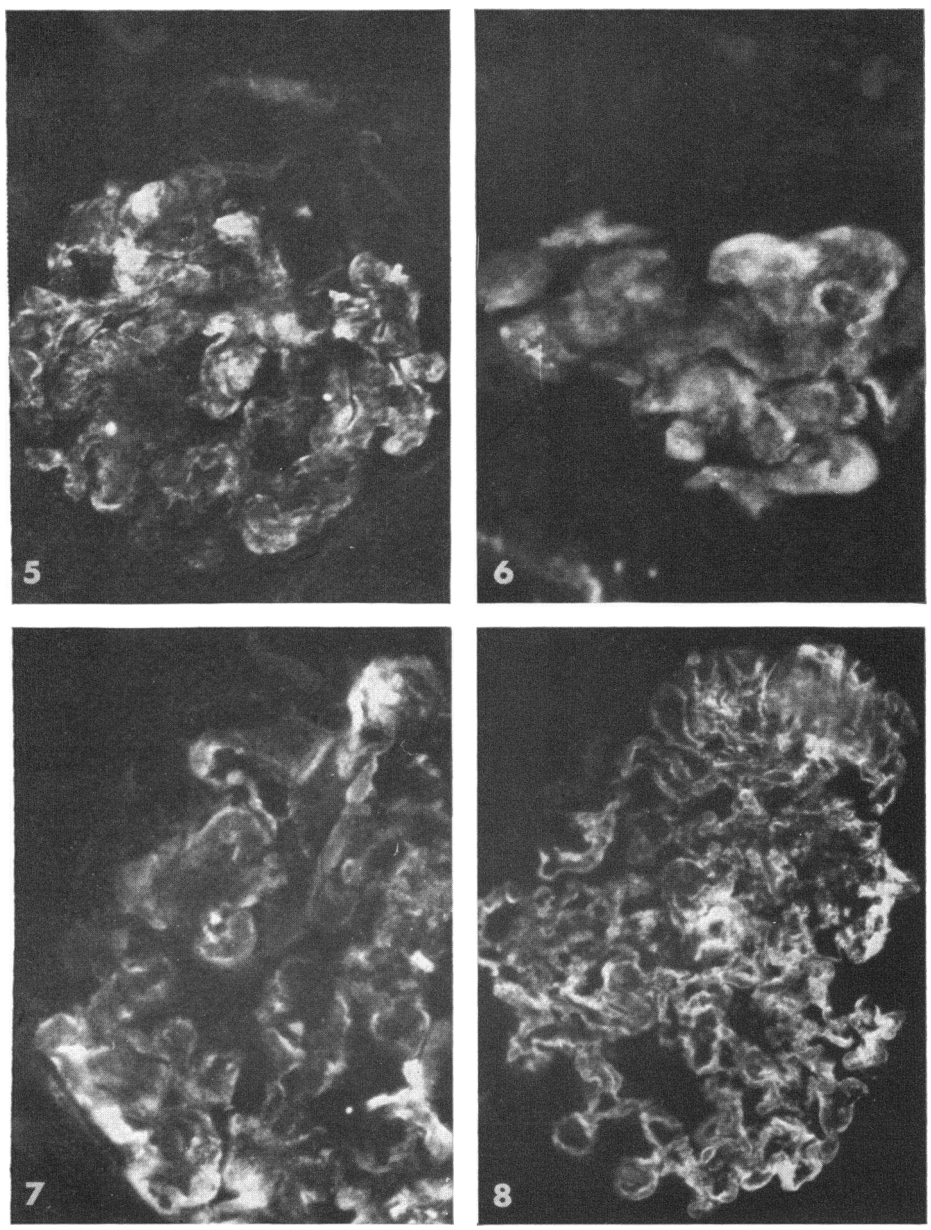


FIGURE 5 Patient LS. Localization in GCW of Fl-anti-T. × 300.
FIGURE 6 Same patient. Localization in a glomerulus of Fl-anti-C. × 300.
FIGURE 7 Same patient. Fl-anti-IgM localized in GCW. × 350.
FIGURE 8 Same patient. Fl-anti-C'1q localized similarly in GCW. × 350.

the preparation of antibodies against nucleosides, and it was not significantly bound except in one instance (LS).

Prior incubation of renal sections from this group of patients with unlabeled anti-T or anti-C blocked the subsequent staining with the respective Fl-Abs. The staining capacity of the labeled antibodies was removed or greatly reduced by absorption with the homologous antigen, T (Fig. 13) or C (Fig. 10), or with denatured DNA (Figs. 11 and 14), whereas it was not affected by cross-absorption, i.e., labeled anti-T with C or anti-C with T.

