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Immunofluorescent Localization of Immunoglobulins, Complement, and Fibrinogen in Human Diseases. I. Systemic Lupus Erythematosus *

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Immunological mechanisms have been considered to play a major role in the pathogenesis of systemic lupus erythematosus (SLE). Although γ-globulin and complement have been demonstrated in the renal lesions of SLE (1–10), the specific immunoglobulins have not been characterized. The presence of fibrin has been previously noted (8, 10, 11), but the relationship of glomerular fibrin deposition to renal damage has not been ascertained. Therefore, the fluorescent antibody technique was employed to study the localization of the following proteins in tissue lesions: *1*) immunoglobulins (γ_2-, γ_{1M}-, and γ_{1A}-globulins), *2*) β_{1C}-globulin (complement), *3*) fibrinogen, and *4*) other plasma proteins (α_2-macroglobulin and albumin).

Methods

Necropsy specimens of kidney, liver, spleen, and heart from 16 patients with SLE and 4 normal kidneys from patients with no evidence of renal disease were frozen in dry ice-isopentane and stored at −20° C. Clinically characteristic cases of SLE with positive LE cell tests were chosen. The pertinent clinical and pathologic features are summarized in Table I. All specimens from patients with SLE showed a moderate to severe nephritis with typical wire loop lesions, focal necrosis, and occasional hematoxylin bodies. Arteriolar fibrinoid necrosis was present in the kidneys and spleen and occasionally in the heart and liver.

Formalin fixed paraffin sections were stained with hematoxylin-eosin, Gomori's elastica stain, phosphotungstic acid-hematoxylin (PTAH), and Lendrum's fibrin stain (12) and were subjected to the periodic acid Schiff reaction after diastase treatment.

Antisera against the following human plasma antigens were prepared in rabbits: *a*) γ_2-globulin prepared by DEAE cellulose fractionation (13); *b*) γ_{1M}-globulin isolated by water precipitation from a patient with Waldenström's macroglobulinemia (14) and further purified by column chromatography on DEAE cellulose (15); *c*) β_{1C}-globulin (complement) prepared by the method of Müller-Eberhard, Nilsson, and Aronsson (16); *d*) fibrinogen (Fraction I);[1] *e*) albumin isolated by ammonium sulfate precipitation (17).

Antisera to γ_{1A}-globulin[2] and to α_2-macroglobulin[3] were purchased. Purified antisera were titered against human serum or plasma by a semimicroprecipitin method (18); specificity of antisera was determined by immunoelectrophoresis (19) and the agar double diffusion method (20). Antisera against γ_2-globulin, γ_{1A}-globulin, β_{1C}-globulin, albumin, and α_2-macroglobulin showed only one precipitin line. Gamma$_{1M}$-globulin antiserum was absorbed with cord blood (21), and antifibrinogen antiserum[4] with human serum until only one reacting line against human plasma was seen on immunoelectrophoresis. Anti-γ_{1A}-serum was absorbed with purified γ_2-globulin.

Globulin fractions of antisera and normal rabbit serum were fluoresceinated (22) and incubated with acetone-fixed cryostat sections as previously described (23). The controls in the fluorescent antibody studies were the following: *a*) use of fluoresceinated normal rabbit globulin; *b*) incubation of fluoresceinated antisera with normal tissues (kidney, liver, spleen, and heart); *c*) blocking of the fluoresceinated antisera binding by prior application of nonfluoresceinated antisera. In addition, sections treated with anti-γ_{1M}-globulin followed by fluoresceinated anti-γ_2-globulin, or with anti-γ_2-globulin followed by fluoresceinated anti-γ_{1M}-globulin antiserum, were used to further assess the specificity of these antisera, and *d*) plasma cells stained with anti-γ_2-serum were not stained by anti-γ_{1M}-serum, and staining of plasma cells from a patient with macroglobulinemia with fluoresceinated anti-γ_2-serum could be blocked by prior application of anti-γ_{1M}-serum.

