LABORATORY INVESTIGATION

153

Vol. 18, No. 2, p. 159, 1968

Human Renal Transplants

II. Immunofluorescent and Immunoferritin Studies

K. A. PORTER, M.D., G. A. ANDRES, M.D., M. W. CALDER, M.B., B.S., J. B. DOSSETOR, M.D., K. C. HSU, PH.D., JANE M. RENDALL, BEATRICE C. SEEGAL, M.D., AND T. E. STARZL, M.D.

Department of Pathology, St. Mary's Hospital, London, W.2., England; Department of Renal and Urological Research, Royal Victoria Hospital, Montreal, Canada; Department of Surgery, University of Colorado Medical Center, Denver, Colorado 80220; Instituto Patologia Medica, Universita di Roma, Rome, Italy; and Department of Microbiology, Columbia University, New York, New York 10032

Seventy-one human renal allografts treated with immunosuppressive agents and two renal isografts were biopsied 18 days to 8 years after transplantation. The tissue obtained was studied by immunofluorescent and immunoferritin techniques using antisera to human immunoglobulin G (IgG), immunoglobulin M, (IgM), β1C/β1A-globulins, C'1q and fibrinogen, and also to type 12 streptococci.

Deposits of amorphous material were detected by electron microscopy on the subendothelial aspects of the glomerular capillary basement membranes of 54 (76 per cent) of the 71 allografts. Three of the grafts with subendothelial deposits also showed focal collections of electron-dense material on the epithelial surfaces of the capillary basement membranes (subepithelial "humps"). Mesangial deposits were common in those grafts that contained capillary basement membrane accumulations.

In 49 (90 per cent) of the 54 allografts with subendothelial deposits, immunofluorescent examination showed IgM distributed along the glomerular capillary walls in a linear or finely granular pattern. The IgM was accompanied by complement (β1C/β1A-globulins or C'1q) in 35 (65 per cent) of the grafts, by IgG in 16 (30 per cent), and by fibrinogen in 12 (22 per cent) of the grafts. The distribution of IgG and complement was similar to that for IgM, but fibrinogen was distributed in a focal mesangial pattern. The use of ferritin-conjugated antisera showed that the IgM and complement were localized in the amorphous deposit on the endothelial aspect of the glomerular capillary basement membranes, in the inner part of the lamina densa, and in the foreign material in the mesangium.

Upon immunofluorescence the three renal allografts with subepithelial humps exhibited additional, distinctive, nodular, "lumpy-bumpy" deposits of IgG, IgM, and complement along the epithelial side of the glomerular capillary basement membranes.

Immunoglobulins, complement, and fibrinogen were absent from the glomeruli of 17 of the 18 renal allografts with normal capillary basement membranes and from the glomeruli of one of the renal isografts. In the other isograft there was binding of fluorescein-labeled antibody to IgM, C'1q, and fibrinogen in several of the glomeruli, and ultrastructurally there was a thin linear subendothelial deposit on some of the capillary basement membranes.

It is suggested that the subendothelial deposits of IgM and complement found in the glomerular capillary walls of many of the allografts occurred through the reaction of circulating antibodies with antigens in the capillary basement membranes of the graft. These deposits resembled in many ways those produced by antiglomerular antibodies in nephrotoxic serum nephritis.

By contrast, the large lumpy-bumpy subepithelial deposits of IgG, IgM, and complement in three of the grafts were probably caused by transmission of active glomerulonephritis from the recipient to the allograft. Similar lesions are produced by circulating antigen-antibody complexes in chronic serum sickness nephritis and are regularly seen in acute poststreptococcal glomerulonephritis.

The changes in one of the two renal isografts are discussed but cannot be explained adequately.

Additional key words: Kidney, Homograft, Allograft, Isograft, Glomerulus, Glomerulonephritis transmission, Immunoglobulin M, Complement.

Recently we reported that, of 50 human renal allografts biopsied 43 days to 2 years 3 months after transplantation, 37 showed subendothelial accumulations of amorphous material on the glomerular capillary basement membranes.[37] In 27 of the kidneys these deposits were large, and the basement membrane thickening was obvious under light microscopy; in 10 they were small and only demonstrable with the electron microscope. It was suggested that these lesions were caused by deposition of circulating antibody directed specifically against

the graft. Two of the transplants were unusual in that they showed additional, characteristic, subepithelial "humps,"[32] and in these cases it was considered probable that there had been transmission of the recipient's active glomerulonephritis to the allograft. All of the patients were receiving immunosuppressive treatment with azathioprine which was either used alone or in combination with prednisone.

It is the purpose of the present communication to report the frequent occurrence of immunoglobulin M (IgM) in these glomerular lesions. The immunofluorescent findings are described in 51 human renal allografts with subendothelial glomerular capillary basement membrane deposits, in 3 similar grafts with subepithelial "humps," in 17 allografts that did not show any capillary basement membrane changes, and in 2 renal isografts. Immunoferritin studies were undertaken in some of the transplants.

MATERIALS AND METHODS

PATIENTS

Seventy patients with renal allografts were studied: 41 from the University of Colorado Medical Center, Denver, Colorado; 10 from the Royal Victoria Hospital, Montreal, Canada; and 19 from St. Mary's Hospital, London, England. The Denver patients had received kidneys from living donors and each recipient is denoted by a number preceded by the letters LD (Tables 2 to 4). The same code has been used previously,[37, 41, 42, 44] and further details of any of the patients can be obtained by referring to these publications. The Montreal and London patients had all received kidneys from cadavers. The Montreal patients are denoted by a number prefixed by the letter M; the London patients by their initials. All of the patients had had their own kidneys removed and splenectomy had been performed on the Denver and St. Mary's patients. The thymus had been removed from 11 of the Denver patients, six before (LD65, LD67, LD68, LD70, LD107, and LD109) and five from 250 to 520 days after transplantation (LD30, LD40, LD41, LD50, and LD54). At the time of biopsy all of the patients were receiving 50 to 200 mg. of azathioprine and 2.5 to 25 mg. of prednisone per day. Thirty-four patients were being treated with one or more of the antihypertensive drugs—chlorothiazide, hydralazine, reserpine, and methyldopa. Eight patients (LD107 to LD114) were receiving equine anti-human lymphocyte globulin.[43]

Two patients with renal isografts were also studied (Table 1).[7, 41] Both individuals had undergone bilateral nephrectomy but not splenectomy or thymectomy. At no time had these patients been treated with immunosuppressive or antihypertensive drugs.

TISSUE PROCESSING

Tissue was obtained from each renal graft by open biopsy after transplantation at the times indicated in Tables 1 to 4. Two allografts (MS and M30) were rebiopsied 14 months after the first sample had been obtained. Each biopsy specimen was divided into three or four pieces. One portion was fixed in 10 per cent neutral formalin or buffered formol-saline, embedded in paraffin wax, serially sectioned, and stained with hematoxylin and eosin, periodic acid-Schiff reagent, Weigert's stain for elastic tissue, and methyl green-pyronin. Another part of the biopsy was immediately cut into small cubes, fixed in Palade's buffered osmium tetroxide, and embedded in Epon 812. Sections, 0.5 μ thick, cut from these blocks were stained with azure II and examined by light microscopy. Very thin sections for electron microscopy were stained with lead hydroxide, uranyl acetate, or a combination of both, and examined in a Siemens-Elmiskop IA. The third piece of tissue was snap-frozen in Dry Ice and alcohol or in isopentane in liquid nitrogen and sectioned in a cryostat at 4 μ, and the unfixed tissue was stained by the direct immunofluorescent method with fluorescein-labeled antisera.[27, 40] In some biopsies a fourth part was used for ferritin-labeled antibody studies.

PREPARATION OF ANTISERA FOR IMMUNOFLUORESCENCE

Antisera, labeled with fluorescein isothiocyanate, were prepared against the following: human IgG and IgM, both of which were isolated by diethylaminoethyl (DEAE) Sephadex A-50 chromatography;[9] human β1C/β1A-globulins (parts of C'3), which were prepared by a column chromatographic method;[31] human C'1q (11 S protein) purified according to the procedure of Morse and Christian;[30] human fibrinogen isolated by the method of Laki;[24] human albumin; rabbit γ-globulin; horse γ-globulin; and a heat-killed suspension of group A type 12 streptococci. The antisera to human IgG, IgM, and β1C/β1A-globulins were prepared in goats and rabbits; the antiserum to rabbit γ-globulin was prepared in a duck; and those to human C'1q, human albumin, equine globulin, and type 12 streptococci were prepared in rabbits. Most of these antisera yielded single precipitin lines against their antigens in whole human serum by immunoelectrophoresis and by the agar double gel diffusion method.[26] The fluorescein labeling was performed as described by Riggs *et al.*[39] The labeled antisera were passed through a Sephadex G-25 column before storing and immediately before use were absorbed with mouse liver powder. The antisera to human albumin and to rabbit γ-globulin were used as control stains, and in no instance was positive glomerular binding obtained. The specificity of the fluorescence was established by using an inhibition test in which the sections were pretreated with unlabeled antisera. Sections were also treated with conjugated antisera that had been absorbed with their specific antigens. In a few instances sections were treated with acid buffers[34] in an attempt to elute γ-globulin from the lesions in the renal allografts.

