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Human Renal Transplants

III. Immunopathologic Studies

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Tissues obtained from 34 human renal allografts by biopsy, 1 to 31 months after transplantation, were studied by histologic, immunofluorescence, and immunoferritin techniques. Our purpose was to gain more precise information concerning the localization of immunoglobulins and complement in glomeruli and to describe the associated tubular and vascular changes.

1. Localization of fluorescein-labeled antibodies (Fl-Abs) to IgG, IgM, β1C, and C'1q was seen in glomeruli from 25 allografts and showed the following distribution of patterns: (a) diffuse linear, four; (b) diffuse granular, four; (c) focal linear, six; (d) focal granular, six; and (e) indeterminate, five. Ferritin-conjugated antibodies (Fer-Abs) localized in 21 allografts. Electron microscopic findings correlated with the foregoing immunofluorescence patterns. (a) Fer-Abs were bound on the endothelial side of the glomerular basement membrane and in the subendothelial space. (b) Fer-Abs were bound in subendothelial and subepithelial deposits similar to those seen in experimental acute or chronic serum sickness. (c and d) Fer-Abs were seen in the subendothelial space and mesangial matrix. Deposits were present in these areas and some bound Fer-Abs. The two groups were not readily separable on the basis of immunopathologic data. (e) Fer-Abs were mostly seen in the mesangial area.

2. Nine allografts displaying little or no binding of Fl-Abs failed to bind Fer-Abs.

3. Small amounts of fibrinogen were found in only six allografts, three of which showed diffuse granular fluorescence.

4. In 12 allografts either Fl-Abs or Fer-Abs or both were bound in walls of blood vessels. The position of these reactants was in foreign deposits and in basement membranes of arteries and veins.

The immunopathologic findings seem to support best the hypothesis that damage to allografts is associated with deposition, in glomerular and vascular structures, of circulating antigen-antibody complexes which might contain, in part, transplantation antigens.

Additional key words: Human renal allografts, Transplantation complexes, Glomerulonephritis transmission, IgG, IgM, Complement, Immunofluorescence, Immunoferritin.

Recently an immunofluorescence study of 71 human renal allografts treated with immunosuppressive agents and two renal isografts has been reported.[36] The occurrence of glomerular localization of fluorescent antibodies to IgG and IgM, to fractions of human complement, and to fibrinogen was described. The findings, correlated with light and electron microscopic studies, were consistent with the hypothesis that circulating immunoglobulins, complement, and fibrinogen may play a role in human chronic allograft rejection.

The purpose of the present immunopathologic studies by morphologic, immunofluorescence, and immunoferritin techniques of 34 human renal allografts is to gain more precise information concerning the localization of immunoglobulins and complement in glomeruli and to describe some tubular and vascular changes which also are associated with the presence of immunoglobulins and complement. The data obtained are useful in assessing possible pathogenetic mechanisms responsible for damage of the renal grafts.

MATERIALS AND METHODS

PATIENT MATERIAL

Biopsy specimens from 34 human renal allografts have been studied by light and electron microscopy and by immunofluorescence and immunoferritin techniques. Of the allografts 20 were from the University of Colorado Medical Center, Denver, Colorado, 9 from the St. Mary's Hospital, London, England, and 5 from the Royal Victoria Hospital, Montreal, Canada. Preliminary immunofluorescent findings of 15 allografts, LD109, LD108, LD107, LD110, LD114, LD71, LD70, LD68, M31, M32, M33, M8, M30, AF, and RM, have

been reported.[36] The Denver patients, indicated by the letters LD followed by a number, received kidneys from living donors. The Montreal patients, identified by the letter M before the number, and London patients, designated with their initials, were given kidneys from cadavers. The spleens of all of the Denver and London patients had been removed. The thymus was removed in nine of the Denver patients. At the time of the biopsy, the allografted patients were receiving 50 to 200 mg. of azothioprine and 2.5 to 25 mg. of prednisone a day. Fourteen patients were treated with one antihypertensive drug or more (chlorothiazide, hydralazine, reserpine, and methyldopa). Five patients were receiving antihuman lymphocyte globulin.[39] Tables 1 and 2 contain additional data.

Antisera Used in Immunofluorescent and Immunoferritin Studies

The following antisera used for fluorescein or ferritin labeling were either kindly supplied by investigators or were purchased from commercial laboratories. Rabbit antiserum to human IgG was supplied by Dr. A. J. L. Strauss;[41] goat antiserum to human IgG, by Dr. C. L. Christian;[29] rabbit antiserum to human fibrinogen, by Dr. F. Gorstein;[17] rabbit antiserum to human C'1q, by Drs. J. H. Morse and C. L. Christian;[29] rabbit or goat antiserum to human IgM, from Hyland Laboratories, Los Angeles, California; and rabbit antiserum to human β1C and β1A, from Hoechst Pharmaceuticals. The globulin fractions were separated from these antisera and from normal rabbit and goat sera and were conjugated with fluorescein[42] or ferritin[4] according to methods previously described.

All ferritin-conjugated globulins were subjected to immunoelectrophoretic tests with antibody to rabbit or goat globulin to determine successful conjugation. They were also tested for their immunologic reactivity with their specific antigens before use in electron microscopic studies, either by immunoelectrophoresis or by the Ouchterlony technique.[4] Ferritin-labeled normal rabbit and goat globulins were used in control tests.

Tissue Processing for Light and Electron Microscopy

Tissue was obtained from each renal graft by open biopsy after transplantation at the times indicated in Tables 1 and 2. Each tissue was divided into four parts. The first portion was fixed in 10 per cent neutral formalin or buffered formol saline, was embedded in paraffin wax, and was serially sectioned and stained with hematoxylin and eosin, periodic acid-Schiff reagent, Weigert's stain, and methyl green-pyronin. The second part of the biopsy was immediately 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. Thin sections for electron microscopy were stained with lead hydroxide, uranyl acetate, or a combination of both and were examined with a Siemens-Elmiskop IA or with a Philips EM-300 electron microscope. The third portion of tissue was quick frozen in a bath of alcohol and Dry Ice or in liquid nitrogen. Frozen sections 4 μ thick were cut in a cryostat and stained with fluorescein-conjugated antisera according to techniques already described.[42] The fourth portion of tissue was immediately treated with ferritin-conjugated antibodies while fresh, before processing for electron microscopy.[4] Prefixation and the particular handling of the tissue necessary for the preservation of the antigenicity and permeability to ferritin-antibody conjugates account for the presence of artifacts in many of the electron micrographs.

RESULTS

Tissue sections from biopsies of the 34 allografts examined in these studies were stained with fluorescein- and ferritin-labeled antibodies to IgG, IgM, β1C, C'1q, and fibrinogen in order to determine which of these antigens were present in excess of normal amounts in the glomeruli. It was found that: (1) 25 of the tissues bound two of the antisera or more in various areas of the glomeruli; and (2) among the other nine tissues, there were six which bound none of the antisera, whereas the remaining three bound only one or two antisera in trace amounts.

Twenty-Five Allografts Showing Localization of Immunoglobulins and Complement in Glomeruli

The 25 biopsies which bound labeled antisera have been subdivided into five groups on the basis of the pattern of localization of the fluorescein-labeled antibodies. The frozen sections from the first 10 allografts (Table 1) bound the antisera in the glomeruli with linear distribution. (1) In four instances, LD84, RM, LD93, and LD7, there was diffuse linear staining. The allografts from patients LD84, RM, and LD93 had only slight or moderate glomerular lesions characterized by diffuse, fine, linear subendothelial changes which are shown in Figure 1, a micrograph of a biopsy from LD84 taken 2½ years after transplantation. The subendothelial space contains fine deposits of material morphologically similar to the basement membrane. Figure 2, *inset,* displays the appearance of linear fluorescence in a piece of the same biopsy stained with fluorescein-labeled antibody to IgG, whereas in Figure 2, ferritin-conjugated antibody to IgG is present on the endothelial side of the basement membrane and in the subendothelial space. This localization may account for a linear fluorescent pattern which was not as sharp as that seen in sections from patients with Goodpasture's disease. Fluorescein- and ferritin-labeled antibodies to IgG showed the strongest glomerular binding. Linear staining of tubular basement membranes also was observed in allografts LD93 and RM. The fourth allograft with linear fluorescence, LD7, had more severe subendothelial and mesangial changes. (2) In six allografts, LD114, LD107, AE, LD71, M8, and LD102, there was focal linear fluorescence. The distinction between focal linear and granular staining was often difficult. As a rule, the cases in which even a few granules could be detected were believed to be granular. The severity of the glomerular changes could not be correlated with the intensity of the fluorescence, which

TABLE 1. HUMAN RENAL ALLOGRAFTS[a]