In vitro complement fixation was carried out, utilizing the method of Lachmann, Müller-Eberhard, Kunkel, and

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[2] Mann Research Laboratories, New York, N. Y.

[3] Lloyd Brothers, Cincinnati, Ohio.

[4] Since fibrinogen cannot be distinguished immunologically from fibrin, the term "fibrinogen" was used to indicate both.

Paronetto (8). Sections were treated with acid buffers as previously described (5, 23) in an attempt to elute γ-globulin from immune complexes. Fluorescence microscopy was performed with a Leitz Ortholux microscope, using two BG12 exciter filters and one OG4 or OG5 barrier filter. Pictures were taken with Anscochrome T/100 tungsten film or Kodachrome X and then converted to black and white negatives.

Results

Immunohistochemical observations in the kidneys (Table II). Gamma$_2$-globulin was localized in the glomeruli in all specimens (Figure 1A). The most frequent pattern was a diffuse membranous staining (Figure 1B), but occasionally a beaded pattern was noted (Figure 1C). Staining of the intercapillary space was frequent. Bowman's capsule also contained focal deposits of γ-globulin. Staining was absent in glomeruli showing partial or total hyalinization. Areas of fibrinoid necrosis in arterioles (Figure 1B), renal tubular casts, and hyaline droplets of the tubular epithelium contained γ_2-globulin. Scattered nuclear fluorescence, mainly of the epithelial tubular cells, was observed after direct staining with fluorescein labeled antihuman γ_2-globulin in three specimens (Patients No. 8, 13, 14).

Gamma$_{1M}$-globulin was seen in a pattern similar to that of γ_2-globulin, but fewer glomeruli and arterioles were stained. Nuclear staining was seen in the three specimens that showed γ_2 localization.

Gamma$_{1A}$-globulin was present in small focal deposits in rare glomeruli and arterioles, but did not show a membranous staining pattern (Figure 2A). The tubular epithelium in most cases showed a pale, diffuse staining (Figure 2A). Occasional casts were also stained. The nuclear staining was similar to that observed with γ_2- and γ_{1M}-antisera (Figure 2B).

Complement (β_{1C}-globulin) was detected in all specimens studied. Its deposition in renal glomeruli and occasionally in small arterioles paralleled that of γ_2- and γ_{1M}-globulin (Figure 3). No increased fluorescence was noted after incubation of sections with fresh human serum before fluores-

TABLE I

Clinical and pathological data of patients with systemic lupus erythematosus (SLE)

								Kidney					Spleen
									Gross	Microscopy			
Patient no.	Age	Sex	Race	Duration of illness	Proteinuria	BUN*	Blood pressure	Weight combined	Surface	Wire loops†	Focal necrosis†	Fibrinoid necrosis of arterioles†	Periarterial fibrosis†
				years		*mg/100 ml*	*mm Hg*	*g*					
1	39	F	W	5	4+	184	160/100	360	Pale, smooth, fleabites	+++	++	+	+
2	40	F	W	3	2+	74	190/100	460	Pale, smooth	+++	++	++	++
3	36	F	W	14	4+	17	170/110	390	Pale, smooth	++	+	±	+
4	17	F	W	2	1+	141	110/70	600	Pale, smooth	+	±	±	±
5	42	F	N	3	2+	14	105/75	395	Pale, smooth	±	±	±	+
6	22	M	W	3	3+	150	180/90	390	Pale, smooth	±	±	±	±
7	36	F	W	7	4+	31	180/110	350	Pale, smooth	++	±	+	+
8	44	F	W	13	4+	58	100/60	420	Pale, smooth, fleabites	+	±	+	±
9	14	F	W	2.5	3+	29	190/125	410	Pale, smooth, fleabites	+++	++	++	++
10	12	F	N	.5	3+	96	150/90	224	Granular, fleabites	+++	++	++	++
11	21	F	W	11	3+	350	180/130	225	Granular, fleabites	+	±	±	+
12	12	F	W	2	4+	132	250/150	400	Pale, smooth, fleabites	+++	++	+	+
13	38	F	N	.25	2+	34	240/130	290	Pale, smooth	++	+	±	++
14	39	F	W	1	4+	62	160/100	450	Pale, smooth	+++	++	++	++
15	36	F	N	3				440	Pale, smooth	+++	++	+	++
16	36	F	W	.25	2+	106	185/105	120	Granular	+	±	±	+