Unfixed sections from three apparently normal unused donor kidneys from very recently dead subjects were treated with the fluorescein-labeled antisera. There was no glomerular localization of immunoglobulins, complement, or fibrinogen.

FERRITIN LABELING

The method for conjugating antisera globulins with ferritin has been previously described.[2, 38] Fragments of the renal biopsies, after fixation for 1 hour at 0° C. in 5

TABLE 1. IMMUNOFLUORESCENT FINDINGS IN GLOMERULI OF TWO HUMAN RENAL ISOGRAFTS

Recipient			Donor	Time biopsy taken after transplant	Renal function at time of biopsy		Fluorescent microscopy[a]				
Patient no.	Sex and age (yr.)	Disease[b]			Ccr[c]	Urinary protein	IgG	IgM	Complement		Fibrinogen
									βIC/βIA	C'1q	
					ml./min.	*mg./100 ml.*					
IDT2	M, 18	CPGN	Identical twin	2 yr. 3 mo.	115	37	0	0	0		0
MIT1	F, 15	?CPyN	Identical twin	8 yr.	90	0	0	+	0	+	+

[a] 0, Negative; +, slight in amount.
[b] CPGN, Chronic proliferative glomerulonephritis; CPyN, chronic pyelonephritis.
[c] Ccr, Creatinine clearance.

per cent formalin, buffered with phosphates at pH 7.2, were washed and then cut into very small pieces under the dissecting microscope in a cold room. The specimens were then immersed in ferritin-conjugated antibody for 20 minutes at room temperature, washed three times in buffer, fixed in osmic acid, and embedded in Araldite or Epon 812. Some fragments were exposed to ferritin-labeled antiserum to goat globulin and to purified ferritin alone in order to check the specificity of ferritin binding in the tissues.[1, 40]

RESULTS

RENAL ISOGRAFTS (TABLE 1)

ELECTRON MICROSCOPY. In one of the transplants (MIT1) several glomeruli were completely fibrosed and a few showed increased amounts of mesangial matrix. The surviving glomeruli and all of those in the other transplant (IDT2) were hypertrophied. Ultrastructurally, the capillary basement membranes were generally normal, although in both kidneys there were small subendothelial thickenings and irregularities. A thin linear subendothelial deposit (Fig. 1) was present in a few glomerular capillary loops of one of the grafts (MIT1). There were focal areas of fusion of the visceral epithelial foot processes in both renal isografts and mesangial cell hyperplasia in one of the kidneys (MIT1). The endothelial cells were normal and the capillary lumina were patent and contained red cells and occasional platelets.

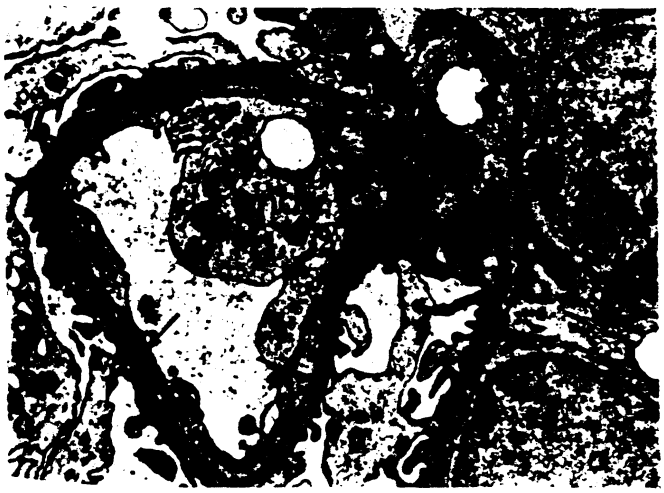

FIG. 1. Electron micrograph of part of a glomerulus from a renal isograft (MIT1) 8 years after transplantation. There is a fine linear subendothelial deposit (*arrows*) on the capillary basement membrane. There is slight fusion of epithelial foot processes and hyperplasia of the mesangial cells (*mc*). *end*, Endothelial cell; *ep*, epithelial cell. Lead hydroxide; ×7000.

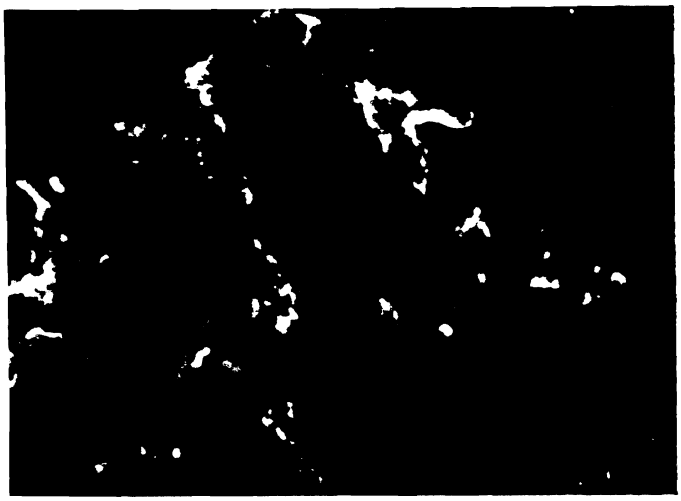

FIG. 2. Same renal isograft as in Figure 1. Frozen section treated with fluorescein-labeled goat antibody to human IgM. Finely granular deposits of IgM are present in some of the capillary walls and in the mesangium. ×600.

IMMUNOFLUORESCENCE. In one of the isografts (MIT1) there was binding of fluorescein-labeled antibody to IgM, C'1q, and fibrinogen in several of the glomeruli (Table 1). The IgM and C'1q were distributed in a finely granular capillary pattern (Fig. 2). The fibrinogen was distributed in a predominantly mesangial pattern. The second isograft (IDT2) showed no glomerular binding of fluorescein-labeled antisera.

RENAL ALLOGRAFTS

From 71 allografts, 73 biopsies were available for analysis. After electron microscopic examination the biopsies were divided into three groups: those with normal glomerular capillary basement membranes, those with subendothelial glomerular capillary deposits, and those with subepithelial humps[32] as well as subendothelial basement membrane accumulations.

RENAL ALLOGRAFTS WITH NORMAL GLOMERULAR CAP-

TABLE 2. CLINICAL DATA ON 18 PATIENTS WHOSE RENAL ALLOGRAFTS SHOWED NO GLOMERULAR CAPILLARY BASEMENT MEMBRANE DEPOSITS

Recipient			Donor, sex, age (yr.), and relationship	Time biopsy taken after transplant	Renal function at time of biopsy	
Patient no.	Sex and age (yr.)	Disease[a]			Ccr[b]	Urinary protei
					ml./min.	*mg./100 ml.*
LD17	F, 15	CPyN	F, 44, mother	2 yr. 1 mo.	71	8
LD25	M, 35	CPGN	M, 29, brother	2 yr.	84	5
LD33	F, 18	CPGN	F, 42, mother	1 yr. 11 mo.	184	0
LD34	F, 8	CPGN	F, 30, mother	1 yr. 11 mo.	59	0
LD39	M, 17	CPGN	F, 48, mother	1 yr. 10 mo.	87	20
LD42	F, 19	CPGN	M, 47, father	1 yr. 9 mo.	84	9
LD58	M, 27	CPGN	F, 23, sister	1 yr. 9 mo.	114	4
LD65	F, 35	CPGN	F, 41, sister	2 yr. 1 mo.	80	0
LD108[c]	M, 33	CPGN	M, 34, brother	4½ mo.	126	10
LD112	M, 29	CPGN	F, 45, mother	3½ mo.	134	10
M8	M, 24	Hydr	F, 53, cadaver	1 yr. 2 mo.	50	100
M15	M, 22	CPGN	M, 34, cadaver	8 mo.	35	80
M21	M, 37	CPGN	M, 22, cadaver	7 mo.	80	80
M29	M, 25	CPGN	M, 17, cadaver	79 da.	45	40
JJ	F, 29	CPyN	M, 9, cadaver	96 da.	55	0
EJ[d]	F, 44	CMGN	F, 22, cadaver	23 da.	25	0
JK	M, 20	CPGN	F, 53, cadaver	104 da.	7	10
DP	F, 24	CPGN	M, 13, cadaver	11 mo.	96	5

[a] CPyN, Chronic pyelonephritis; CPGN, chronic proliferative glomerulonephritis; Hydr, hydronephrosis; CMGN, chronic mem branous glomerulonephritis.

[b] Ccr, Creatinine clearance.

[c] Binding of fluorescein-labeled antibody to IgM and C'1q.

[d] Second renal allograft. The first transplant is listed in Table 3.

ILLARY BASEMENT MEMBRANES. There were biopsies from 18 transplants in this group (Table 2). Only one (LD108) showed glomerular binding of fluorescein-labeled antisera; in this kidney IgM and C'1q were deposited in a granular capillary pattern. Five of these transplants showed more glomerular mesangial matrix than normal.