Recipient			Donor sex, age (yr.), and relationship	Time of biopsy taken after transplant	Renal function at time of biopsy		Glomerular pathology by electron microscopy				Localization in glomerular capillary walls									
											Immunofluorescence microscopy					Immunoelectron microscopy				
Patient No.	Sex and age (yr.)	Disease			Ccr ml./min.	Urinary protein gm./24 hr.	Mesangial changes	Subendothelial changes	Proliferative and exudative changes	Subepithelial deposits	Fluorescence pattern	IgG	IgM	Complement		Fibrinogen	IgG	IgM	Complement		Fibrinogen
														β1C	C'1q				β1C	C'1q	
LD 84	F 20	CPGN	F, 23, sister	2 yr. 6 mo.	90	0	0	±	0	0	Lin D	+++	0	0	0	0	+++	+	−	0	−
RM	M 17	CLGN	M, 38, cadaver	1 yr.	65	6.00	±	+	0	0	Lin D	++	++	0	0	0	0	++	−	+	−
LD 93	F 8	CPGN	F, 37, mother	2 yr. 2 mo.	12	3.20	±	±	0	±	Lin D	++	*(+)	*(+)	*(+)	0	±	+	−	+	−
LD 7	F 11	—	M, 37, cadaver	1 yr. 9 mo.	55	0.30	+	+	++	±	Lin D	+	±	0	0	0	+	0	0	+	−
LD 114	M 34	CPGN	M, 38, brother	3½ mo.	75	0.10	+	+	0	0	Lin F	+	+	0	0	±	+	−	0	−	−
LD 107	F 15	CPGN	M, 21, brother	5 mo.	119	0.30	±	±	0	0	Lin F	±	+**	0	++**	0	+	+	+	−	−
AE	F 24	CPyN	M, 23, cadaver	1 yr.	104	1.00	+	+	+	±	Lin F	±	+	+	+	0	−	±	−	+	−
LD 71	M 37	CPGN	M, 43, unrelated	1 yr. 10 mo.	72	0.20	++	++	+	0	Lin F	±	+**	0	+**	0	±	+*	+	−	0
M 8	M 24	Hydr	F, 53, cadaver	2 yr. 5 mo.	50	2.00	+	+	±	0	Lin F	0	++	±	+	0	0	0	0	−	0
LD 102	F 20	CPGN	F, 40, mother	1 yr. 8 mo.	16	1.20	+++	+++	±	0	Lin F	±**	*(±)	*(±)	0	0	±**	+**	−	−	−
LD 89	M 23	CMGN	M, 56, father	2 yr. 4 mo.	29	2.00	++	++	++	+	GD	++**	+++**	++++**	++++**	+	++	+	−	++	−
DH	M 33	CLGN	M, 28, cadaver	1 yr. 9 mo.	70	3.50	++	+	++	+++	GD	++	++	++	0	0	++	++	++	+	−
LD 85	M 18	CPGN	F, 21, sister	2 yr. 6 mo.	78	2.10	+	±	+++	+++	GD	+**	+	++**	++++**	±	±**	++**	−	+++**	0
LD 110	M 36	CPGN	F, 34, sister	4 mo.	71	0.10	±	+	+	0	GD	0	+**	++**	+	+**	+	−	+	−	−
LD 106	F 15	CPGN	F, 40, mother	1 yr. 6 mo.	51	0.20	+	+	0	0	GF	+	+	0	±	0	+**	++	−	+	−
LD 90	F 18	CPGN	F, 38, mother	2 yr. 3 mo.	63	0.02	±	±	0	0	GF	+	+	±	+	0	0	+	−	−	−
LD 94	F 26	CPGN	M, 50, father	2 yr. 2 mo.	63	0.10	±	++	0	0	GF	+	+	+	±	0	0	+	−	+	−
LD 68	F 24	CPyN	F, 23, sister	1 yr. 11 mo.	81	0.04	±	+	0	0	GF	±	+	0	0	0	0	+	0	−	−
LD 70	F 27	CPGN	M, 40, unrelated	1 yr. 10 mo.	43	0.04	++	++	++	0	GF	±	+	0	++	0	±	+	±	−	−
M 31	M 42	CPGN	M, 20, cadaver	4½ mo.	55	1.00	+	0	0	0	GF	0	+	0	+	0	0	0	0	−	−
LD 109	M 31	CPGN	M, 34, brother	4½ mo.	107	0	+	+	+	0	Ind.	0	+	0	+	0	0	−	0	−	−
LD 108	M 33	CPGN	M, 34, brother	4½ mo.	126	0	+	0	0	0	Ind.	0	+	0	0	±	0	−	0	−	−
BF	F 32	CPGN	F, 35, cadaver	11 mo.	110	0.30	+	±	0	0	Ind.	0	±	0	±	0	0	±	+	−	−
M 30	M 29	CPGN	M, 57, cadaver	1 yr. 6 mo.	74	2.00	±	+	0	0	Ind.	0	±**	+**	*(+)	0	+	±	±	−	0
M 33	M 21	Good.	F, 65, cadaver	1 mo.	0	0	+	++	+	0	Ind.	0	±	0	+	0	0	0	0	−	0

[a] Abbreviations are: Ccr, creatinine clearance; 0, negative; −, not tested; ±, minimal in amount; +, slight amount; ++, moderate amount; +++, marked in amount; ++++, very marked in amount; *, only in vessel walls' and sign within ( ) indicate amount; **, also in vessel walls; Hydr., hydronephrosis; CPGN, chronic proliferative glomerulonephritis; CPyN, chronic pyelonephritis; CLGN, chronic lobular glomerulonephritis; CMGN, chronic membranous glomerulonephritis; Lin D, linear diffuse; Lin F, linear focal; GD, granular diffuse; GF, granular focal; Ind, indeterminate; and Good., Goodpasture's disease.

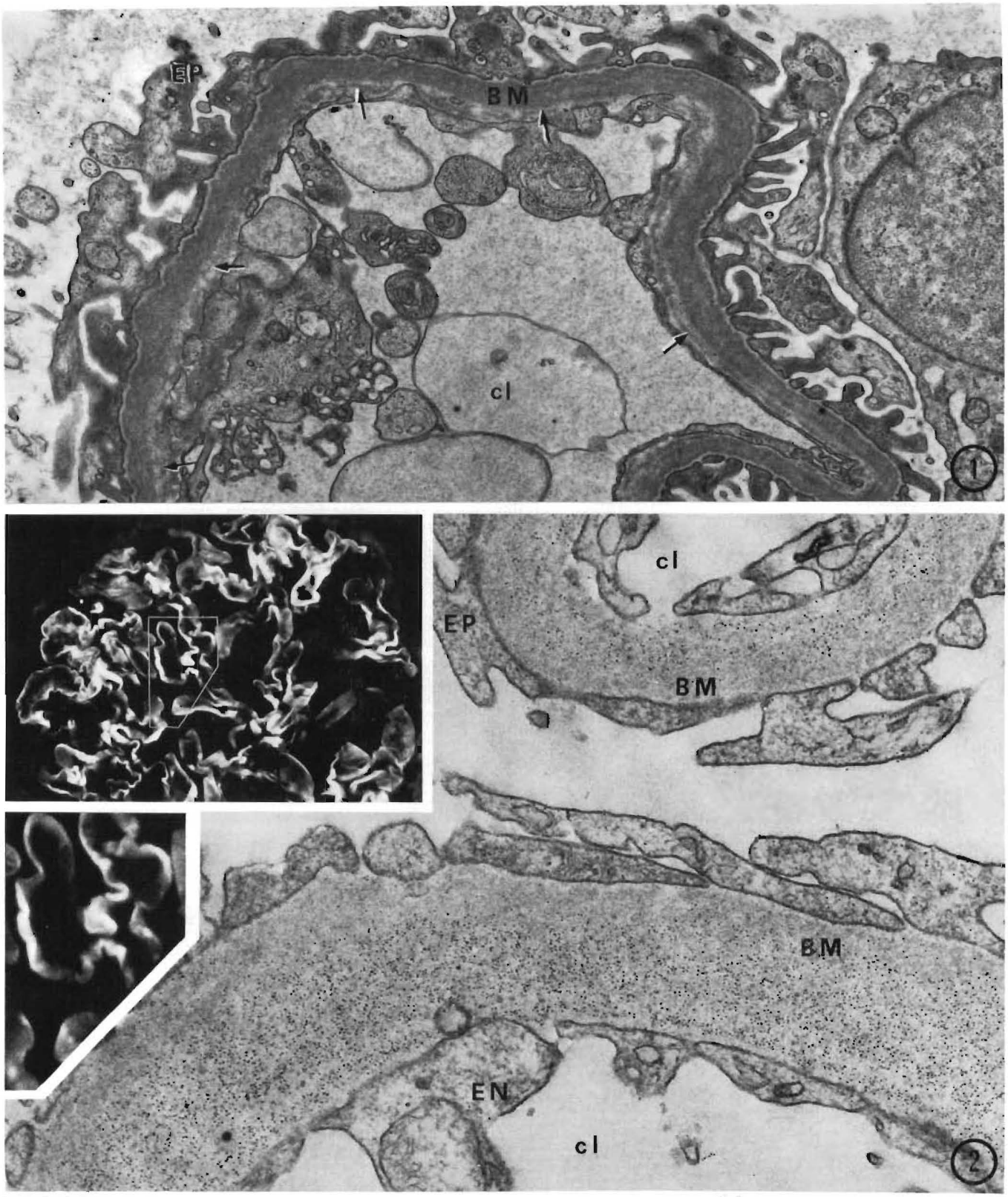
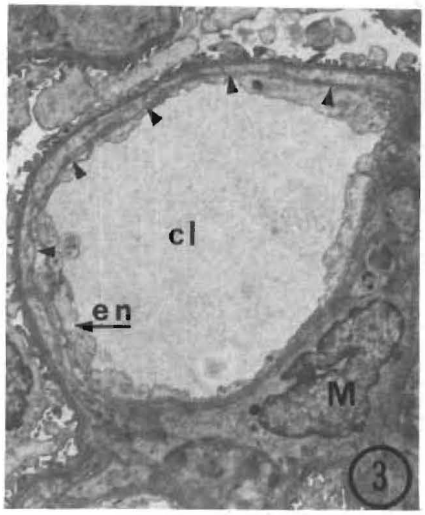


FIG. 1. Kidney biopsy of allograft LD84, 2 years and 6 months after transplantation. The subendothelial space of the glomerular capillary wall contains fine deposits of material (*arrows*) morphologically similar to the basement membrane (*BM*). *cl*, Capillary lumen; *EP*, epithelium. ×17,000.