* BUN = blood urea nitrogen.
† ± = rare; + = few; ++ = moderate; +++ = many.

TABLE II

Renal localization of immunoglobulins, complement, and fibrinogen in systemic lupus erythematosus

Patient no.	Structures stained	γ_2-Globulin*	γ_{1M}-Globulin*	γ_{1A}-Globulin*	β_{1C}-Globulin (complement)*	Fibrinogen*
1	Glomeruli	+++				
	Vessels	++				
2	Glomeruli	+++				
	Vessels	++				
3	Glomeruli	+++				
	Vessels	+				
4	Glomeruli	+++				±
	Vessels	+				±
5	Glomeruli	++				±
	Vessels	++				±
6	Glomeruli	++	+		+	±
	Vessels	+	+		±	0
7	Glomeruli	+			+	0
	Vessels	++			+	0
8	Glomeruli	+++			++	+
	Vessels	++			+	±
9	Glomeruli	+++	+		++	++
	Vessels	+	+		±	±
10	Glomeruli	++	+		+	+
	Vessels	++	++		±	+
11	Glomeruli	++	+		++	±
	Vessels	+	±		++	±
12	Glomeruli	+++	++	+	+++	++
	Vessels	++	++	0	+	+
13	Glomeruli	+++	+++	0	+++	±
	Vessels	++	++	0	0	0
14	Glomeruli	+++	++	0	++	+
	Vessels	++	++	±	++	0
15	Glomeruli	+++	++	±	+++	++
	Vessels	++	+	±	±	+
16	Glomeruli	+	+	0	+	±
	Vessels	+	+	0	0	±

* 0 = negative; ± = rare; + = few; ++ = moderate; +++ = many. A blank space indicates that staining was not done.

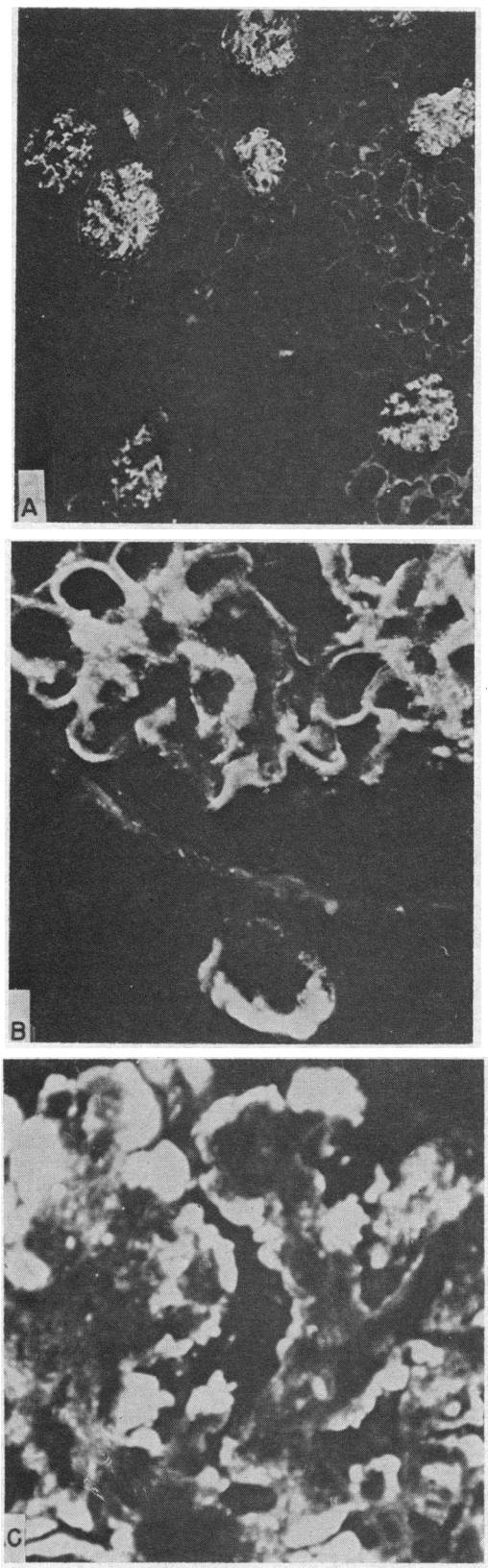
ceinated anti-β_{1C}-globulin. A rare nucleus that showed immunoglobulin deposition also exhibited bright staining.