RENAL ALLOGRAFTS WITH SUBENDOTHELIAL DEPOSITS ON THEIR GLOMERULAR CAPILLARY BASEMENT MEMBRANES. There were 52 biopsies in this group from 51 renal allografts (Table 3). One of the kidneys (M8) had shown no deposits when first biopsied, but developed glomerular lesions in the following 15 months. Another allograft (M30) that had been biopsied on two occasions showed subendothelial glomerular deposits in both specimens.

Light Microscopy. Thickening of the glomerular capillary basement membranes was detectable in 28 of the biopsies. This thickening was generally focal within the glomerular tufts; less frequently it was a diffuse process (Fig. 3). Sometimes it was accompanied by localized areas of increased tuft cellularity and adhesions between tuft and capsule. Epithelial capsular crescents and lobular lesions containing trapped polymorphonuclear leukocytes were rare. The amount of mesangial matrix was always increased, often markedly so.

Electron Microscopy. In all 52 biopsies the glomerular capillary basement membranes were altered by subendothelial accumulations of amorphous material.[37] The deposits were marked in amount in 3 of the specimens, moderate in 16, slight in 16, and minimal in 17 of the biopsies. The density and compactness of the deposit varied; in four kidneys it was as though the lamin densa had been split and a broad band of loosely ar ranged material inserted between the two layers. Com monly, processes from mesangial cells extended into th thickened basement membranes.[20] In all of the biopsie the epithelial and endothelial cells were hypertrophiec and contained increased numbers of cytosomes, cyto segresomes, and ribosomes; increased amounts of rougl endoplasmic reticulum; and enlarged Golgi bodies. Multi vesicular bodies and endothelial cytofolds[10] were com mon; centrioles were prominent and frequent. There wa fusion of the epithelial foot processes, and in 18 of the al lografts there was hyperplasia of the mesangial cells. Epi thelial microvilli were present in several of the kidneys In the more severely affected transplants the enlarged en dothelial cells caused narrowing and obliteration of some capillary lumina. Intracapillary groups of monocytes and lymphoid cells were frequent, and in the seven biopsies taken during a rejection episode, capillary loops were blocked by aggregated masses of platelets. In three of the allografts many of the platelets were intermixed with fibrin.

Immunofluorescence. IgM was present in the glomeruli of 46 of the renal allografts. It was distributed in a capillary pattern, outlining the walls of the peripheral parts of the capillaries and occurring in the mesangium. In the majority of the biopsies the fluorescence was focal within the glomeruli; diffuse staining was only found in the more severely damaged renal allografts. The staining of the capillary walls was either linear, forming an uniterrupted

TABLE 3. IMMUNOFLUORESCENT FINDINGS IN GLOMERULI OF 51 HUMAN RENAL ALLOGRAFTS WITH SUBENDOTHELIAL CAPILLARY BASEMENT MEMBRANE (bm) DEPOSITS VISIBLE ON ELECTRON MICROSCOPY (EM)

Recipient			Donor sex, age (yr.), and relationship	Time biopsy taken after transplant	Renal function at time of biopsy		Amount of glomerular capillary bm deposit on EM[a]	Fluorescent microscopy[a]				
Patient no.	Sex and age (yr.)	Disease[b]			Ccr[c]	Urinary protein		IgG	IgM	Complement		Fibrinogen
										βIC/βIA	C'1q	
					ml./min.	*mg./100 ml.*						
LD41	M, 3	CPGN	F, 37, mother	1 yr. 10 mo.	33	11	+++	+	++	+		+
LD54[d]	M, 21	CPGN	M, 38, unrelated	1 yr. 10 mo.	89	200	+++	+	+++	+		++
LD63	M, 35	CPGN	M, 33, unrelated	1 yr. 10 mo.	95	16	+++	++	+++	+		++
LD18	M, 39	CMGN	F, 44, sister	2 yr. 2 mo.	61	13	++	+	+	+		0
LD27[d]	F, 20	CMGN	M, 22, unrelated	2 yr.	59	90	++	+	+	0		0
LD30	M, 40	CMGN	M, 28, unrelated	1 yr. 11 mo.	70	310	++	+	++	+		0
LD40	F, 21	CMGN	F, 57, mother	1 yr. 10 mo.	62	18	++	+	++	+		0
LD45	M, 35	CPGN	F, 29, sister	2 yr. 1 mo.	65	20	++	0	+	+		0
LD51	M, 18	CPGN	F, 56, aunt	1 yr. 11 mo.	92	4	++	+	+	+		0
LD60	M, 21	CPGN	M, 23, cousin	1 yr. 10 mo.	90	5	++	+	++	+		0
LD70	F, 27	CPGN	M, 40, unrelated	1 yr. 10 mo.	43	0	++	0	++	0	++	0
LD71	M, 37	CPGN	M, 43, unrelated	1 yr. 10 mo.	72	0	++	0	+	0	+	0
LD111	F, 27	CPGN	M, 25, brother	4 mo.	87	12	++	0	+	0	0	0
LDBS	M, 16	CPGN	F, 40, mother	2 yr. 5 mo.	10	200	++	+	++	+		0
M13	M, 28	CPyN	F, 29, cadaver	10 mo.	50	0	++	0	++	+		0
DB	M, 37	Poly	M, 43, cadaver	1 yr. 7 mo.	73	150	++	0	++	+		0
EN	F, 24	CPyN	M, 21, cadaver	1 yr. 5 mo.	112	30	++	0	++	0		0
AR[d]	M, 37	CPGN	F, 41, cadaver	43 da.	4	200	++	+	+	+		+
PW	F, 22	CPyN	F, 15, cadaver	1 yr. 8 mo.	61	200	++	0	+	0	+	0
LD22	F, 15	Poly	F, 41, mother	2 yr. 1 mo.	66	34	+	0	+	0		0
LD48	M, 34	CPGN	F, 29, sister	2 yr.	58	105	+	0	+	0		0
LD55	M, 21	CPGN	M, 49, father	1 yr. 11 mo.	82	14	+	0	+	0		0
LD67	M, 36	CPGN	M, 28, unrelated	2 yr.	54	300	+	0	0	0	0	0
LD68	F, 24	CPyN	F, 23, sister	1 yr. 11 mo.	81	0	+	0	+	0	0	0
LD75	M, 19	CPGN	M, 38, father	1 yr. 9 mo.	78	12	+	+	±	0	±	+
LD110	M, 36	CPGN	F, 34, sister	4 mo.	71	0	+	0	+	++	+	+
LD113	M, 24	CPGN	F, 22, sister	3½ mo.	137	10	+	0	+	±	+	0
LD114	M, 34	CPGN	M, 38, brother	3½ mo.	75	0	+	0	+	0	0	±
M8[e]	M, 24	Hydr	F, 53, cadaver	2 yr. 5 mo.	51	6	+	0	++	±	+	0
M30	M, 29	CPGN	M, 57, cadaver	4 mo.	60	70	+	0	+	+		0
M30[e]				1 yr. 6 mo.	74	63	+	0	+	+	+	0
AE	F, 24	CPyN	M, 23, cadaver	1 yr.	68	30	+	0	+	+	+	0
RE	M, 18	CPGN	M, 20, cadaver	1 yr. 2 mo.	59	30	+	0	+	0	+	0
RM	M, 8	CPyN	M, 13, cadaver	11 mo.	40	0	+	+	+	+	+	0
RM	M, 17	CLGN	M, 38, cadaver	1 yr.	65	100	+	0	++	0	0	0
M33[d]	M, 21	Good	F, 65, cadaver	1 mo.	0	0	±	0	+	0	+	0
LD37	M, 21	CPGN	F, 43, mother	1 yr. 10 mo.	159	22	±	0	0	0		0
LD49	M, 32	CMGN	F, 41, sister	1 yr. 10 mo.	70	4	±	0	+	+		0
LD50	F, 16	CPGN	M, 38, father	2 yr.	121	2	±	0	+	0		0
LD52	F, 15	CPGN	M, 56, father	1 yr. 11 mo.	103	8	±	0	0	0		0
LD53	M, 15	CPGN	M, 35, uncle	1 yr. 11 mo.	101	3	±	0	0	0		0
LD107	F, 15	CPGN	M, 21, brother	5 mo.	119	10	±	0	+	0	++	0
LD109	M, 31	CPGN	M, 34, brother	4½ mo.	107	0	±	0	+	0	+	0
M31	M, 42	CPGN	M, 20, cadaver	4½ mo.	55	50	±	0	+	0	+	0
M32	F, 13	CPGN	M, 38, cadaver	4 mo.	50	56	±	0	±	0	0	±
CB[d]	M, 25	CPGN	M, 43, cadaver	11 mo.	33	0	±	0	+	0		0
KF	M, 17	CPyN	F, 41, cadaver	1 yr. 10 mo.	124	0	±	0	0	0		0
DL	F, 30	CPyN	M, 44, cadaver	1 yr. 8 mo.	85	159	±	0	+	0	0	0
EJ[d]	F, 44	CMGN	F, 38, cadaver	1 mo.	20	50	±	0	+	+		+
AW[d]	M, 16	CPGN	M, 17, cadaver	18 da.	5	0	±	0	+	+		0
PL	M, 24	Poly	F, 34, cadaver	1 yr. 8 mo.	72	0	±	0	+	±	+	0
BM	M, 32	CPGN	M, 17, cadaver	10 mo.	98	0	±	0	+	0	0	0