FIG. 2. Allograft LD84. The *upper inset* displays a diffuse, linear binding of fluorescent antibody to IgG in capillary walls of one glomerulus. The *lower inset* illustrates a few loops of the same glomerulus at higher magnification. In the electron micrograph, parts of two glomerular capillary loops from tissue treated with ferritin-conjugated antibody to IgG are seen. Ferritin granules are mainly localized on the endothelial side of the basement membranes (*BM*). *cl*, Capillary lumen; *EP*, epithelium; *EN*, endothelium. Figure 2, ×30,000; *upper inset*, ×300; *lower inset*, ×600.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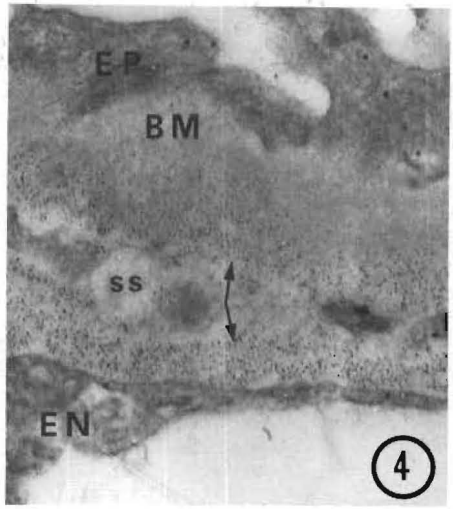
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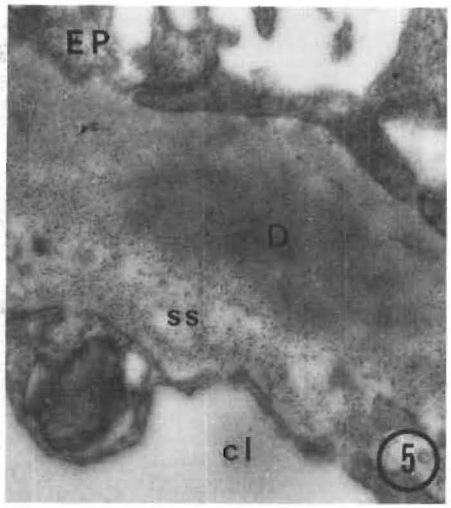
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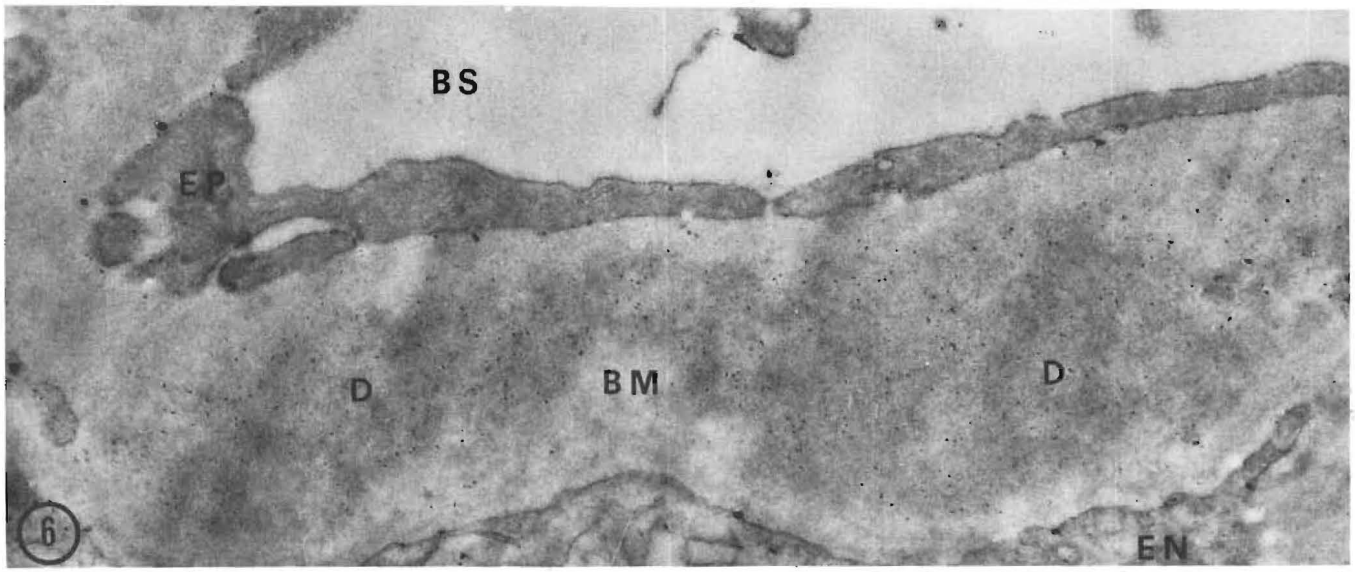
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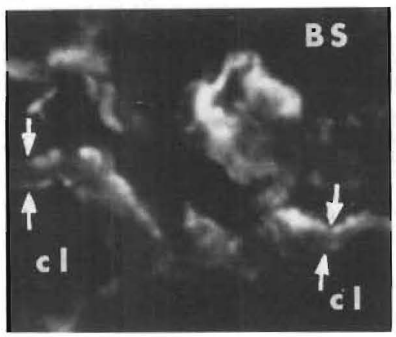
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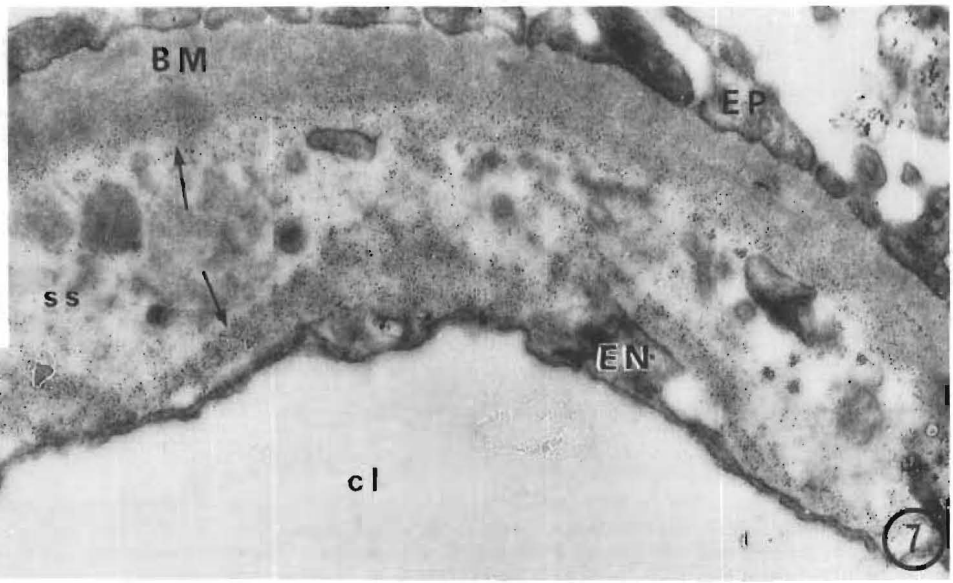
BM
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FIGS. 3 TO 7

was usually slight or moderate. In the most severe cases, LD71 and LD102, pseudopods of the mesangial cells extended into the subendothelial space, and morphologic similarities between the material present in the subendothelial space and mesangial matrix were seen. In some capillary loops, LD102, a continuous band of newly formed basement membrane-like material lay close to the endothelial cytoplasm (Fig. 3). Ferritin-conjugated antibodies, bound to the endothelial side of the basement membrane and in the newly formed basement membrane-like material (Fig. 4), as well as in the mesangial matrix, showed focal distribution. In the basement membrane and in the mesangium, some electron-dense deposits bound ferritin-conjugated antibodies, whereas others did not (Fig. 5). This finding may be due to the problem of penetration by the labeled antibody or to variation of the composition of the deposits, the latter of which may explain the difficulty in separating allografts with focal linear from those with focal granular fluorescence.

Fluorescein-labeled antibodies to IgG and IgM and to complement showed granular localization in glomerular structures of 10 allografts listed in Table 1. (3) Four of these, LD89, DH, LD85, and LD110, had a diffuse granular fluorescence. The immunoelectron microscopic data suggested that the granular fluorescent pattern was due to localization of labeled antibodies in aggregates of foreign material forming deposits in different parts of the glomeruli. Thus in allografts LD89 and LD110, electron-dense deposits binding the ferritin-conjugated antibodies were seen within the basement membrane (Fig. 6) or on its endothelial side only. In addition, subendothelial and mesangial changes, similar to those already described in allografts with linear fluorescence (Figs. 1 to 5), were present (Fig. 7). In contrast, the other two allografts with diffuse granular fluorescence, LD85 and DH, exhibited a pathology characteristic of acute proliferative, LD85 (Fig. 8), or membranous, DH, glomerulonephritis. Fluorescein- or ferritin-conjugated antibodies to IgG and IgM and to complement were localized in subepithelial and subendothelial deposits. The *inset* of Figure 9 reveals a section of the allograft of LD85 stained with fluorescein-labeled antibody to β1C. Fluorescent deposits are in the walls of capillaries in a glomerulus and also in Bowman's capsule. In Figure 9 there is binding of ferritin-conjugated antibody to C'1q in subepithelial deposits. The glomerular basement membranes, the basement membrane of the Bowman's capsule, and the mesangial matrix did not contain ferritin granules. In the same two allografts, deposits present within or on the outer side of Bowman's capsule (Fig. 10) bound fluorescein- (Fig. 11) and ferritin-conjugated (Fig. 12) antibodies. Similar binding was observed in precipitates contained in the tubular lumina, LD85 (Figs. 13 to 16), and in deposits present within or on the outer side of tubular basement membranes, LD85, LD89, and DH (Figs. 17 to 19). The tubular deposits were not autofluorescent,[7] and in no instances were there fibrils similar to those of amyloid observed by electron microscopy.

In allograft LD89, removed 2 months after biopsy, it was found that immunoglobulins and complement were no longer bound to the tissues. (4) Six allografts, LD106, LD90, LD94, LD68, LD70, and M31, had focal granular fluorescence with subendothelial and mesangial changes which were indistinguishable from those of allografts with focal linear fluorescence. Ferritin-conjugated antibodies to IgG and IgM and to complement were localized in electron-dense aggregates present in the subendothelial space and in the mesangium, indicating that the immunofluorescent pattern seen resulted from binding of the conjugate in the same areas. In addition, ferritin-labeled antibodies were also seen in the subendothelial space and in the mesangial matrix.

In the last five allografts, the immunofluorescent pattern was considered indeterminate. (5) The distribution of the conjugated antibody to IgM and complement in glomerular capillary walls in LD109, LD108, BF, M30, and M33 was neither linear nor granular. Ferritin-labeled antibody was seen only in allografts of BF and M30 and was mainly present in the mesangial area.

Nine Allografts with Little or No Localization of Immunoglobulins or Complement

The renal biopsies from the nine patients listed in Table 2 revealed histopathologic changes in glomeruli which were little more than minimal, although similar in kind, if not in amount, to some of the allografts presented in Table 1. Subendothelial accumulations of amorphous material similar to the mesangial matrix and increased amount of mesangial matrix were observed. Six allografts, GW, LD78, LD87, DP, CJ, and VA, were

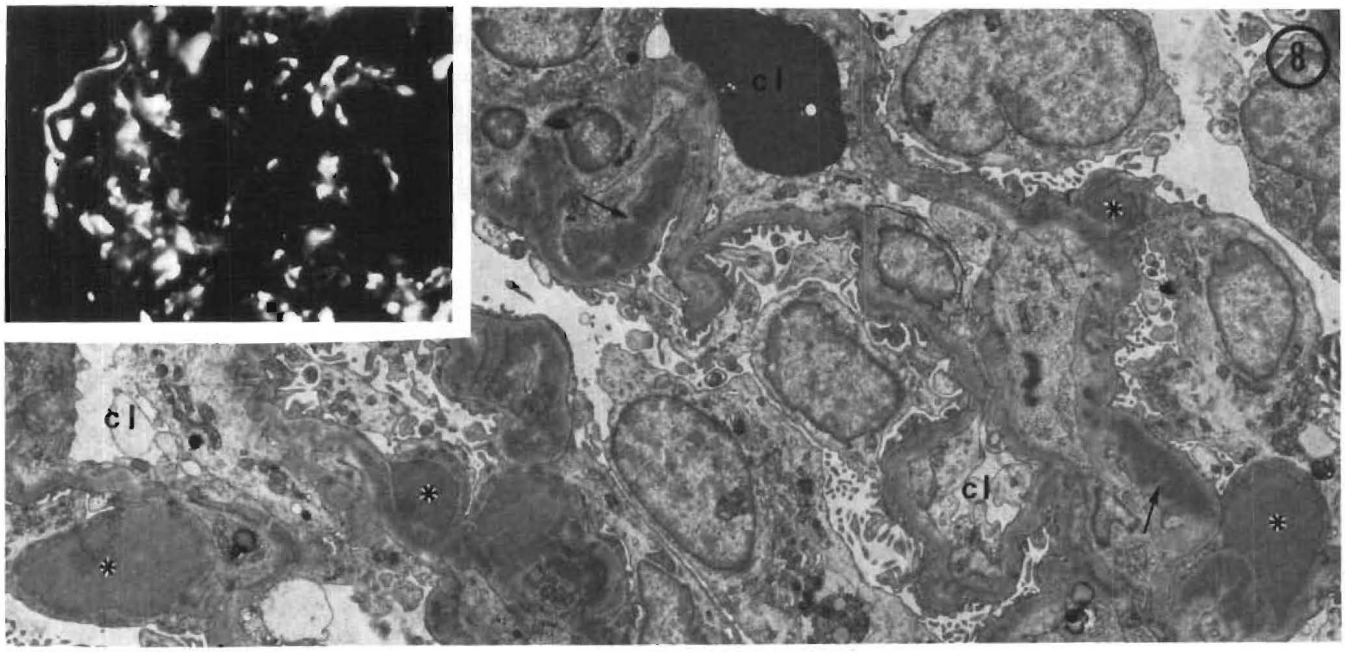
Fig. 3. Kidney biopsy of allograft LD102, 1 year and 8 months after transplantation. The subendothelial space of the basement membrane is occupied by foreign material which is more electron-dense (*arrowheads*) in proximity of the endothelial cytoplasm (*en*): *M*, Mesangial cell nucleus; *cl*, capillary lumen. ×2,900.