Fibrinogen was present in the hyaline thrombi of glomerular loops, and scattered glomeruli showed a diffuse deposition of fibrinogen in a membranous pattern (Figure 4) similar to γ_2- and γ_{1M}-globulins and complement. Arterioles occasionally showed bright fluorescence in areas of fibrinoid necrosis. Tubular epithelium was not stained except for hyaline droplets, but some tubular casts fluoresced brightly. Nuclear staining was not observed. Phosphotungstic acid hematoxylin (PTAH) and Lendrum's fibrin stains demonstrated hyaline thrombi and occasional interstitial glomerular deposits of fibrin, but failed to detect the diffuse pattern of glomerular fibrin deposition noted by the fluorescent antibody technique.

Albumin and α_2-macroglobulin were localized infrequently only in hyaline thrombi and tubular casts.

Elution procedures. Treatment of sections with acid buffers markedly decreased the renal glomerular fluorescence associated with γ_2- and γ_{1M}-globulins in all specimens studied, but the fluorescence observed in tubular epithelium and casts associated with γ_{1A}-globulin was not decreased.

Staining of normal kidney sections revealed no glomerular localization of immunoglobulins, complement, or fibrinogen in unfixed or acetone fixed sections. Faint staining of tubular epithelium with anti-γ_{1A}-serum was noted.