The renal tissues of two SLE patients (CC and PP) with chronic nephritis, characterized by diffuse glomerular sclerosis, did not bind significant amounts of Fl-Abs to immunoglobulins, complement, T, and C. The same findings were obtained in two SLE patients (SA and PC) without glomerulonephritis.

(*b*) Tests for the localization of Fl-anti-T and Fl-anti-C were made also in tissues from 53 biopsies and one

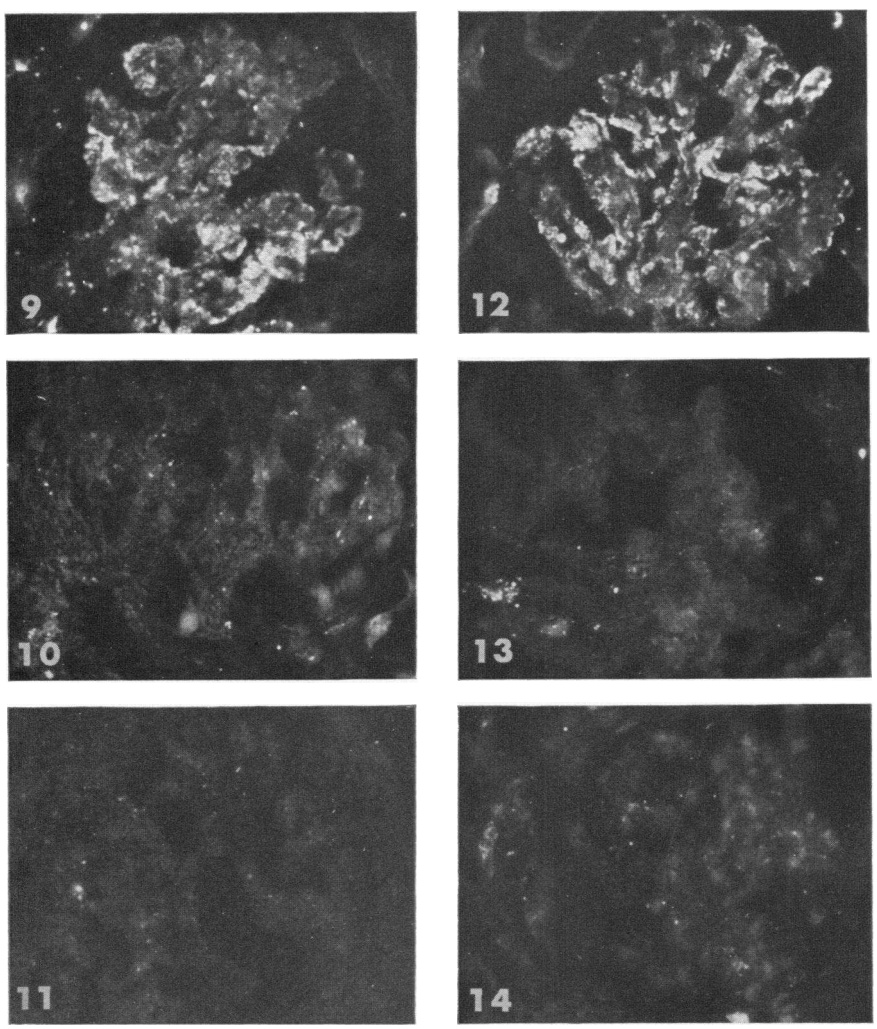


FIGURE 9 Patient ID. Granular localization of Fl-anti-C in GCW. × 100.
FIGURE 10 Same patient. Treated with Fl-anti-C absorbed with C. The staining capacity of this fluorescence is almost completely removed. × 100.
FIGURE 11 Same patient. Treated with Fl-anti-C absorbed with denatured DNA. No fluorescence is seen. × 100.
FIGURE 12 Same patient. Granular localization in GCW of Fl-anti-T. × 100.
FIGURE 13 Same patient. The staining capacity of Fl-anti-T is completely abolished by absorption with T. × 100.
FIGURE 14 Same patient. Treated with Fl-anti-T absorbed with denatured DNA. Very little fluorescence can be seen. × 100.