Fig. 4. Allograft LD102. Ferritin-conjugated antibody to IgM (*arrows*) is localized on the endothelial side of the basement membrane (*BM*) and in the newly formed basement membrane-like material close to the endothelial cytoplasm (*EN*). Organelles present in the subendothelial space (*ss*) do not bind the ferritin conjugate. *EP*, Epithelium. ×32,000.

Fig. 5. Allograft LD102. Ferritin-conjugated antibody to IgM is seen in the subendothelial spaces (*ss*). The electron-dense deposits (*D*) do not contain ferritin granules. *cl*, Capillary lumen; *EP*, epithelium. ×28,000.

Fig. 6. Kidney biopsy of allograft LD89, 2 years and 4 months after transplantation. The picture illustrates two electron-dense deposits (*D*) within the thickened glomerular basement membrane (*BM*). Ferritin-conjugated antibody to IgM is present in the deposits, but not in the basement membrane. *BS*, Bowman's space; *EP*, epithelium; *EN*, endothelium. ×30,000.

Fig. 7. Allograft LD89. The *inset* illustrates a frozen section stained with fluorescein-conjugated antibody to IgM which is localized in the capillary walls forming two parallel lines (*arrows*) corresponding to the "splitting" of glomerular basement membrane shown in Figure 3. *cl*, Capillary lumen; *BS*, Bowman's space. In the electron micrograph ferritin-conjugated antibody to IgM is localized (*arrows*) on the endothelial side of the basement membrane (*BM*) and in the newly formed basement membrane-like material close to the endothelial cytoplasm (*EN*). The organelles in the subendothelial space (*ss*) do not bind ferritin. *EP*, Epithelial foot processes. Figure 7, ×28,000; *inset*, ×1,100.



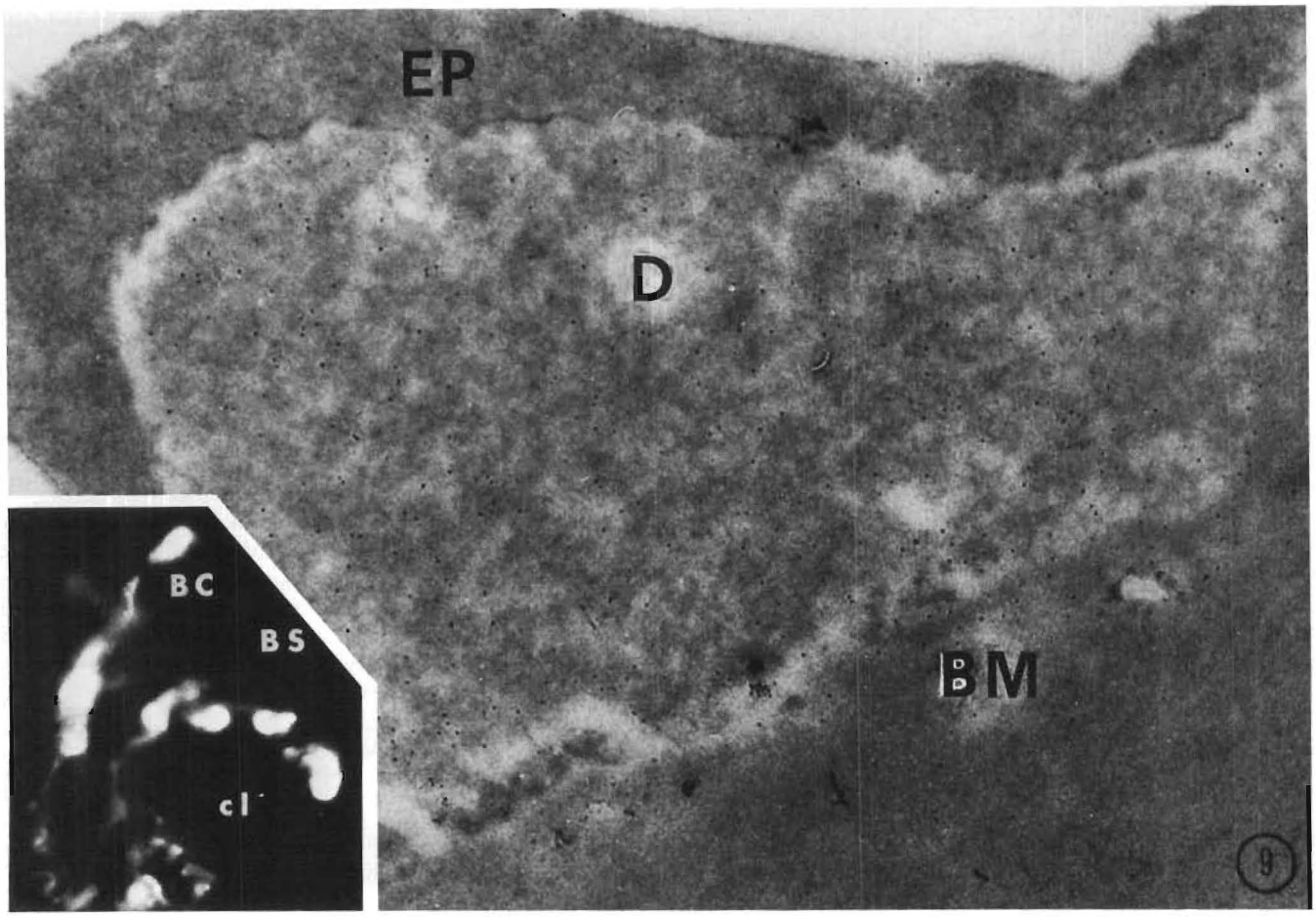


FIG. 8. Kidney biopsy of allograft LD85, 2 years and 6 months after transplantation. Proliferating cells partially obliterate the capillary lumina (*cl*). Subendothelial (*arrows*) and focal subepithelial (*asterisks*) deposits are visible. In the *inset* there is granular fluorescence resulting from staining of one glomerulus of the same allograft with fluorescein-conjugated antibody to β1C. Figure 8, ×5,000; *inset*, ×400.

FIG. 9. Allograft LD85. The *inset* illustrates part of a frozen section stained with flourescein-labeled antibody to β1C. Fluorescent granular deposits are seen along the wall of a glomerular capillary (*cl*). Other deposits are present in Bowman's capsule (*BC*). *BS*, Bowman's space. The electron micrograph displays part of the same tissue treated with ferritin-conjugated antibody to C'1q. Ferritin granules are localized in the subepithelial deposits (*D*), whereas they are not bound in the basement membrane (*BM*). *EP*, Epithelium. Figure 9, ×50,000; *inset*, ×1,000.