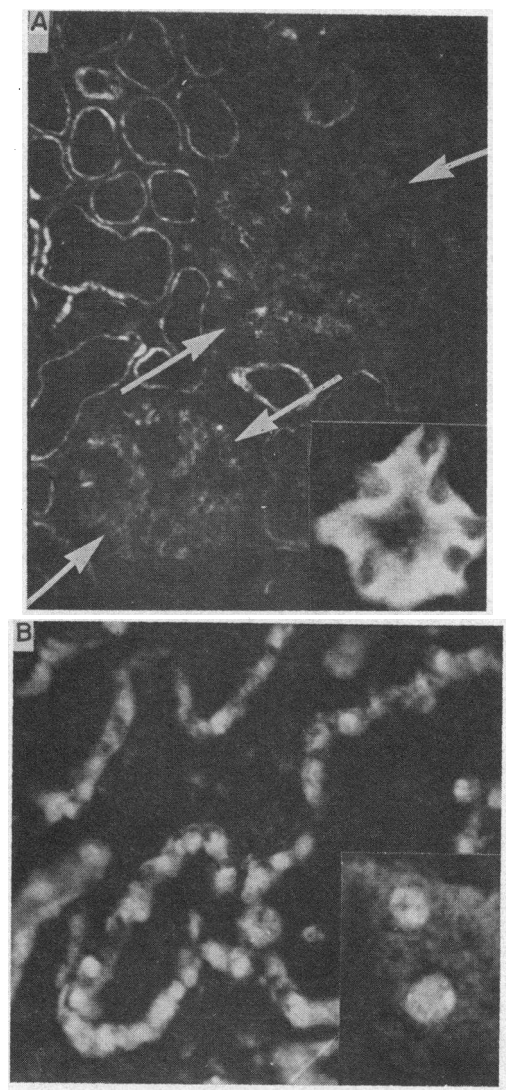


FIG. 2. KIDNEY SECTION FROM PATIENT NO. 13 TREATED WITH FLUORESCEINATED ANTI-γ_{1A}-GLOBULIN ANTISERUM. A. Absence of glomerular fluorescence (arrows) and positive staining of tubules (× 100). Exposure time doubled to emphasize tissue structure. Insert (lower right) shows diffuse epithelial cytoplasmic fluorescence (× 560). B. Nuclear staining of tubular epithelial nuclei (× 250) of diffuse type (insert × 960).

Immunohistochemical observations of spleen, heart, and liver. The spleen showed numerous

FIG. 1. SECTIONS OF KIDNEY FROM A PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) (No. 14), TREATED WITH FLUORESCEINATED ANTI-γ_2-ANTISERUM. A. Diffuse staining of multiple glomeruli and tubular epithelium containing hyaline droplets (× 35). B. Homogeneous diffuse fluorescence of wire loop lesion of glomerulus and adjacent arteriole (× 560). C. Beaded pattern of fluorescence in wire loop lesion (× 560).

plasma cells exhibiting γ_2-globulin, several cells containing γ_{1M}-globulin, and rare cells with γ_{1A}-globulin. Gamma$_2$-globulin deposition was prominent in the follicular arterioles (Figure 5) associated with small quantities of complement, fibrinogen, and γ_{1M}-globulin. Many arterioles showing "onion skin" lesions contained fibrinogen in the periarterial connective tissue and lesser amounts of γ-globulin.

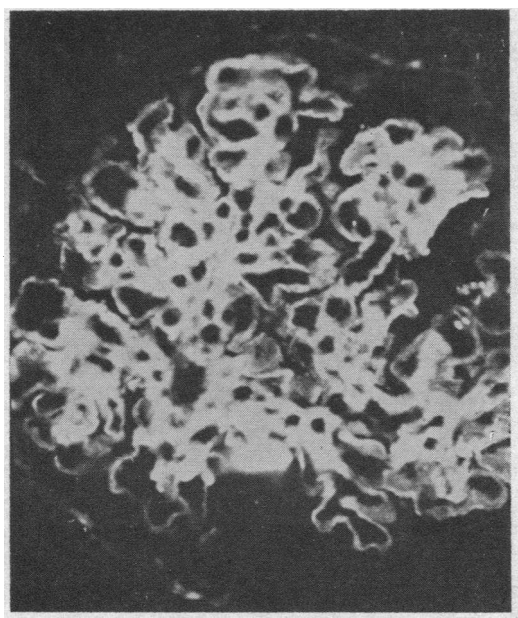


FIG. 3. KIDNEY SECTIONS FROM PATIENT NO. 13 STAINED WITH FLUORESCEINATED ANTI-β_{1C}-GLOBULIN ANTISERUM SHOWING DIFFUSE FLUORESCENCE OF BASEMENT MEMBRANES OF GLOMERULUS (× 250).

Occasional arterioles in the heart and liver with fibrinoid necrosis showed protein depositions similar to those in the arterioles of the kidney. No nuclear staining was encountered in these organs.

Discussion

Previous immunohistochemical investigations have demonstrated γ-globulin and complement in the kidneys, heart, spleen, and skin of patients with SLE (1–10, 24–28). Fibrinogen and other plasma proteins, however, have not been consistently demonstrated (Table III). The present study, which extends these observations, indicates that γ_2- and γ_{1M}- but not γ_{1A}-globulin are usually present in renal and vascular lesions of SLE.