[a] 0, Negative; ±, minimal in amount; +, slight in amount; ++, moderate in amount; +++, marked in amount.
[b] CPGN, Chronic proliferative glomerulonephritis; CMGN, chronic membranous glomerulonephritis; CPyN, chronic pyelonephritis; Poly, bilateral polycystic disease; Hydr, hydronephrosis; CLGN, chronic lobular glomerulonephritis; and Good, Goodpasture's syndrome.
[c] Ccr, Creatinine clearance.
[d] Biopsied during rejection episode.
[e] Second biopsy.

smooth line, or granular, or a combination of the two (Figs. 4 and 5). In 24 of the transplants βIC/βIA-globulins were present; they were distributed in a focal, capillary, linear, or granular pattern (Fig. 6). In 16 of 24 kidneys tested with antibody to C'1q, this fraction of complement was present and the staining pattern was the same as for IgM. IgG was found in 13 of the allografts, but staining was only pronounced in one of these: fluorescence within the glomeruli was capillary in pattern, diffuse, and granular. Fibrinogen was present in nine of the transplants and showed a focal mesangial pattern, staining few capillary walls. Labeled antisera to type 12 streptococci and to equine globulin gave no glomerular localization.

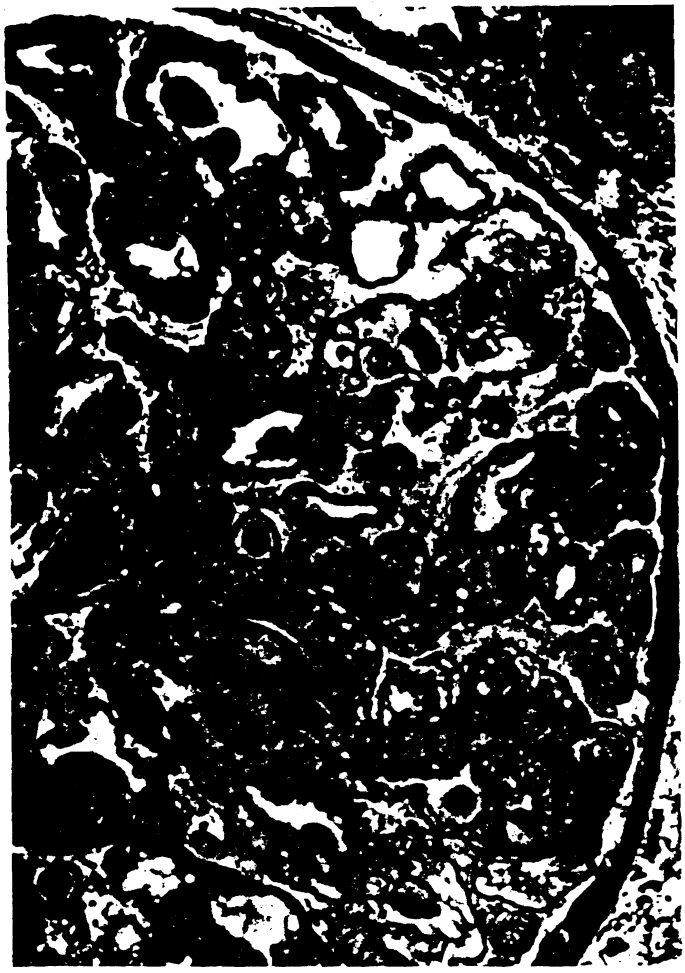

FIG. 3. Biopsy of renal allograft 2 years 1 month after transplantation (LD45). In this Epon-embedded, 0.5-μ section, there are more mesangial cells and matrix than normal and the endothelial cells are prominent. The capillary basement membrane thickening is uneven and subendothelial. Several of the thickened basement membranes are split by mesangial cell extensions. Azure II; ×1000.

Treatment of sections from four allografts (LD41, LD54, LD63, and LDBS) with acid buffers almost abolished the renal glomerular fluorescence for IgG and IgM.

Immunoferritin. Treatment of glomerular tissue from 24 renal allografts with ferritin-labeled antisera demonstrated that IgM, β1C/β1A-globulins, and C'1q were all localized in the collections of amorphous material that were seen by electron microscopy. Ferritin molecules were densely clustered in the linear subendothelial capillary basement membrane deposits (Fig. 7), and in the innermost part of the lamina densa. Ferritin was not found in the outer part of the lamina densa or in the lamina externa rara. The matrix-like foreign material in the mesangium also became heavily labeled with ferritin. In two patients IgG was demonstrated in the capillary basement membrane and mesangial deposits. The controls were consistently negative.

RENAL ALLOGRAFTS WITH SUBEPITHELIAL HUMPS AS WELL AS SUBENDOTHELIAL DEPOSITS ON THEIR GLOMERULAR CAPILLARY BASEMENT MEMBRANES. There were three kidneys in this group (Table 4).

Light Microscopy. All of the glomeruli were abnormal. Many showed accentuated tuft lobulation and marked capillary basement membrane thickening (Figs. 8 and 9). Many of the tufts were hypercellular and some of the increased cellularity was caused by polymorphonuclear leukocytes. Epithelial capsular crescents were frequent. In the renal allograft from patient RT, crescents were so common as to give the picture of a rapidly progressive acute glomerulonephritis. Lobular and basement membrane lesions predominated in the kidneys from patients M17 and LD73.

Electron Microscopy. There were dense hemispherical humps of homogeneous, electron-dense material on the epithelial surface of the glomerular capillary basement membrane in each of the kidneys (Fig. 10). A few of these focal deposits lacked a lamina rara externa and resembled Kimmelstiel's "variant A."[32] Impacted neutrophil polymorphonuclear leukocytes in various stages of disintegration and surrounded by fibrin and strands of basement membrane-like material were common in the capillary loops and mesangium (Fig. 11). In addition there were subendothelial basement membrane deposits and the same endothelial, epithelial, and mesangial changes as were present in the other renal allografts (Fig. 12).

Immunofluorescence. Intense staining for IgM, IgG, complement, and fibrinogen was present in all of the glomeruli of these three allografts. The IgM and IgG

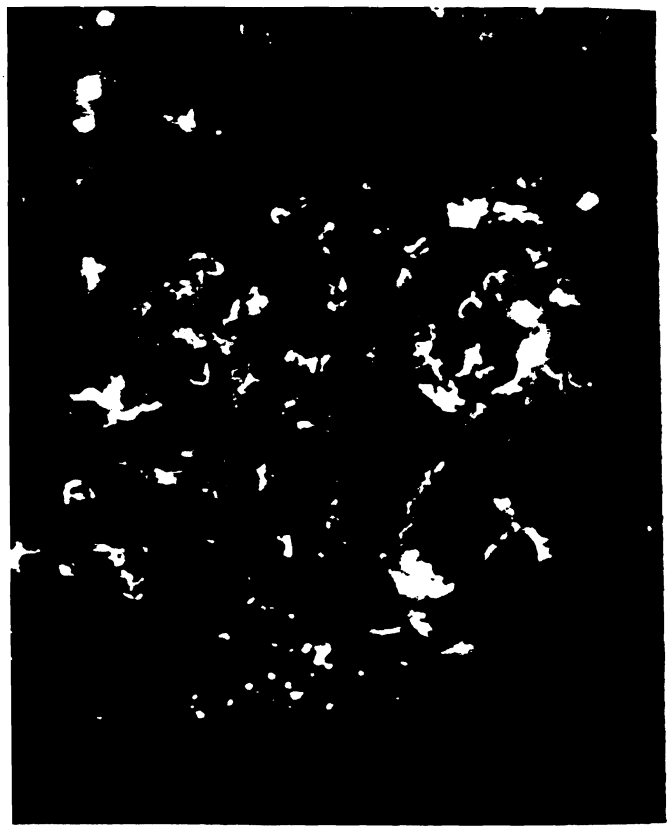

FIG. 4. Biopsy of a renal allograft 3½ months after transplantation (LD114). Frozen section treated with fluorescein-labeled goat antibody to human IgM. There is localization of IgM in the capillary walls of a glomerulus and in the cytoplasm of a few infiltrating mononuclear cells that are lying in the interstitium. ×200.

were distributed in a diffuse, capillary, coarsely granular pattern which gave a beaded or "lumpy-bumpy" [15] appearance. In some capillary loops the immunoglobulin deposits were clearly on the epithelial side of the basement membrane (Figs. 13 and 14). β1C/β1A-globulin and C'1q staining was in a combined capillary, linear, and beaded pattern (Fig. 15). The fluorescence for fibrinogen was mesangial and focal. There was no staining with fluorescein-labeled antisera to type 12 streptococci.

DISCUSSION

Earlier we described the frequent occurrence of subendothelial collections of amorphous material on the glomerular capillary basement membranes of human renal allografts.[37] That paper was based upon an examination of biopsies from 50 allografts and one isograft. The present report gives the immunofluorescent findings in 40 of these same grafts, but also includes the fine structural changes and immune deposits encountered in a further 32 renal allografts, in another renal isograft, and in repeat biopsies of two of the original allografts taken after an interval of 14 months.