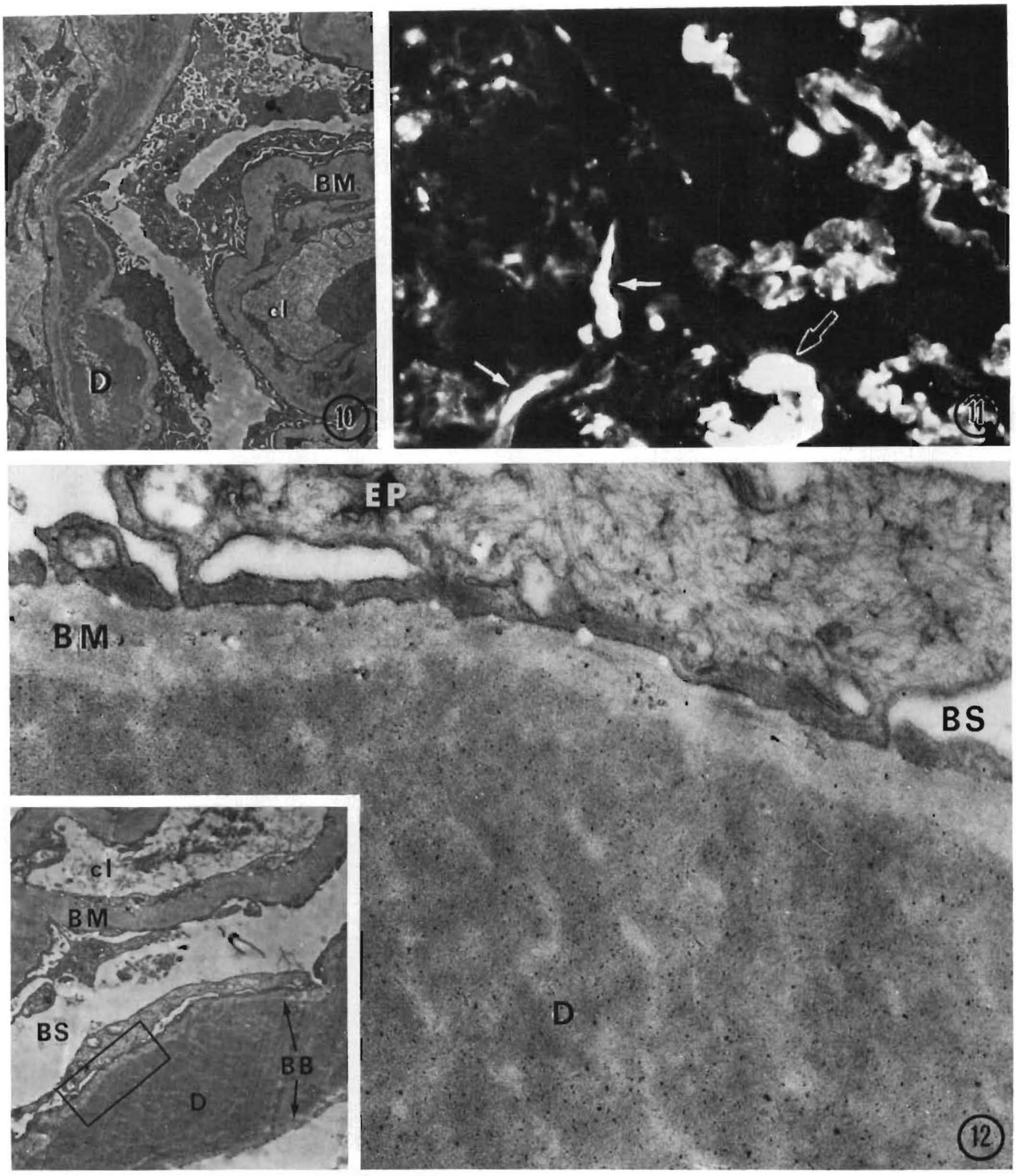
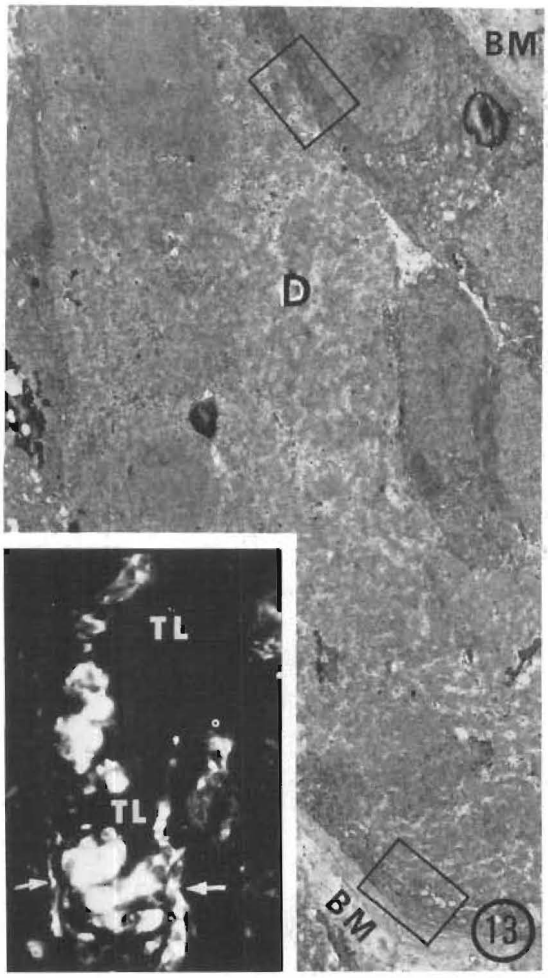
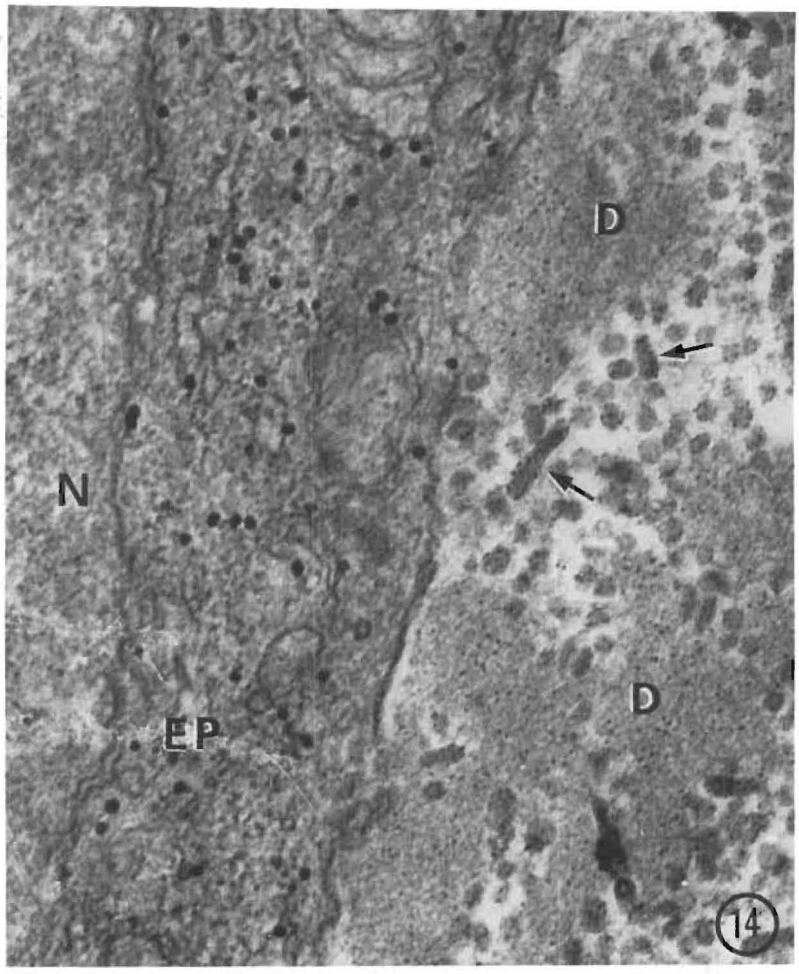


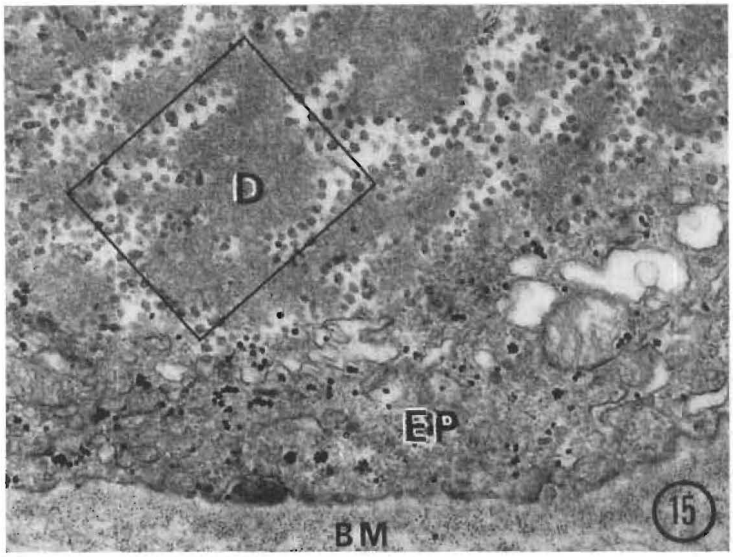
FIG. 10. Allograft LD85. Deposits (*D*) of foreign material are present within the basement membrane of Bowman's capsule. The glomerular basement membranes (*BM*) are thickened. *cl*, Capillary lumen. ×3,000.

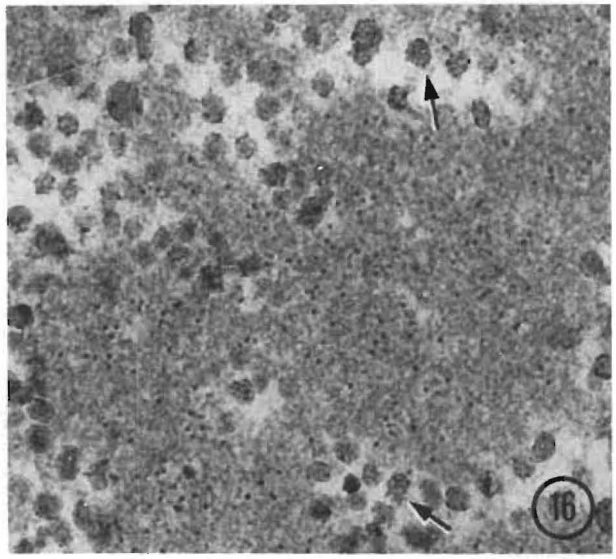
FIG. 11. Frozen section of allograft LD85 treated with fluorescein-conjugated antibody to β1C. The *small arrows* point to deposits in the Bowman's capsule, probably similar to those seen in Figure 10. The fluoresceinated antibody is also bound in the wall of the afferent arteriole (*large arrow*) and in the walls of some tubules. ×450.

FIG. 12. Allograft LD85. Tissue treated with ferritin-conjugated antibody to C'1q. In the *inset*, a deposit (*D*) is localized within the basement membrane of the Bowman's capsule (*BB*). *BS*, Bowman's space; *cl*, the lumen of a glomerular capillary; *BM*, a glomerular basement membrane. In the enlarged electron micrograph, ferritin granules are bound in the foreign deposit (*D*). The basement membrane (*BM*) of the Bowman's capsule and the parietal epithelium (*EP*) do not bind ferritin. *BS*, Bowman's space. Figure 12, ×35,000; *inset*, ×4,500.









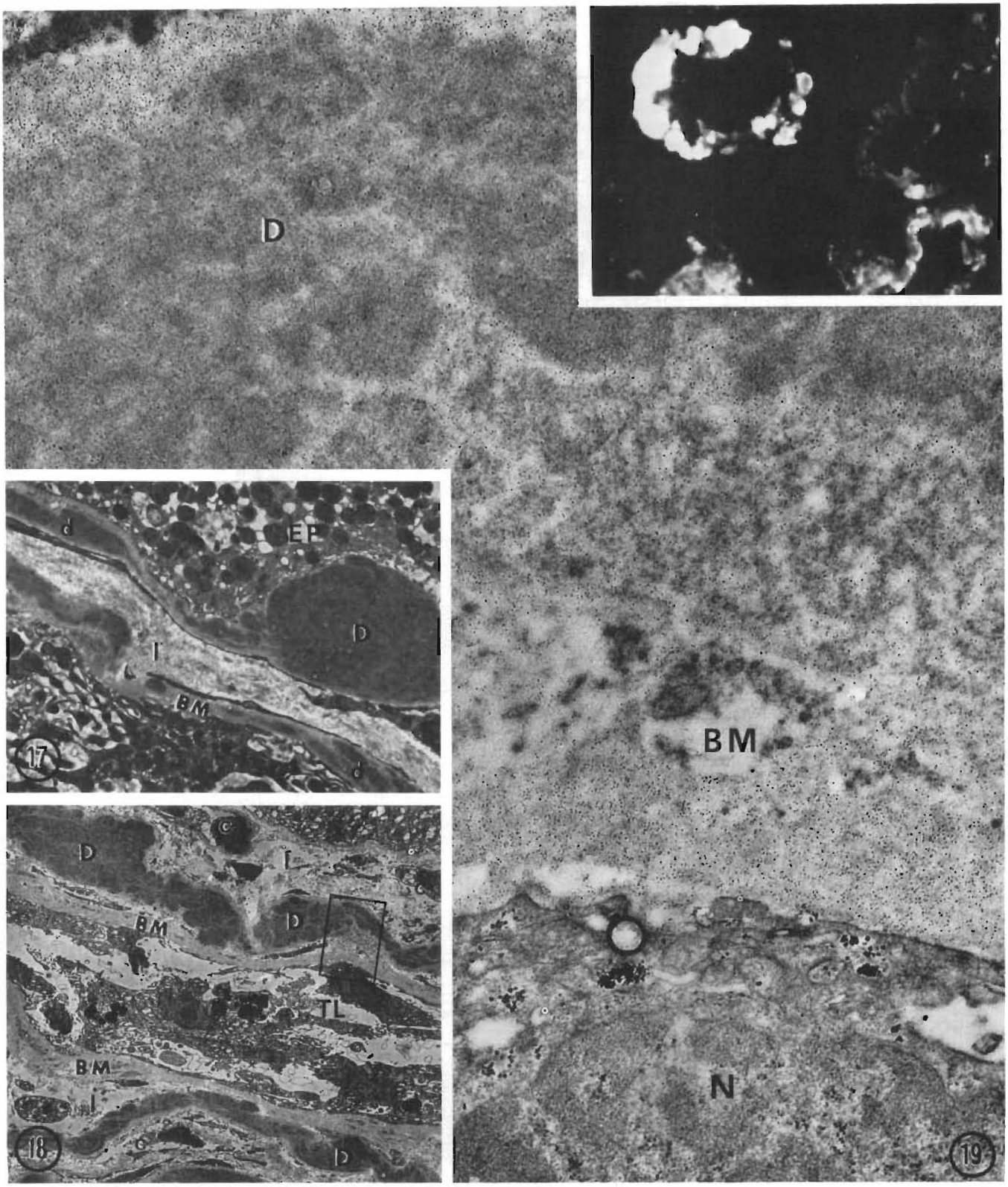
Note: Figures 13 to 16 illustrate cross-sections of renal tubules of allograft LD85.