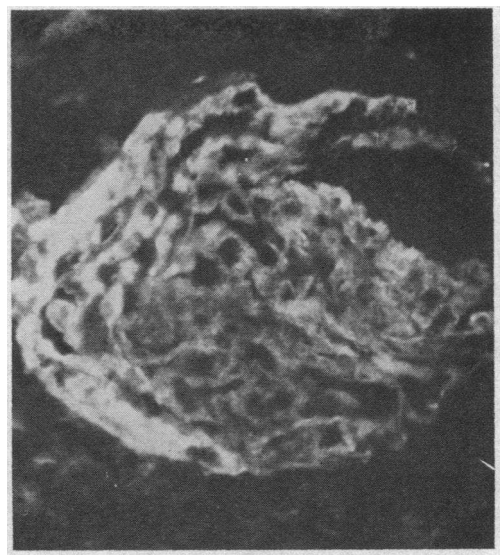


FIG. 4. KIDNEY SECTION FROM PATIENT NO. 14 SHOWING A PARTIALLY HYALINIZED GLOMERULUS WITH FIBRINOGEN IN BASEMENT MEMBRANE AND MORE INTENSE STAINING OF A CRESCENT (FLUORESCEINATED ANTIFIBRINOGEN ANTISERUM) (× 250).

The localization of complement and immunoglobulins in similar areas suggests the presence of immune complexes or aggregated γ-globulin (29, 30). The role of the latter in eliciting renal glomerular damage has been recently considered (27, 31). The elution of γ_2 and γ_{1M} at acid pH, how-

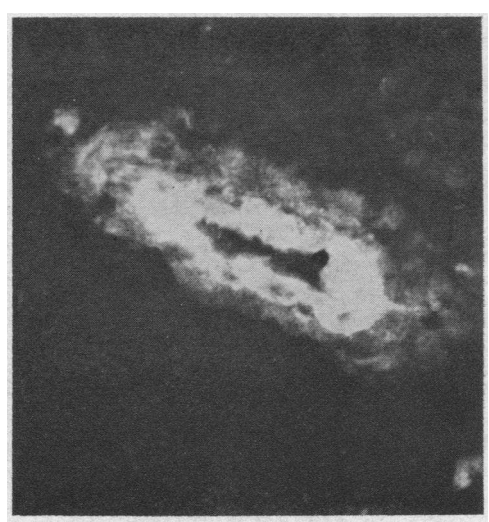


FIG. 5. SECTION OF SPLEEN FROM PATIENT NO. 15 SHOWING STAINING OF ARTERIOLE WALL AND FAINT FLUORESCENCE OF PERIARTERIAL "ONION SKIN" LESION, TREATED WITH FLUORESCEINATED ANTI-γ_2-GLOBULIN ANTISERUM (× 250).

ever, suggests that antibody is present as a component of an antigen-antibody complex. Aggregated γ-globulins are not eluted from tissue sections by a similar procedure (32). The consistent demonstration of γ_{1M} in areas of tissue damage indicates that this protein is of pathogenetic importance, in addition to γ_2-globulin.

The absence of α_2-macroglobulin and γ_{1A}-globulin from renal glomeruli indicates that macromolecules and immunoglobulins are not secondarily deposited in damaged tissues. By contrast, it appears that γ_{1A}-globulin localized in the renal tubular epithelium and not associated with *in vivo* or *in vitro* complement fixation is not part of a cytotoxic immune complex. This is consistent with the *in vitro* observation that γ_{1A}-globulin antibody does not fix complement (33).