Our previous results obtained by electron microscopy are confirmed in the new material. Subendothelial deposits were present on the glomerular capillary basement membranes of 54 of the 71 allografts in this series. In 28 of the kidneys the basement membrane thickening was obvious under light microscopy; this is a lower percentage than in the first series, partly because of the exclusion of several of the more severely affected allografts, and partly because of the inclusion of 15 biopsies obtained in the first 5 months after transplantation.

The frequent finding by immunofluorescent and immunoferritin methods of IgM and complement in the linear subendothelial deposits of the affected renal allografts was unexpected. There have been few immunofluorescent and no immunoferritin investigations of transplanted human kidneys. Hamburger, Crosnier, and Dormont,[19] when first reporting that severe glomerular lesions may occur in longer lived renal allografts, stated that "fluorescent antiglobulins showed an elective fixation on the glomerular tuft(s) . . . " of the transplant in their patient 8. Recently Hadley and Rosenau[16] found IgG, β1C-globulin, and fibrin deposited along the glomerular capillary basement membranes and in the mesangium in two of six human renal allografts biopsied during a rejection episode. Those kidneys had been transplanted for periods ranging from 3 to 18½ months. Fish, Herdman, Kelly, and Good,[12] however, failed to find any localization of IgG, IgM, IgA, or fibrin in the glomeruli of seven treated human renal allografts when they were biopsied 1 to 3 years after transplantation, even though glomerular capillary basement membrane thickening was present in six of their patients and β1C-globulin was demonstrated in the glomeruli of three of the kidneys. Only Williams and his colleagues[30] mentioned that IgM may be deposited (together with IgA) in the glomeruli, but they found that these immunoglobulins were difficult to demonstrate 1 or more years after transplantation.

Deposits of amorphous material along the endothelial surface of the glomerular capillary basement membranes can be produced experimentally by antiglomerular antibodies. In nephrotoxic serum nephritis[11] and in experimental allergic glomerulonephritis induced by immunization with glomerular capillary basement membranes,[45] the lesions resemble those which occur in human renal allografts. There are endothelial, mesangial, and epithelial cell changes but no subepithelial deposits. However, immunofluorescence shows that these changes are accompanied by a uniform ribbon-like accumulation of γ-globulin and complement along the inner aspect of the glomerular capillaries[46] which lacks the finely granular character of the deposit found in transplanted kidneys.

FIG. 5. Same renal allograft as in Figure 4. Frozen section treated with fluorescein-labeled goat antibody to human IgM. Higher power view of finely granular deposit of IgM in glomerular capillary walls. ×500.

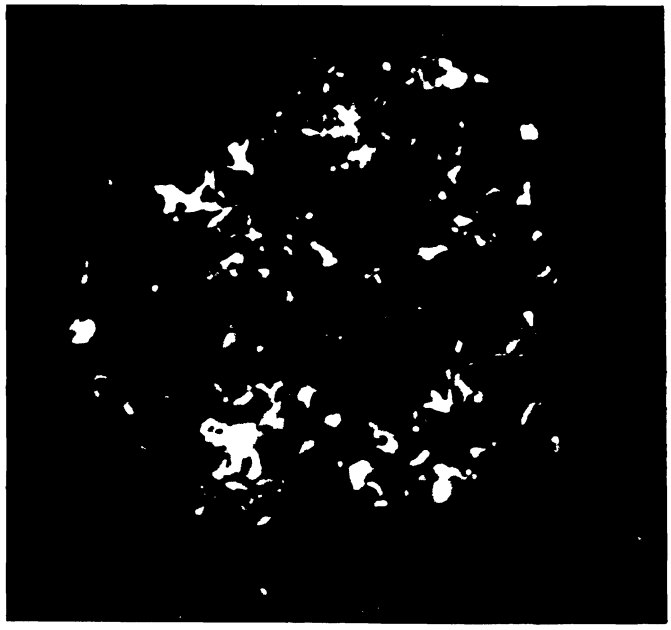

FIG. 6. Biopsy of a renal allograft 4 months after transplantation (LD110). Frozen section treated with fluorescein-labeled goat antibody to human β1C/β1A-globulins. There is localization of these globulins in the capillary walls and mesangium of a glomerulus. ×250.

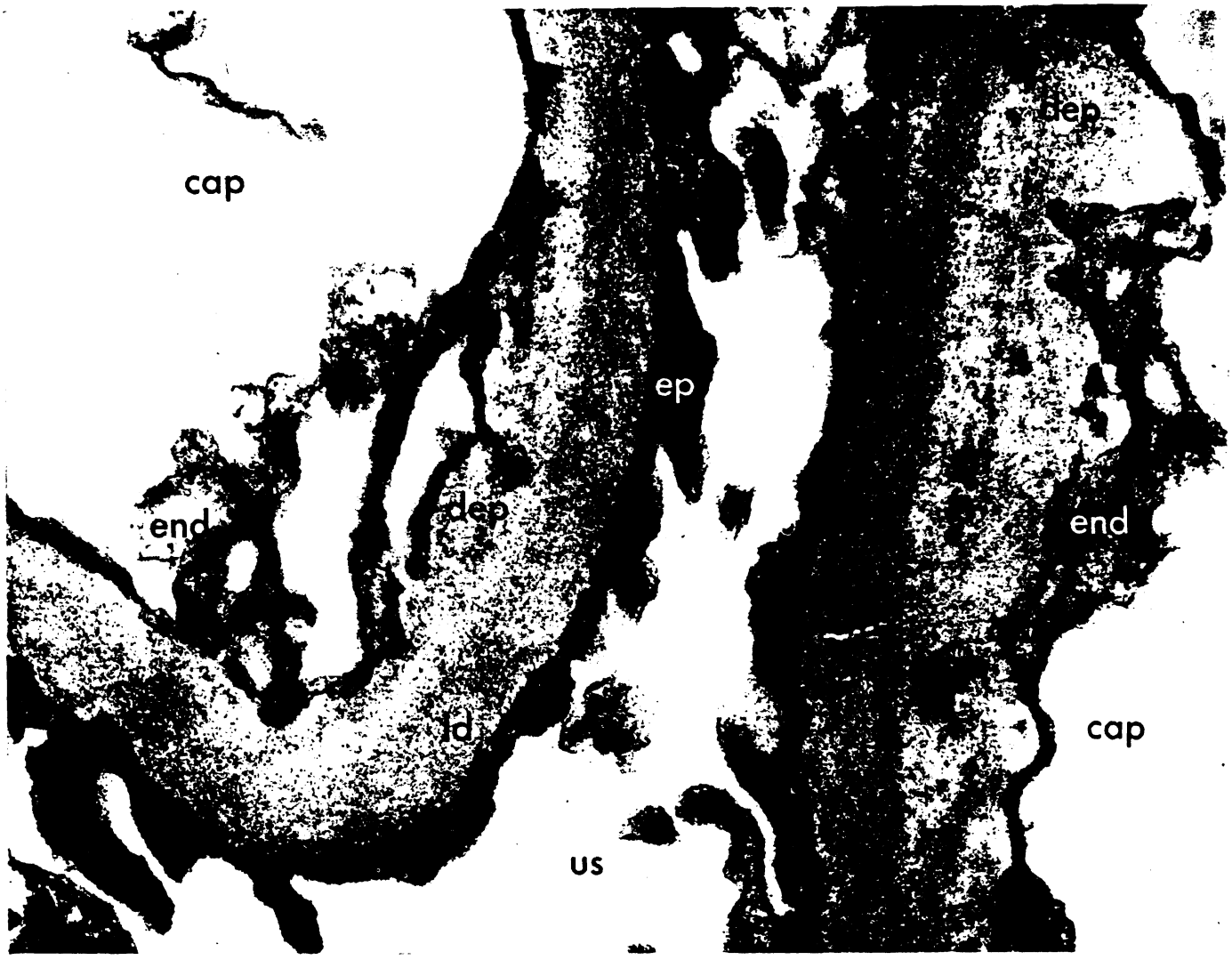

FIG. 7. Biopsy of a renal allograft 1 year after transplantation (RM). The tissue has been treated with ferritin-conjugated goat antibody to human IgM. Ferritin molecules are present in the linear deposits (*dep*) on the endothelial aspects of the basement membranes of two glomerular capillary loops. Ferritin is also present in the inner part of the lamina densa (*ld*). *ep*, Epithelial foot processes; *end*, endothelium; *cap*, capillary lumen; *us*, urinary space. ×42,000.