FIG. 13. In the *inset*, a frozen section was stained with fluorescein-conjugated antibody to β1C. Aggregates of material present within the tubular lumen (*TL*) and parts of the tubular basement membrane (*arrows*) are stained. In the electron micrograph of Figure 13, the lumen of a tubule is almost completely obliterated by granular material (*D*). *BM*, Parts of tubular basement membrane. The two *boxed areas* of Figure 13 are illustrated at higher magnifications in Figures 14 to 16. Figure 13, ×4,000; *inset*, ×250.

FIG. 14. An enlargement of the *boxed area* at the *top* of Figure 13, ferritin-conjugated antibody to C'1q is bound to aggregates of electron-dense material (*D*) present within the tubular lumen. *N*, Part of the nucleus of an epithelial cell (*EP*). ×45,000.

FIG. 15. The ferritin conjugate is bound to aggregates of material contained in the tubular lumen (*D*) and to the tubular basement membrane (*BM*). *Square boxed area* is detailed in Figure 16. *EP*, Epithelial cell. ×20,000.

FIG. 16. Detail of *square boxed area* in Figure 15. The nature of the circular or elongated profiles, 600 to 800 Å in diameter, visible in Figures 14 to 16 (*arrows*) is unknown. ×65,000.



Note: Figures 17 to 19 are of allograft LD85. The electron micrographs illustrate sections of tissue treated with ferritin-conjugated antibody to C'1q.

FIG. 17. Electron-dense deposits (*d*) are present within the tubular basement membrane (*BM*). In this picture, the eliptical deposit (*D*) seems located between the tubular basement membrane and the epithelial cytoplasm (*EP*). At higher magnification, however, this deposit proved to be intramembranous. *I*, Interstitium. ×2,800.

FIG. 18. Electron-dense deposits (*D*) lie in the interstitium (*I*), along tubular basement membranes (*BM*). *TL*, Tubular lumen; *c*, three peritubular capillaries. ×1,500.

FIG. 19. At higher magnification this figure reveals the part of the tissue included in the *boxed area* of Figure 18. Ferritin-labeled antibody is localized in the deposit (*D*) and in the basement membrane (*BM*). *N*, Nucleus of an epithelial cell. In the *inset*, a frozen section of the same allograft is stained with fluorescein-labeled antibody to β1C. Granular staining of tubular walls is visible. Figure 19, ×30,000; *inset*, ×250.

TABLE 2. HUMAN RENAL ALLOGRAFTS[a]

Recipient			Donor sex, age (yr.), and relationship	Time of biopsy taken after transplant	Renal function at time of biopsy		Glomerular pathology by electron microscopy				Localization in glomerular capillary walls									
											Immunofluorescence microscopy					Immunoelectron microscopy				
Patient No.	Sex and age (yr.)	Disease			Ccr. ml./min.	Urinary protein gm./24 hr.	Mesangial changes	Subendothelial changes	Proliferative and exudative changes	Subepithelial deposits	IgG	IgM	Complement		Fibrinogen	IgG	IgM	Complement		Fibrinogen
													β1C	C'1q				β1C	C'1q	
GW	M 14	CPGN	M, cadaver	1 yr. 9 mo.	72	−	±	±	0	0	0	0	0	0	0	0	0	−	0	−
M 32	F 13	CPGN	M, 38, cadaver	4 mo.	50	1.20	±	±	0	0	0	±	0	0	±**	0	0	0	−	0
LD 78	M 34	CPGN	M, 30, unrelated	2 yr. 7 mo.	88	0.04	+	±	0	0	0	0	0	0	0	−	0	−	0	0
LD 87	F 33	Hydr.	M, 24, unrelated	2 yr. 5 mo.	83	0.10	±	±	0	0	0	0	0	0	0	0	0	−	0	−
LD 103	M 36	CPGN	F, mother	1 yr. 7 mo.	82	0.10	±	±	0	0	0	±**	0	0	0	0	0	−	0	−
DC	M 22	CPGN	M, 50, cadaver	1 yr. 4 mo.	31	0.90	+	+	0	0	0	0	±**	0	0	0	0	−	0	−
DP	F 24	CPGN	M, 13, cadaver	11 mo.	96	0.10	±	0	0	0	0	0	0	0	0	−	0	−	0	−
CJ	M 35	CPyN	M, 52, cadaver	1½ mo.	31	0.80	+	+	0	0	0	0	0	0	0	−	0	−	0	−
VA	F 32	CPGN	F, 35, cadaver	11 mo.	55	0.50	±	±	0	0	0	0	0	0	0	0	0	−	−	−

[a] Abbreviations are: Ccr, creatinine clearance; 0, negative; −, not tested; ±, minimal in amount; +, slight amount; ++, moderate amount; **, also in vessel walls; Hydr., hydronephrosis; Good., Goodpasture's disease; CPGN, chronic proliferative glomerulonephritis; and CPyN, chronic pyelonephritis.

negative for all five immunofluorescent sera tested, and staining was minimal in the other three, M32, LD103, and DC. Ferritin-conjugated antibodies were not bound in the renal tissue of any of the nine allografts.

Presence of Fibrinogen in Allografts

Fluorescein-labeled antibody to fibrinogen was bound in only six instances in the glomeruli of the 34 allografts. In four the binding was minimal; in the other two it was rated only one plus. The greatest concentration of fibrinogen was observed in the allografts with generalized granular distribution of immunoglobulins and complement. It was present mainly in the mesangial areas of LD85 and LD110. Ferritin-labeled antibody to fibrinogen was tested in seven cases but did not localize in these tissues.

Localization of Immunoglobulins and Complement in Vessel Walls

Morphologic and immunologic changes were frequently observed in glomerular and interstitial arterioles. In 12 allografts fluorescein- and/or ferritin-labeled immunoglobulins and complement were demonstrable in these areas, as is illustrated in Figures 20 to 29. The *inset* of Figure 20 exhibits a section of allograft LD71 (1 year and 10 months old) in which the wall of an afferent arteriole was stained with fluorescent antibody to IgM. Figures 20 and 21 display an arteriole from the same kidney in which ferritin-conjugated antibody to IgM has been bound in the basement membrane. Conjugated antibodies to immunoglobulins and complement were seen in the basement membranes of arterioles, in the normal or increased amount of basement membrane-like material lying between the cells of the media, and in the basement membranes of interstitial capillaries and venules (Figs. 22 to 24). The antibody to complement gave more uniform localization. In two allografts, LD85 and LD89, granular deposits (Fig. 25), binding fluorescein- (Fig. 26) and ferritin-conjugated antibody (Figs. 27 to 29) to C1q were present among the smooth muscle cells of the media or near the walls of a vein and arteries. Control tests were consistently negative (Fig. 30) in all instances.

DISCUSSION

Examination of biopsies from 34 allografts by immunofluorescence and immunoferritin techniques leads to a separation of the tissues into two major groups. The first contained 25 allografts in which the presence of excess immunoglobulins and complement was demonstrated in the glomerular capillary walls, whereas the second consisted of nine tissues in which there was minimal or no evidence for the presence of these proteins. The major group was subdivided on the basis of the distribution of the immunoglobulins and complement in the glomeruli.

When tested with fluorescein-labeled antibody, 10 of the tissues showed linear localization in the capillary loops, which was widely dispersed in four and was focal in distribution in six. Another 10 showed granular distribution in capillary loops, which also was widely dispersed in four and focal in six. The other five tissues could not be classified on the basis of what was seen in the fluorescent microscope. The localization appeared to be mainly in the mesangial area.

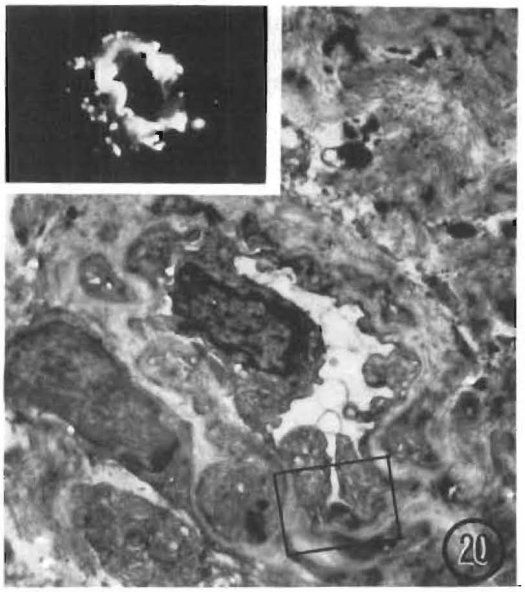
The electron microscopic studies gave precise information concerning the lesions in these allografts and the specific area of localization of the immunoglobulins and complement. In allografts of the first four patients (Table 1), the most characteristic glomerular changes consisted of fine subendothelial deposits, subendothelial accumulation of material morphologically similar to mesangial matrix, and increase of the mesangial matrix. Immunoglobulins and complement were localized, by the ferritin-labeled antisera, on the endothelial side of the basement membrane and in the subendothelial deposits.

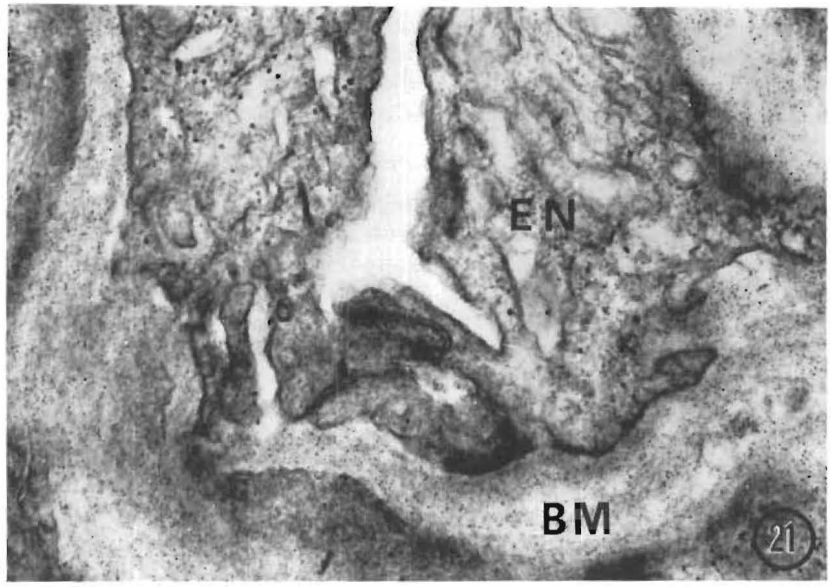
In four allografts, LD89, DH, LD85, and LD110, granular localization of fluorescent antibodies to immunoglobulins and complement was also diffuse. The granular appearance of the fluorescence was reminiscent of pathology produced by circulating antigen-antibody complexes.[6, 11, 12, 14] By the immunoferritin technique, it was seen that in allografts LD110 and LD89 there were no subepithelial deposits, and the immunoferritin studies showed that the granular pattern seen by immunofluorescence resulted from staining of immune deposits present within glomerular basement membranes, in subendothelial spaces, between proliferating cells, and in the mesangial areas. By contrast, in allografts LD85 and DH subepithelial deposits binding the labeled antibodies characteristic of acute, LD85, or chronic, DH, serum sickness were also present.