The role of fibrinogen deposition in hyaline thrombi, basement membranes of renal glomeruli, and vessel walls has not been clarified. It has been suggested that intraglomerular fibrin deposition in rabbits may induce glomerular sclerosis (34),

TABLE III

Previous immunohistochemical investigations in systemic lupus erythematosus

No. of patients	Source of specimen*	Organs	Structures stained	Localization of†				Reference no.
				γ-Globulin	Complement	Albumin	Fibrinogen	
4	N	Kidney	Glomeruli	0		0	+	11
			Vessels	0		0	+	
		Spleen	Vessels	0		0	+	
		Heart	Artery	0		0	+	
			Valve pocket	0		0	+	
2	N	Kidney	Glomeruli	+				1
			Vessels	+				
3	N	Kidney	Glomeruli	+		0	0	2–4
			Vessels	+		0	0	
2	N	Spleen	Vessels	+		0		
10	B	Kidney	Glomeruli	+ 6				3
8	B	Kidney	Glomeruli	+ 7				5
			Vessels	+ 3				
15	B	Kidney	Glomeruli	+ 8				6
8	B	Kidney	Glomeruli	+	+			7
			Vessels	0	+ 2			
6	B	Skin	Dermal-epidermal junction	+			0	24
2	N	Kidney	Glomeruli	+	+		+	8
			Vessels	+	+			
2	B	Kidney	Glomeruli	+	+ 1			
			Vessels	+ 1	+ 1			
2	N	Spleen	Vessels	+	+			
2	N	Heart	Amorphous bodies	+ 1	+ 1			
7	N	Kidney	Glomeruli	+ ‡				9
			Vessels	+ 6				
2	B	Skin	Dermal-epidermal junction	+				25
2	B	Skin	Dermal-epidermal junction		+			26
Not given	B	Skin	Dermal-epidermal junction, clamps, nuclei	+	+			27–28
1§	N	Kidney	Glomeruli	+	+	0	0	10
			Vessels	+	+	+	+	
		Spleen	Vessels	+	0	0	0	
		Liver	Vessels	+	+	+	+	

* B = biopsy; N = necropsy.

† 0 = negative; + = positive; number after + indicates positive cases; a blank space indicates that the substance was not mentioned.

‡ Guinea pig complement was fixed *in vitro* in the same area.

§ This patient had lesions resembling those of periarteritis nodosa and malignant nephrosclerosis.

and that it may play a role in immunologically induced renal diseases (35). The diffuse deposition of fibrinogen in renal glomeruli of patients with SLE may contribute to the glomerular damage. The localization of fibrinogen in the renal glomeruli and vessels may result from an independent alteration of the coagulation system, or it may be secondary to vascular injury.

In vivo nuclear localization of γ-globulin has been previously described in the kidney (7) and skin (27, 28). The infrequent demonstration of *in vivo* nuclear γ-globulin or β_{1C}-globulin in the kidneys of our patients argues against a major autoaggressive pathogenetic role in renal lesions of antinuclear antibodies, although these antibodies may combine with nuclei of damaged cells. DNA and other tissue antigens may be components of antigen-antibody complexes localized in the kidney (36). Attempts to localize DNA antigen with human sera containing anti-DNA antibodies have been unsuccessful (32). The *in vivo* nuclear deposition of all three immunoglobulins is in agreement with the finding that antinuclear antibodies are present in the γ_2-, γ_{1M}-, and γ_{1A}-globulin fractions of serum (37).

The present study suggests that antibodies in the γ_2- and γ_{1M}-globulin fractions are components of complement fixing cytotoxic antigen-antibody complexes responsible for the renal and vascular lesions in systemic lupus erythematosus.

Summary

Immunofluorescence studies on tissues of patients with systemic lupus erythematosus (SLE) revealed γ_2- and γ_{1M}-immunoglobulins, complement, and fibrinogen localized in renal glomeruli and vessels of kidney, spleen, heart, and liver. $Alpha_2$-macroglobulin, albumin, and γ_{1A}-globulin were absent from glomeruli, but the latter was visualized in tubular epithelium. $Gamma_{1M}$- and γ_2-globulins were eluted by acid buffers. Nuclear localization of all immunoglobulins was seen in the renal tubular epithelium in only 3 of the 16 patients investigated. These findings support the hypothesis that γ_2- and γ_{1M}-globulins are antibody components of immune complexes localized in the vascular and glomerular lesions of SLE. Fibrinogen deposition may contribute to renal glomerular damage.

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