TABLE 4. IMMUNOFLUORESCENT FINDINGS IN GLOMERULI OF THREE HUMAN RENAL ALLOGRAFTS WITH SUBEPITHELIAL HUMPS AS WELL AS SUBENDOTHELIAL DEPOSITS VISIBLE ON THE CAPILLARY BASEMENT MEMBRANES (bm) BY ELECTRON MICROSCOPY (EM)

Recipient			Donor sex, age (yr.), and relationship	Time biopsy taken after transplant	Renal function at time of biopsy		Amount of glomerular capillary bm deposit on EM[a]	Fluorescent microscopy[a]				
Patient no.	Sex and age (yr.)	Disease[b]			Ccr[c]	Urinary protein		IgG	IgM	Complement		Fibrinogen
										β1A/β1C	C'1q	
					ml./min.	*mg./100 ml.*						
LD73	M, 26	CLGN	F, 32, sister	1 yr. 9 mo.	34	310	+++	++	+++	++	++	+++
M17	M, 18	APGN	M, 8, cadaver	7 mo.	45	0	++	++	++	++		++
RT	M, 14	RPGN	F, 34, cadaver	1 yr.	30	10	++	++	++	++		++

[a] ++, Moderate in amount; +++, marked in amount.

[b] CLGN, Chronic lobular glomerulonephritis; APGN, active proliferative glomerulonephritis; RPGN, rapidly progressive acute glomerulonephritis.

[c] Ccr, Creatinine clearance.

Linear subendothelial deposits of immunoglobulins and complement have also been found in the glomeruli of kidneys from patients suffering from Goodpasture's syndrome,[8] systemic lupus erythematosus,[33] and certain forms of glomerulonephritis.[23] IgM has been identified in some of these lesions.[23, 33]

In nephrotoxic serum nephritis and in experimental allergic nephritis, the deposits result from the reaction of circulating antibodies with antigens which are attached to or are part of the glomerular capillary basement membrane. We think it probable that a similar mechanism accounts for the glomerular lesions in treated human renal allografts, but conclusive supporting evidence is lacking. Indeed, the role of immunoglobulins at any stage in the rejection of treated or untreated renal allografts is unsettled. Their involvement is suggested by the studies of

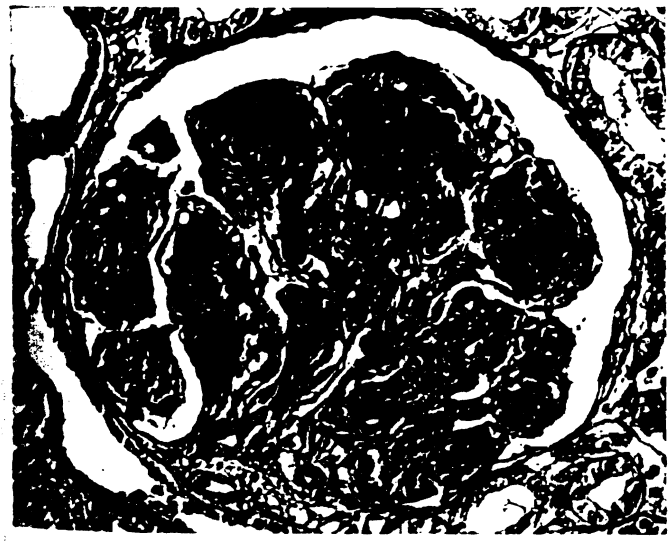

FIG. 8. One of diseased kidneys of patient LD73 removed at time of renal transplantation. The glomerulus is affected by lobular glomerulonephritis. Hematoxylin and eosin; ×380.

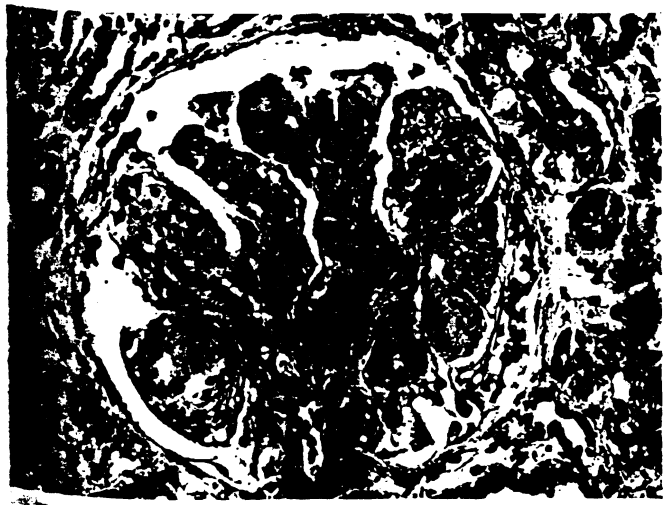

FIG. 9. Biopsy of renal allograft 1 year 9 months after transplantation into patient LD73, one of whose own diseased kidneys is shown in Figure 8. The glomerular tuft is lobulated and more cellular than normal, and the capillary basement membranes are thickened. The changes resemble those seen in the patient's own kidneys. Hematoxylin and eosin; ×350.

FIG. 10. Electron micrograph of same renal allograft as in Figure 9. Part of a glomerular capillary loop in which the basement membrane is thickened by an amorphous subendothelial deposit. There are also two dense hemispherical humps (*hu*) on the epithelial side of the capillary basement membrane. *mc*, Mesangial cell; *ep*, epithelial cell. Lead hydroxide; ×11,400.

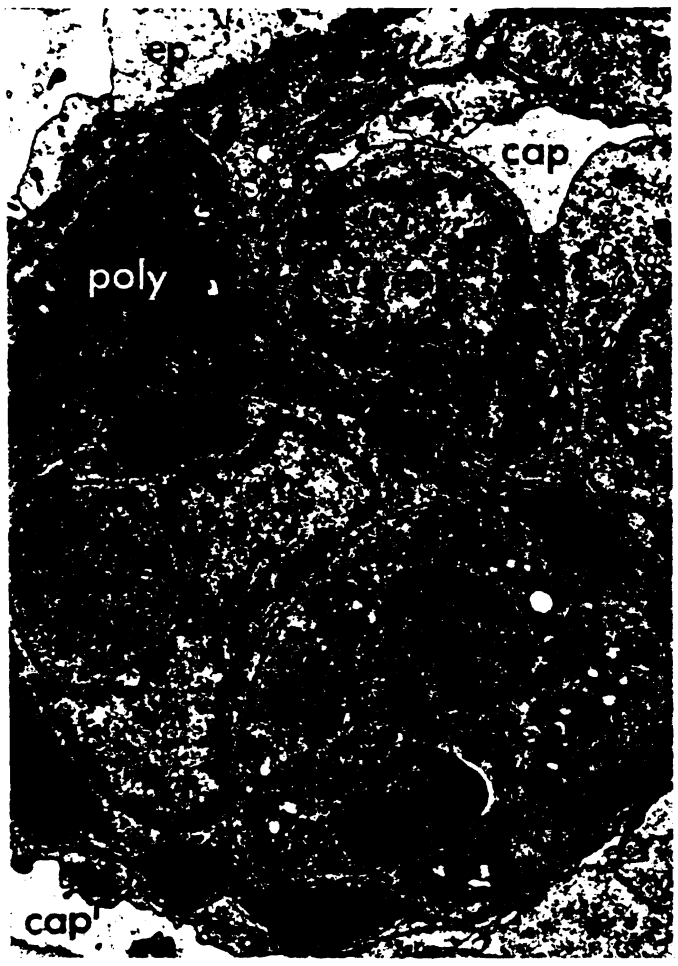

FIG. 11. Electron micrograph of same renal allograft as in Figures 9 and 10. There are two neutrophil polymorphonuclear leukocytes (*poly*) in the mesangium. The endothelial cells (*end*) lining the adjacent glomerular capillary (*cap*) are enlarged. *cap'*, Capillary lumen; *mc*, mesangial cell; *mm*, mesangial matrix; *ep*, fused epithelial foot processes. Lead hydroxide; ×6710.

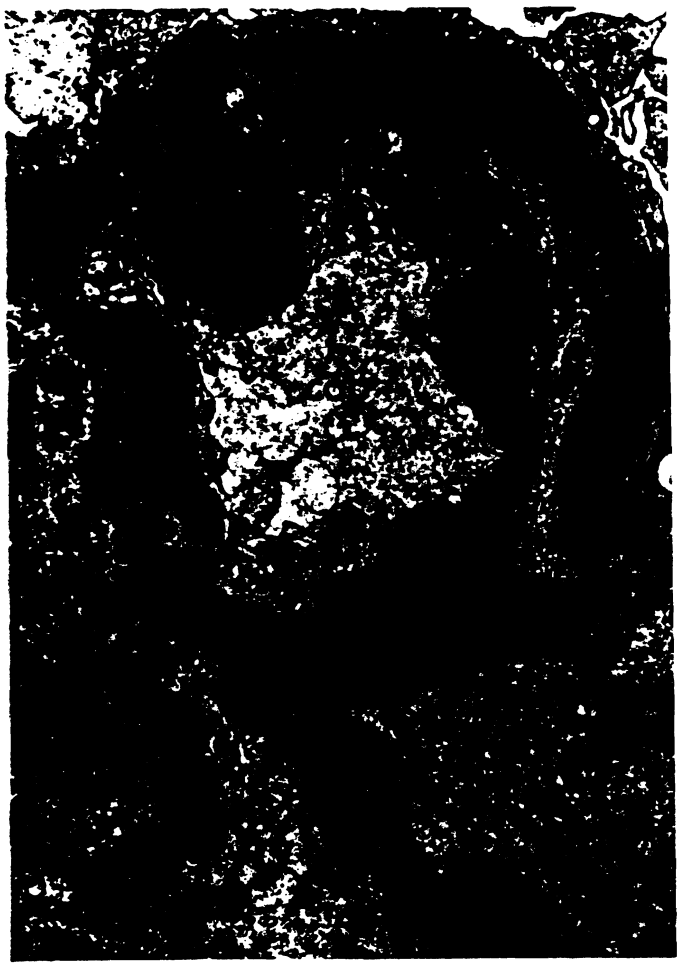

FIG. 12. Another electron micrograph of same renal allograft as in Figures 9 to 11. The wall of a glomerular capillary loop is greatly thickened by a massive deposit (*dep*) on the basement membrane. Most of the deposit is subendothelial. The mesangial matrix (*mm*) is increased in amount, and processes (*cp*) from the mesangial cells (*mc*) extend into parts of the thickened capillary wall. *cap*, Capillary lumen; *ep*, epithelial cell with fused foot processes. Lead hydroxide; ×8000.