TABLE III

Results of Tests for the Binding of Fl-Anti-T and Fl-Anti-C in Glomeruli of Kidneys from 53 Patients with Glomerular Diseases other than SLE GN

Disease	Number of cases	LE test and anti-nu.Ab	Number of binding A-T/A-C	Presence of immunoglobulins and complement
Acute poststreptococcal GN	12	both neg	0	Varying amounts of immunoglobulins and C′ were bound in glomeruli.
Idiopathic membranous nephropathy	11	both neg	1	All had large amounts of immunoglobulins and C′ in glomeruli. One had +binding with anti-T and anti-C.
Chronic nephritis	9	—	0	Small amounts of immunoglobulins and C′ in glomeruli.
Renal allografts	10	—	0	Some with large amounts of immunoglobulins and C′ in GCW. All contained some immunoglobulins.
Diabetic nephropathy	2	—	0	(Tissue from one obtained at autopsy). One had C′ in GCW. The second was negative.
Pyelonephritis	3	—	0	None or little immunoglobulins and C′ in glomeruli.
Polyarteritis	1	both neg	—	Severe renal disease with immunoglobulins and C′ in glomeruli and vessels.
Goodpasture's disease	2	—	0	Marked localization of immunoglobulins and C′, linear distribution in GCW.
Renal vein thrombosis with neph. syn.	2	both neg.	0	Marked localization of immunoglobulins and C′. Granular distribution in glomeruli.
Familial GN	1	—	0	Moderate amount of immunoglobulins and C′.

Abbreviations: GN, glomerulonephritis; C′, complement.

autopsy of patients with glomerular diseases other than SLE nephritis (Table III). All the tests for nucleosides were negative except for one tissue obtained at autopsy from a patient with idiopathic membranous nephropathy, in which both LE cell and antinuclear antibody tests were negative. In this case, a one plus binding of anti-T and anti-C in the GCW was detected after elution with citrate buffer. The 53 renal tissues also were examined with antibodies to immunoglobulins, complement, fibrinogen, and BSA. There were numerous positive stainings of GCW similar in distribution and intensity to those seen in the nine SLE patients with active glomerulonephritis. This suggested that binding of anti-T and anti-C was specific for active lupus nephritis, whereas the other antibodies investigated were bound frequently by tissues obtained from patients with other nephropathies.

DISCUSSION

During the last 12 yr, a number of studies have contributed to a better understanding of immunological disorders characteristic of SLE in man. Several circulating antibodies reactive with native or altered nuclear antigens have been described (30–33). One potential antigen, DNA, has been found in the sera of patients with active SLE nephritis (34). Immunoglobulins and complement are localized in GCW (4–6) with a granular distribution resembling the pathology produced by circulating antigen-antibody complexes (35). Antinuclear antibodies are also concentrated in the glomerular lesions together with the antigen, DNA. The presence of DNA was demonstrated by means of anti-DNA antibody isolated from the serum of a patient with SLE. These last findings give strong support to the hypothesis that SLE nephritis in man is provoked by renal deposition of circulating antigen-antibody complexes formed by DNA, specific antinuclear antibodies, and complement (11).

The purpose of this paper is to report the localization in glomerular lesions of human SLE nephritis of fluorescein-conjugated antibodies of defined specificity. These antibodies are specific for pyrimidine bases of DNA, and react with denatured, but not with native, DNA. The specificity of localization was demonstrated by blocking experiments and also by the removal of the localizing activity by absorption with the homologous nucleoside-protein conjugate. The results reported in human SLE

nephritis are similar to those found in a study of lupus-like nephritis of NZB/NZW F1 mice (36).

The data obtained suggest the presence of denatured DNA in the foreign material forming granular deposits in the GCW (1–3) of eight SLE patients with active glomerulonephritis. Only one patient with active glomerulonephritis failed to localize the Fl-Abs to T and C. Failure of staining in two SLE patients with diffuse glomerular sclerosis and in two SLE patients with normal glomerular structures may be explained by the lack of reactive antigen in these areas. It is difficult to evaluate the role of denatured DNA in the pathogenesis of the disease. The presence in the same areas of immunoglobulins and of complement could be the result of accumulation of immune complexes formed by denatured DNA, antibodies to nucleosides, and complement, implying the presence of circulating denatured DNA, possibly as a result of a viral infection (38). Antibodies to denatured DNA have been described in SLE serum (27); and recently, in a study of a large series of SLE sera with the complement fixation text, Seligmann and Arana have shown that antibodies to denatured DNA are found more frequently than those to native DNA (37).

Another possible interpretation of the observations is that native DNA subsequent to deposition in GCW becomes denatured to a degree that permits interactions with anti-T and anti-C. In membranous nephropathies, morphologic appearances and immunologic reactivity of material contained in the foreign deposits may undergo changes during the course of the disease. This was also observed in the third biopsy of patient EC, since glomerular lesions of membranous transformation (29) appeared, and the intensity of staining for immunoglobulins and for T and C were markedly decreased. The determination of the specificity of antibodies eluted from the glomeruli of patients with SLE nephritis may present further evidence in elucidating the role of denatured DNA in the pathogenesis of the disease.

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