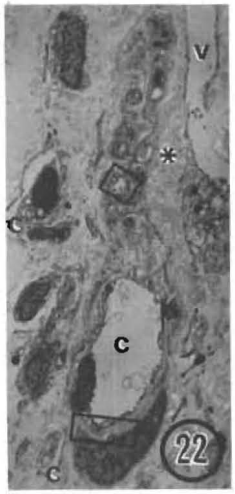
The separation of six allografts with focal linear fluorescence from six with focal granular fluorescence was often difficult, and the changes detected by light and electron microscopy were not distinctive. Subendothelial and basement membrane deposits were present in both groups. Some of the deposits bound ferritin-conjugated antibodies, whereas others did not. This may be due to the lack of penetration of ferritin conjugates or to the variation in the composition of the deposits. The variable capacity of the foreign deposits to bind the conjugates may account for the difficulty in separating allografts with focal linear from those with focal granular fluorescence.

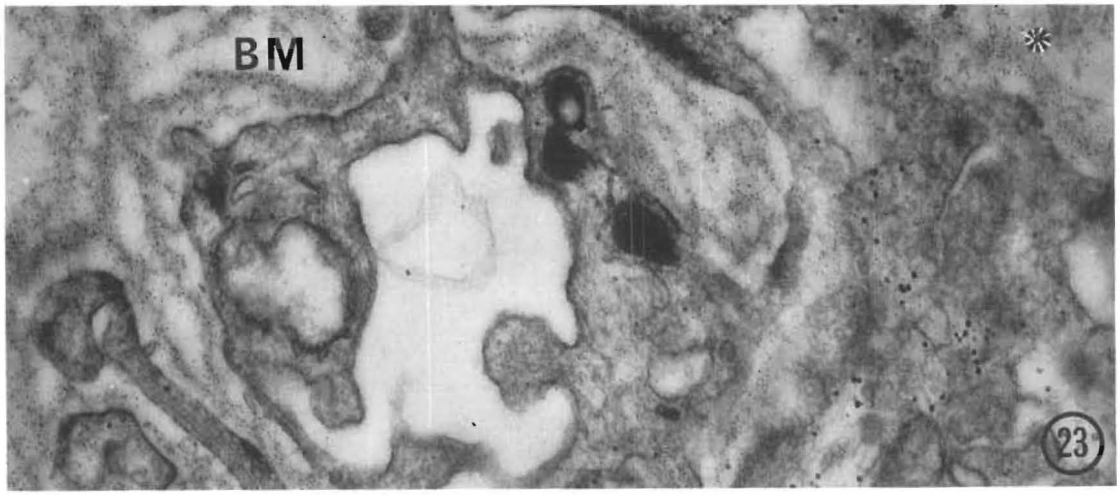
Two of the five allografts in which localization of fluorescein-labeled antibodies could not be classified as linear or granular also bound ferritin-labeled antibody to immunoglobulins and complement. The localization of these antibodies was mainly in the mesangial area.

Three of the nine allografts (Table 2) bound only minimal amounts of antibodies to immunoglobulins or complement in glomerular structures by the immunofluorescence technique. Ferritin-conjugated antibody was not found in any of the tissues. These allografts, however, had mesangial and subendothelial changes as well as vascular changes similar to slight or moderate changes seen in some of the allografts in which immune globulins and complement were bound. It has been proposed that nonimmunologic factors may be partially or wholly responsible for chronic renal graft rejection.[15, 37] How-









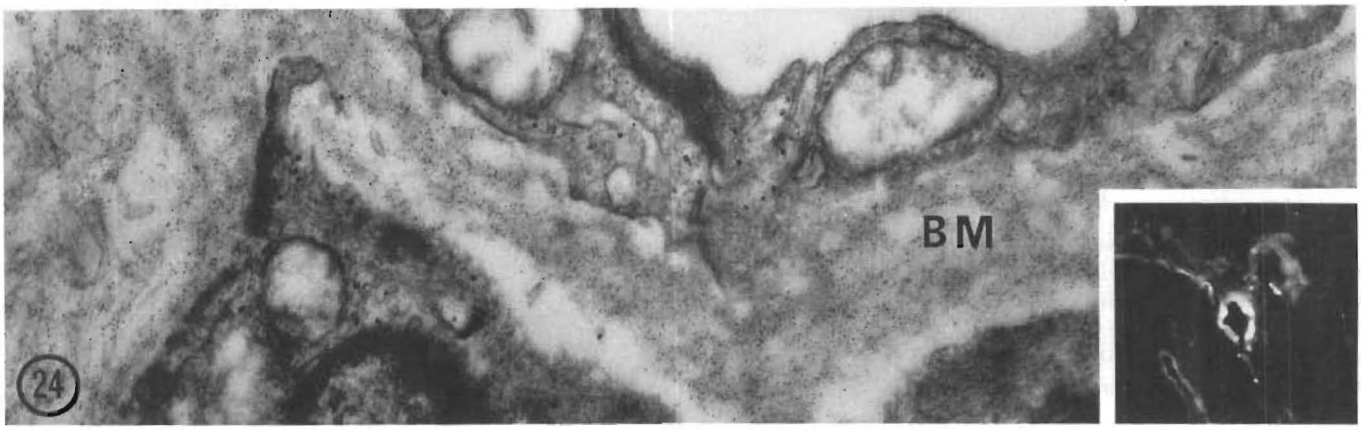
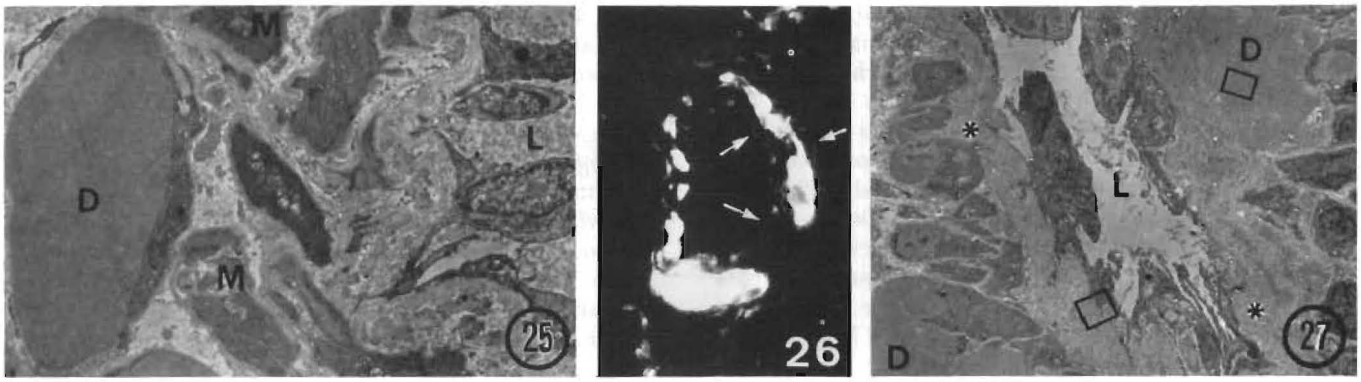


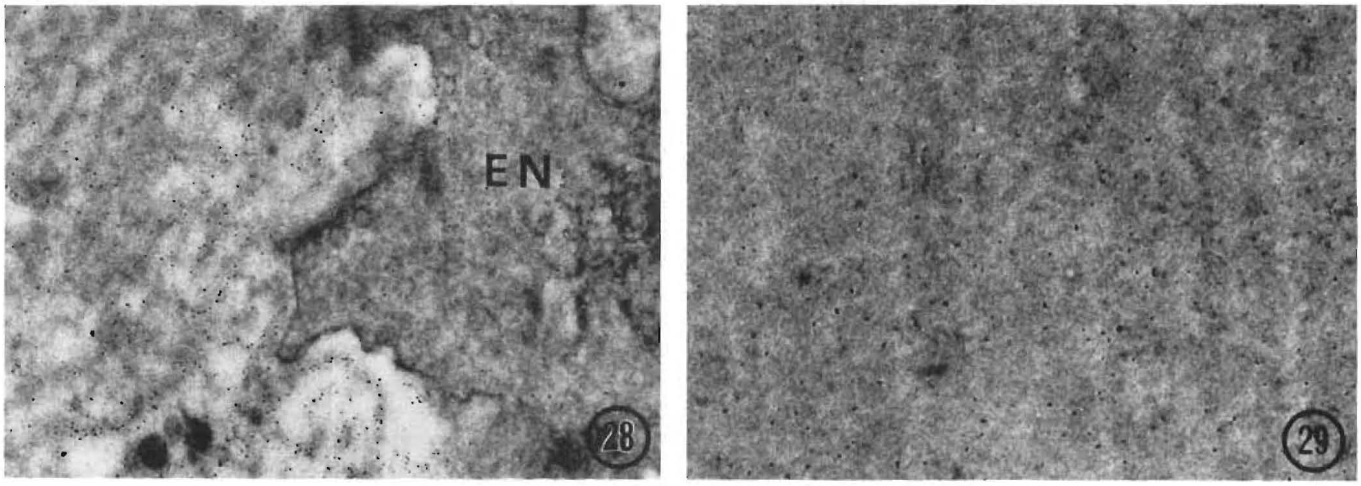
FIG. 20. Biopsy of allograft LD71, 1 year and 10 months after transplantation. The *inset* illustrates the localization of fluorescent antibody to IgM in the wall of an afferent glomerular arteriole. Fluorescent antibody was not seen in the glomerulus. The electron micrograph displays a cross-section of an afferent arteriole in the same allograft. Tissue treated with ferritin-conjugated antibody to IgM. The *boxed area* is shown at higher magnification in Figure 21. Figure 20, ×2,500; *inset*, ×250.

FIG. 21. Allograft LD71. Higher magnification of the *boxed area* of Figure 20. Ferritin granules are localized in the basement membrane (*BM*) of the arteriole. *EN*, Endothelium. ×33,000.