FIG. 13. Same renal allograft as in Figures 9 to 12. Frozen section treated with fluorescein-labeled goat antibody to human IgM. Large, nodular deposits of IgM are present in parts of the glomerular capillary walls, and smaller amounts of the same immunoglobulin are present in the mesangium. ×100.

FIG. 14. Same renal allograft as in Figures 9 to 13. Frozen secti treated with fluorescein-labeled rabbit antibody to human Ig Finely granular deposits of IgG are present in the capillary wal ×100.

FIG. 15. Same renal allograft as in Figures 9 to 14. Frozen secti treated with fluorescein-labeled rabbit antibody to human C'1 Large, nodular deposits of C'1q are present on the epithelial su of the glomerular capillary walls. ×600.

Hager[17] in which antibody, consisting of a mixture of IgG and IgM, was eluted from unmodified rejecting canine renal allografts 6 to 10 days after transplantation. This author concludes that both groups of immunoglobulin participate in the rejection process. There is also evidence that humoral antibodies are formed by patients with renal allografts, even when they are receiving standard immunosuppressive therapy. Milgrom, Litvak, Kano, and Witebsky,[29] using a mixed agglutination test with cell cultures, found that these antibodies were difficult to demonstrate because they were largely adsorbed from the blood circulation by the renal graft and only appeared in high titers after removal of the transplant. Recently, however, Iwasaki, Talmage, and Starzl[21] have demonstrated, by both direct and indirect antiglobulin consumption tests, antibodies in the sera of eight of nine allografts in the present series, but not in the serum of the identical twin with the renal isograft (IDT2). These antibodies seemed to be IgG, but it is probable that IgM and other classes of antibody are also formed, as IgM synthesis appears to be more resistant than IgG to treatment with 6-mercaptopurine,[4, 25] and presumably also to treatment with azathioprine. Recent evidence from Zühlke and Deodhar[51] lends support to this idea. These investigators followed the serum levels of IgA, IgG, and IgM in 13 patients before and after transplantation of a renal allograft and found that after transplantation the serum IgM level rose and only fell during rejection episodes. The IgA and IgG levels did not change significantly. However, it should be noted that commercial immunoplates were used in this study and that the serum IgM elevation was slight in patients who had undergone splenectomy. It is also significant that rheumatoid factors, which are immunoglobulins of the IgM class with specific reactivity for denatured IgG, are frequently present in patients with renal allografts.[5, 22, 49] The majority of the Denver patients in the present series had positive latex fixation tests, the exceptions being LD41 and the identical twin IDT2.[5] Kano and Milgrom[22] found that after a renal graft rheumatoid factors appeared in 3 to 6 weeks and persisted for periods up to 2 years.

A possible explanation of these events is that the patient initially produces antibodies, predominantly of the IgG class, directed against the foreign histocompatibility antigens of the renal transplant, and that when these immunoglobulins combine with the graft antigens in the glomerular capillary walls, the conformation of the IgG is altered. This leads to production of rheumatoid factor type IgM antibodies and secondary deposition of IgM on the altered capillary basement membranes. IgM antibodies can fix complement and are often more potent than antibodies of other classes. For example, in the rat the IgM type of nephrotoxic antibody is approximately 60 times as effective as IgG type in producing glomerular injury.[47]

The glomerular lesions in the allografts could have been caused by the recipient's original disease. Host glomerulonephritis has been transmitted to renal isografts,[14, 36] and experimental allergic glomerulonephritis has been passively conveyed from one rabbit to another by serum antibody.[48] In the Boston series, 18 patients with renal failure resulting from glomerulonephritis received renal transplants from their presumed identical twins. Eleven redeveloped clinical evidence of glomerulonephritis in the isograft, and seven died with recurrent disease at intervals as long as 8 years after transplantation.[14] Many of the ultrastructural lesions found in these isografts resembled those seen in our allografts, and immunofluorescence revealed glomerular deposits of IgG, β1C-globulin, and fibrinogen.

The minor ultrastructural and immunofluorescent abnormalities found in one of the two renal isografts in the present series are difficult to explain. They could mean that the changes which we are seeing in transplanted kidneys are nonimmunologic and arise from some other unknown cause. It is just possible that the identical twins were not so identical as was originally thought; it even raises the question of the absolute identicality of monozygotic twins. Another explanation is that the patient's original disease was not chronic pyelonephritis as reported,[7] but was glomerulonephritis. The histology of the patient's own kidneys, which were removed some months after renal transplantation, was compatible with, but certainly not diagnostic of, "burned out" chronic glomerulonephritis. The clinical course was suggestive of chronic pyelonephritis.

In the majority of the transplants in this series we do not think that the glomerular lesions were caused by the recipient's original disease, for reasons given previously.[37] In three of the 71 allografts, however, it does seem probable that the changes in the transplant were brought about by transmission of glomerulonephritis from the host. The recipients of these grafts were unusual in that they all were suffering from active glomerulonephritis at the time of renal transplantation. In each of the grafts nodular deposits of IgG, IgM, and complement were present on the subepithelial surfaces of the glomerular capillary basement membranes, giving a lumpy-bumpy effect on immunofluorescence. These changes are similar to those found in the kidneys of patients suffering from acute poststreptococcal glomerulonephritis.[1, 28] Experimentally, this type of glomerular disease occurs in acute and chronic serum sickness nephritis,[3, 6, 13] in which antigen-antibody complexes, not immunologically directed against renal tissue, are filtered from the circulation by the kidneys and accumulate on the outer aspects of the capillary basement membranes. Transmission of glomerulonephritis from the host has been strongly suspected in at least two other treated renal allografts,[18, 35] but it still appears to be a rare complication.

Acknowledgments. The St. Mary's Hospital Medical School authors are indebted to the Anatomy Department for allowing them to use the electron microscope donated by the Wellcome Trust. They would also like to thank Professor W. S. Peart for permission to study his patients. The anti-IgG serum used by the Columbia University authors was kindly supplied by Dr. Arthur J. L. Strauss, the antiserum to fibrinogen by Dr. Görnstein, and the antiserum to C'1q by Dr. C. L. Christian.

Accepted for publication November 6, 1967.

This investigation was supported by grants from the Medical

Research Council of Great Britain, British Heart Foundation, Dr. Clark Memorial Fund, and St. Mary's Hospital Research Fund; by a grant from Consiglio Nazionale Delle Ricerche, Rome; and by United States Public Health Service Grants AM-06283, AM-06344, AM-07772, HE-07735, AI-04152, AI-04527, AI-05474, HE-03929, and FR-00051. The work was further facilitated by the award of a Wellcome Research Travelling Grant to Professor Porter.

REFERENCES

1. Andres, G. A., Accinni, L., Hsu, K. C., Zabriskie, J. B., and Seegal, B. C. Electron microscopic studies of human glomerulonephritis with ferritin-conjugated antibody. Localization of antigen-antibody complexes in glomerular structures of patients with acute glomerulonephritis. *J. Exp. Med. 123:* 399, 1966.
2. Andres, G. A., Morgan, C., Hsu, K. C., Rifkind, R. A., and Seegal, B. C. Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. The basement membranes and cisternae of visceral epithelial cells in nephritic rat glomeruli. *J. Exp. Med. 115:* 929, 1962.
3. Andres, G. A., Seegal, B. C., Hsu, K. C., Rothenberg, M. S., and Chapeau, M. L. Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. Localization of antigen-antibody complexes in rabbit glomeruli following repeated injections of bovine serum albumin. *J. Exp. Med. 117:* 691, 1963.
4. Borel, Y., Fauconnet, M., and Miescher, P. A. Effect of 6-mercaptopurine (6-MP) on different classes of antibody. *J. Exp. Med. 122:* 263, 1965.
5. Bravo, J. F., Herman, J. H., and Smyth, C. J. Musculoskeletal disorders after renal homotransplantation. A chemical and laboratory analysis of 60 cases. *Ann. Intern. Med. 66:* 87, 1967.
6. Dixon, F. J., Feldman, J. D., and Vazquez, J. J. Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J. Exp. Med. 113:* 899, 1961.
7. Dossetor, J. B., MacKinnon, K. J., Luke, J. C., Morgen, R. O., and Beck, J. C. Renal transplantation between identical twins. *Lancet 2:* 572, 1960.
8. Duncan, D. A., Drummond, K. N., Michael, A. F., and Vernier, R. L. Pulmonary hemorrhage and glomerulonephritis. Report of six cases and study of the renal lesion by the fluorescent antibody technique and electron microscopy. *Ann. Intern. Med. 62:* 920, 1965.
9. Fahey, J. L. Chromatographic studies of anomalous γ-, β_{2A}-, and macroglobulins and normal γ-globulins in myeloma and macroglobulinemic sera. *J. Biol. Chem. 237:* 140, 1962.
10. Faith, G. C., and Trump, B. F. The glomerular capillary wall in human kidney disease. Acute glomerulonephritis, systemic lupus erythematosus, and preeclampsia-eclampsia. Comparative electron microscopic observations and a review. *Lab. Invest. 15:* 1682, 1966.
11. Feldman, J. D., Hammer, D., and Dixon, F. J. Experimental glomerulonephritis. III. Pathogenesis of glomerular ultrastructural lesions in nephrotoxic serum nephritis. *Lab. Invest. 12:* 748, 1963.
12. Fish, A. J., Herdman, R. C., Kelly, W. D., and Good, R. A. Glomerular changes in well-functioning human renal homografts. *Transplantation 5:* 1338, 1967.
13. Fish, A. J., Michael, A. F., Vernier, R. L., and Good, R. A. Acute serum sickness nephritis in the rabbit. An immune deposit disease. *Amer. J. Path. 49:* 997, 1966.
14. Glassock, R. J., Feldman, D., Reynolds, E. S., Dammin, G. J., and Merrill, J. P. Recurrent glomerulonephritis in human renal isotransplants. A clinico-pathologic study. In *Proceedings of the First International Congress of the Transplantation Society, Paris, June 26 to 30, 1967.*
15. Good, R. A. Personal communication.
16. Hadley, W. K., and Rosenau, W. Study of human renal disease by immunofluorescent methods. *Arch. Path. (Chicago) 83:* 342, 1967.
17. Hager, E. B. Personal communication.
18. Hallenbeck, G. A., Shorter, R. G., Titus, J. L., Thomford, N. R., Johnson, W. J., and De Weerd, J. H. Apparent glomerulonephritis in a homotransplant. *Surgery 59:* 522, 1966.
19. Hamburger, J., Crosnier, J., and Dormont, J. Observations in patients with a well-tolerated homotransplanted kidney. Possibility of a new secondary disease. *Ann. N. Y. Acad. Sci. 120:* 558, 1964.
20. Ishikawa, E. The kidney in toxemias of pregnancy. *Path. Microbiol. (Basel) 24:* 576, 1961.
21. Iwasaki, Y., Talmage, D., and Starzl, T. E. Humoral antibodies in patients after renal homotransplantation. *Transplantation 5:* 191, 1967.
22. Kano, K., and Milgrom, F. Rheumatoid factor in human kidney homotransplantation. In *Proceedings of the First International Congress of the Transplantation Society, Paris, June 26 to 30, 1967.*
23. Koffler, D., and Paronetto, F. Immunofluorescent localization of immunoglobulins, complement, and fibrinogen in human diseases. II. Acute, subacute and chronic glomerulonephritis. *J. Clin. Invest. 44:* 1665, 1965.
24. Laki, K. The polymerization of proteins. The action of thrombin on fibrinogen. *A. M. A. Arch. Biochem. 32:* 317, 1951.
25. Levin, R. H., Landy, M., and Frei, E., III. The effect of 6-mercaptopurine on immune response in man. *New Eng. J. Med. 271:* 16, 1964.
26. May, J. R., and Rawlins, G. A. A micro method for agar-gel precipitin reactions. *J. Clin. Path. 15:* 186, 1962.
27. Mellors, R. C. Fluorescent-antibody method. In *Analytical Cytology*, edited by Mellors, R. C., p. 1. New York, McGraw-Hill Book Co., 1959.
28. Michael, A. F., Jr., Drummond, K. N., Good, R. A., and Vernier, R. L. Acute poststreptococcal glomerulonephritis. Immune deposit disease. *J. Clin. Invest. 45:* 237, 1966.
29. Milgrom, F., Litvak, B. I., Kano, K., and Witebsky, E. Humoral antibodies in renal homograft. *J. A. M. A. 198:* 226, 1966.
30. Morse, J. H., and Christian, C. L. Immunological studies of the 11S protein component of the human complement system. *J. Exp. Med. 119:* 195, 1964.
31. Müller-Eberhard, H. J., Nilsson, U., and Aronsson, T. Isolation and characterization of two β_1-glycoproteins of human serum. *J. Exp. Med. 111:* 201, 1960.
32. Osawa, G., Beres, J., and Kimmelstiel, P. Glomerulonephritis. Observations by light and electron microscopy. *Amer. J. Clin. Path. 46:* 295, 1966.
33. Paronetto, F., and Koffler, D. Immunofluorescent localization of immunoglobulins, complement, and fibrinogen in human diseases. I. Systemic lupus erythematosus. *J. Clin. Invest. 44:* 1657, 1965.
34. Paronetto, F., Rubin, E., and Popper, H. Local formation of γ-globulin in the diseased liver, and its relation to hepatic necrosis. *Lab. Invest. 11:* 150, 1962.
35. Petersen, V. P., Olsen, S., Kissmeyer-Nielsen, F., and Fjeldborg, O. Transmission of glomerulonephritis from host to human kidney allotransplant. *New Eng. J. Med. 275:* 1269, 1966.
36. Pfeiffer, E. F., and Merrill, J. P. Die Autoaggression in der Pathogenese der diffusen Glomerulonephritis. *Deutsche Med. Wschr. 87:* 934, 1962.
37. Porter, K. A., Dossetor, J. B., Marchioro, T. L., Peart, W. S., Rendall, J. M., Starzl, T. E., and Terasaki, P. I. Human renal transplants. I. Glomerular changes. *Lab. Invest. 16:* 153, 1967.
38. Rifkind, R. A., Hsu, K. C., and Morgan, C. Immunochemical staining for electron microscopy. *J. Histochem. Cytochem. 12:* 131, 1964.
39. Riggs, J. L., Seiwald, R. J., Burckhalter, J. H., Downs, C. M., and Metcalf, T. G. Isothiocyanate compounds as fluorescent labeling agents for immune serum. *Amer. J. Path. 34:* 1081, 1958.
40. Seegal, B. C., Andres, G. A., Hsu, K. C., and Zabriskie, J. B. Studies on the pathogenesis of acute and progressive glomerulonephritis in man by immunofluorescein and immunoferritin techniques. *Fed. Proc. 24:* S100, 1965.
41. Starzl, T. E. *Experience in Renal Transplantation.* Philadelphia, W. B. Saunders Co., 1964.
42. Starzl, T. E., Marchioro, T. L., Faris, T. D., Hutt, M. P., Carey, T. A., Ogden, D. A., Waddell, W. R., and Porter, K. A. The problems and prognosis of the chronically surviving patient after renal homotransplantation. *Ann. N. Y. Acad. Sci. 129:* 398, 1966.
43. Starzl, T. E., Marchioro, T. L., Porter, K. A., Iwasaki, Y., and Cerilli, G. C. The use of heterologous antilymphoid agents in

canine renal and liver homotransplantation and in human renal homotransplantation. *Surg. Gynec. Obstet. 124:* 301, 1967.

44. Starzl, T. E., Marchioro, T. L., Terasaki, P. I., Porter, K. A., Faris, T. D., Herrmann, T. J., Vredevoe, D. L., Hutt, M. P., Ogden, D. A., and Waddell, W. R. Chronic survival after human renal homotransplantation. Lymphocyte-antigen matching, pathology and influence of thymectomy. *Ann. Surg. 162:* 749, 1965.
45. Steblay, R. W. Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane 'GBM' and Freund's complete adjuvant. *J. Exp. Med. 116:* 253, 1962.
46. Unanue, E., and Dixon, F. J. Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. *J. Exp. Med. 119:* 965, 1964.
47. Unanue, E. R., and Dixon, F. J. Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. *J. Exp. Med. 121:* 697, 1965.
48. Unanue, E. R., Dixon, F. J., and Feldman, J. D. Experimental allergic glomerulonephritis induced in the rabbit with homologous renal antigens. *J. Exp. Med. 125:* 163, 1967.
49. Waller, M., Irby, R., Mullinax, F., and Toone, E. C., Jr. Connective-tissue disease and rheumatoid factors in patients with renal transplants. *New Eng. J. Med. 273:* 12, 1965.
50. Williams, G. M., Lee, H. M., Weymouth, R. F., Harlan, W. R., Holden, K. R., Stanley, C. M., Millington, G. A., and Hume, D. M. The etiology of nephrotic syndrome in human renal transplant recipients. *Surgery 62:* 204, 1967.
51. Zühlke, V., and Deodhar, S. D., Nakamoto, S., and Kolff, W. J. Serum immunoglobulin levels following human renal allotransplantation. A preliminary report. *Transplantation 5:* 135, 1967.