FIG. 22. Allograft LD102. Tissue treated with ferritin-conjugated antibody to IgM. The picture exhibits an oblique section of a capillary (*c*) and of a venule (*v*) in the interstitium, and the * indicates basement membrane-like material interposed between capillary and venule. The *square* and *rectangular boxed areas* are illustrated at higher magnifications in Figures 23 and 24, respectively. ×1,000.

FIGS. 23 AND 24. Allograft LD102. Ferritin-conjugated antibody to IgM is localized in the basement membrane-like material interposed between the capillary and the venule (*asterisk*, Fig. 23, ×28,000) and in the basement membrane (*BM*) of the capillary. The *inset* of Figure 24 reveals the localization of fluorescent antibody to IgM in the wall of an intertubular capillary. Figures 23 and 24, ×36,000; *inset* of Figure 24, ×200.





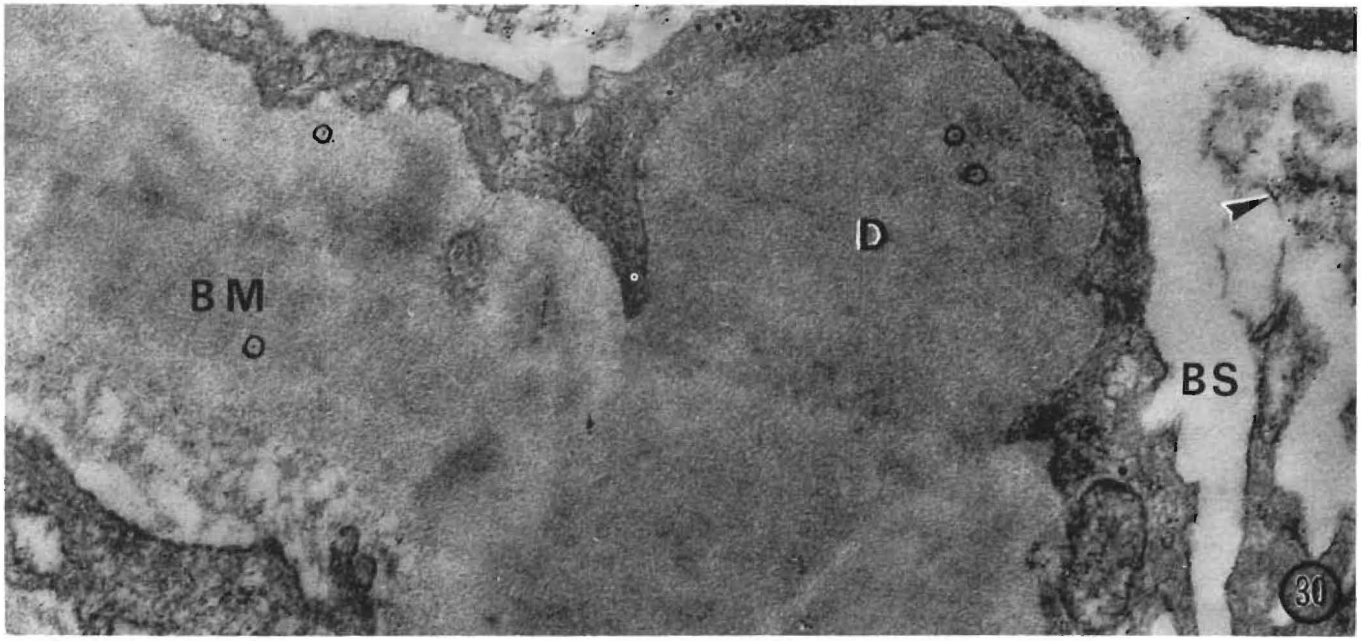


FIG. 25. Allograft LD85. Cross-section of the wall of an artery. *L*, Lumen of the vessel; *D*, a granular deposit between the smooth muscle cells (*M*) of the media. ×2,000.

FIG. 26. Frozen section of allograft LD85 treated with fluorescein-conjugated antibody to C'1q. Granular deposits are localized in the wall of an artery between the autofluorescent elastica interna and elastica externa (*arrows*). ×250.

FIGS. 27 TO 29. Allograft LD85. Tissue treated with ferritin-conjugated antibody to C'1q. Cross-section of an artery. Foreign deposits (*D*) are present between the smooth muscle cells of the media. *L*, Lumen of the vessel; *asterisk*, the thickened subendothelial space which probably contains the basement membrane and the elastica interna. Figures 28 and 29 represent higher magnifications of the two *boxed areas* of Figure 27. *EN*, Endothelium. Ferritin-conjugated antibody to β1C is bound in the subendothelial space and the deposit, respectively. Figure 27, ×1,300; Figures 28 and 29, ×36,000.

FIG. 30. Allograft LD85. Tissue treated with ferritin-conjugated normal goat globulin. Only a few ferritin granules (*circles*) are present in the basement membranes (*BM*) and in the focal subepithelial deposit (*D*). Other ferritin granules (*arrowhead*) are visible in the Bowman's space (*BS*). ×37,000.

ever, failure to demonstrate immune reactants at one time does not rule out previous immunologic activity. It has been repeatedly observed in experimental[26] and human[38] glomerulonephritis, as well as in human renal allografts, LD89, that detectable amounts of immunoglobulins and complement may have a short life. Their disappearance may not necessarily coincide with clinical and morphologic recovery. This may be particularly true in patients treated with immunosuppressive agents.

The present study of human kidney transplants does not contribute information concerning the specificity of the immunoglobulins found. It still is not known which antigen or antigens are involved or if true antigen-antibody complexes are always present.[22] The immunoferritin studies, however, have given precise information as to where the immunoglobulins and complement are found. The data presented here suggest some directions which may be given to hypotheses raised concerning the antigens and/or mechanisms causing the immunologic reactions.

The diffuse linear staining of glomerular capillary walls, obtained with fluorescent antibodies to immunoglobulins and complement in some of the grafts, was similar to that observed in glomerulonephritis produced by antibodies directed against antigens of the basement membrane as in Goodpasture's disease, primary nephrotoxic nephritis, or against antigens fixed to the basement membrane (second phase of nephrotoxic nephritis). An attractive hypothesis to account for the linear staining seen in the biopsies of allografts described here is that antibodies to glomerular basement membrane[27] may be responsible for the subendothelial and mesangial lesions. The staining, however, was not as sharp as that seen in antiglomerular basement membrane pathology.[26] Furthermore, the ferritin studies indicated that the immunoglobulins localized more in the subendothelial space than in the glomerular basement membrane proper, which might rule out basement membrane as the exclusive specific antigen involved in this pathology. There are two clinical observations which also throw doubt on the hypothesis that the reaction between basement membrane and specific antibody is the only cause of the observed linear localization. The first of these is that subendothelial and mesangial lesions were observed as well in allografts of patients who had not suffered from glomerulonephritis as, for example, M8.[36] The second is that significant correlation existed between incompatibility of host and donor and the occurrence of renal damage.[32]

Recently Koffler, Agnello, Carr, and Kunkel[24] reported the results of an immunofluorescence study of renal tissues, obtained either by biopsy or at autopsy from patients with systemic lupus erythematosus. Biopsy specimens from three kidneys out of 19 and both necropsy specimens exhibited linear deposition of antibody to IgG in the glomerular capillary walls. Citrate buffer eluates from biopsy and necropsy specimens did not contain demonstrable antibody to basement membrane. Thus in some phases of systemic lupus erythematosus, a disease probably due to antigen-antibody complexes, linear localization may nevertheless occur. Evidence could not be obtained that this linear localization was associated with antibody to basement membrane.

A second hypothesis to explain the immunologic findings is that the pathology described in this group of allografts is initiated and/or maintained by circulating antigen-antibody complexes. Inasmuch as the antigens which may have participated in the formation of circulating immune complexes are unknown, the possibility that in some cases an immune complex glomerulonephritis of the recipient may have been transmitted to the graft cannot be excluded.[16, 18, 19, 34-36] The theory presents the same etiologic uncertainties which exist concerning the origins of human nephropathies produced by nonglomerular antigen-antibody complexes.[13]

A more likely available complex would be that between histocompatibility antigens and transplantation antibodies.[10, 44] In such a case, one of two possible sequences might occur. (1) Transplantation antigens are as yet only partially characterized both chemically and immunologically.[1, 2, 8, 9, 21, 31, 40] Therefore, weak and widely diffuse transplantation antigens might be present in some components of glomerular and vascular basement membrane, where they react with antibodies of the host.[28] (2) Under conditions of intense immunosuppressive treatment, small amounts of circulating transplantation complexes may localize within vascular structures. An accumulation slower than glomerular catabolism[11] or a qualitatively different type of immune complex with strong affinity for glomerular basement membrane[24] may account for the absence of the type of glomerular pathology usually associated with serum sickness mechanisms.[6, 11, 13, 14] This pathogenesis could be common both to allografts with linear and with granular fluorescence and may explain the pathologic similarities observed in cases with focal linear and with focal granular stainings. The hypothesis that transplantation antigens released from the graft[30] may react with specific antibody and complement and then lodge in the glomeruli appears to be most important to establish. This pathogenetic mechanism could cover the variety of glomerular lesions observed.

In human patients and in rabbits with renal allografts, the formation of kidney-specific antibody and deposition of γ-globulins along tubular basement membranes have been described.[23, 45] The experimental counterparts of tubular lesions similar to those observed in allografts RM, LD93, LD89, DH, and LD85 might be represented by allergic glomerulonephritis induced in rabbits with homologous renal antigens.[43] However, in these studies, the deposits in tubular basement membranes were believed to be the result of a reaction between host antibody and specific antigenic determinants of tubular basement membranes. In allograft LD85 it was shown that aggregates of material binding fluorescein- and ferritin-conjugated antibodies were seen in the glomeruli and also in the tubular lumina. Their accumulation on both sides of tubular basement membranes should be interpreted as owing to reabsorption of immunologic reactants filtered through injured

glomeruli. This hypothesis gains further support from the presence of deposits in Bowman's capsule. Therefore, the present study cannot provide evidence that an autoimmune reaction is responsible for tubular damage.

In 12 allografts (see Tables 1 and 2), antibodies to immunoglobulins and complement localized in basement membranes of arteries and veins and in the increased amount of basement membrane-like material present between the smooth muscle cells of the arteries.[37] These findings are similar to studies on vascular localization of goat radioiodinated transplantation antibodies[33] and on vascular localization of fluorescein-conjugated 19S and 7S globulin fractions eluted from canine renal allotransplants.[20, 25] The localization of immunologic reactants in the walls of glomerular arterioles (also observed in a new and larger series of allografts with acute or chronic rejection, presently under study[3]), suggests that histocompatibility antigens of vascular structures generate a marked antibody response.[33, 46] Such antibodies directed against vessels may also be influential in the production of rejection.

In two allografts, LD89 and LD85, the granular deposits present between the smooth muscle cells of the media were similar to vascular deposits seen in rabbits with chronic serum sickness.[5] Therefore, these lesions were probably produced by prolonged exposure to circulating antigen-antibody complexes.

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