Renal Transplantation between HL-A Identical Donor-Recipient Pairs

FUNCTIONAL AND MORPHOLOGICAL EVALUATION

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ABSTRACT 16 patients underwent renal transplantation from a sibling donor who was prospectively determined to be ABO compatible and HL-A identical with the recipient. Unidirectional mixed leukocyte reactions were performed; in each instance, lymphocyte stimulation in either direction was not observed.

The plasma creatinine 10–68 months after transplantation in these 16 patients ranged between 0.9 and 1.9 mg/100 ml. The creatinine clearance ranged from 48 to 113 ml/min, and the blood urea nitrogen (BUN) ranged between 12 and 35 mg/100 ml. Urine protein excretion varied from 0.11 to 1.86 g/day. Six patients exhibited no detectable clinical episodes of acute rejection; they were treated with azathioprine alone and each of them demonstrated normal or near normal renal histology when biopsy specimens were obtained more than 6 months after transplantation. Nine patients experienced acute rejection episodes that required the use of steroid therapy. The severity of these rejection episodes was variable; they included a mild reduction in renal function with an immediate steroid-induced restoration of function and eventual discontinuance of steroid therapy to severe reduction in function requiring prolonged and moderate doses of steroids without return to normal renal function. Renal histological observations in this group ranged from mild to marked cellular and structural changes which fit the criteria of the rejection. One patient demonstrated a gradual loss of renal function with heavy proteinuria. Biopsy of this allograft demonstrated the recurrence of original disease, i.e., lobular glomerulonephritis.

The marked variability in the clinical course and allograft morphology in these 16 patients could be explained by antigenic differences at non-HL-A loci. The presence of minor histocompatibility loci has been well documented in other mammalian species and they are most certainly present in man. The need for their identification and definition is stressed.

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INTRODUCTION

Initial efforts in histocompatibility testing before renal transplantation have been directed toward the definition of donor-recipient compatibility for the major transplantation antigens with the hope that the adverse clinical expressions of minor incompatibilities might be controlled by immunosuppressive therapy (1). It was established quite early that the red cell isoantigens A and B provided at least one major determinant of histocompatibility and that donor-recipient incompatibility for the minor red cell isoantigens was of lesser importance (2). Complete donor-recipient compatibility for all surface membrane-associated tissue isoantigens would be difficult if not impossible to achieve (except among monozygotic twins), and identification of major antigenic differences between a single donor-recipient pair was recognized as the most that might be achieved realistically.

The genetic loci of the major histocompatibility antigens in man have now been identified and partially defined utilizing serologic techniques (3–6). Present data indicate that this antigenic system designated HL-A, is controlled by two closely linked polymorphic loci on a single pair of autosomal chromosomes. The first locus defines a series of HL-A antigens which includes HL-

A1, 2, 3, 9, 10, and 11. A second series, controlled by the second locus, includes HL-A5, 7, 8, 12, and 13(7). Studies within individual families indicate that a single HL-A antigen is defined by each of the two loci of a single chromosome. The complete HL-A genotype of any individual is therefore composed of four antigens defined by the corresponding first and second loci of the chromosomal pair, the contribution to the genotype by the two loci on a single chromosome being defined as the haplotype.[1] The immunogenicity of individual HL-A antigens has not yet been evaluated completely. However, within a family group, HL-A compatibility between any sibling-sibling or parental-offspring combination can be classified as HL-A identical, different by one HL-A haplotype, or different by both HL-A haplotypes. A positive correlation between these genetic relationships and the survival of test skin grafts exchanged within families has been demonstrated previously (8).

Further, numerous early studies suggested that the mixed leukocyte reaction provided an in vitro measure of antigenic differences under the control of the HL-A loci (9–11). It has been generally accepted that the HL-A antigens, the mixed leukocyte reaction, and the immunological rejection of an allograft are the expression of the same locus. The validity of this statement has been challenged recently by the observation of anomalous mixed leukocyte reactivity between HL-A identical pairs as well as in situations where recombination for the HL-A loci was known to have occurred (12–14). These findings have led Yunis and Amos (15) to suggest that HL-A antigens, the mixed leukocyte reaction, and hypersensitivity delayed reaction may represent the expression of separate but closely linked genetic loci.

It now seems certain that the HL-A loci provide a major determinant of histocompatibility in man. Nevertheless, further studies are needed to assess the role of non-HL-A and other membrane-associated antigenic systems in the determination of histocompatibility, and to identify and define the genetic loci of such factors. Clinical evaluation of the results of renal transplantation between HL-A identical donor-recipient pairs should provide valuable insight into the nature of such factors, facilitate our understanding of the importance of single non-HL-A loci, and permit the detection of possible synergism between two or more non-HL-A loci as shown previously in the mouse non-H-2 system (16).

This report describes clinical observations on 16 patients who underwent renal transplantation after HL-A identity with their respective living donors was established by serotyping, the mixed leukocyte reaction, and the exchange of test skin grafts between selected family pairs. Clinical observations on the structure and function of the renal allograft were made for varying periods of time after renal transplantation in an effort to establish the nature and intensity of non-HL-A determinants of histocompatibility and the relationships between HL-A identity, the subsequent clinical course, and alterations of renal structure and function.

[1] Haplotype refers to the contribution of one member of a pair of homologous chromosomal units to the genotype.

METHODS

16 patients with primary renal disease and chronic uremia and their potential living related donors underwent complete histocompatibility testing before renal transplantation; in each instance, serotypic evidence of HL-A identity was established between the recipient and a medically acceptable and willing donor. The potential recipients ranged in age from 16 to 52 yr; 14 patients were male, and 2 were multiparous females. All recipients had been followed closely at either the Duke Medical Center or the Durham Veterans Administration Hospital for 1–10 months. Each of them required repetitive hemodialysis for periods as long as 25 wk immediately before transplantation. Overall, based on an amalgam of clinical evidence (including morphologic examination of the recipient kidneys after bilateral nephrectomy at the time of transplantation), a diagnosis of chronic glomerulonephritis was made in 13 recipients, malignant nephrosclerosis in 2 recipients, and chronic pyelonephritis in one recipient.

Living donors were selected from one or more siblings of each recipient. The selected sibling donors ranged between 20 and 47-yr of age; 5 were female and 11 were male. In addition to histocompatibility evaluation, each selected donor underwent complete medical and psychiatric evaluation to determine his or her suitability. All were informed and willing volunteers. The specific details of donor selection at our institution have been outlined previously (17).

Histocompatibility testing. Serotyping of all available members of each family was carried out using a panel of antisera to human leukocytes that was capable of detecting each of the 11 HL-A antigens that have been recognized by the World Health Organization (HL-A1–3, 5, and 7–13), as well as additional antigenic specificities that have not yet been recognized officially. A two-stage, semi-micro, cytotoxicity, dye-exclusion test was employed (18). Inheritance of the two HL-A haplotypes from each parental pair was established by haplotype analysis (8).

Test skin grafts (4 mm in diameter) were exchanged between selected family pairs by a method described previously (1). A skin graft was considered rejected when less than 50% of the transplanted tissue was viable by gross and microscopic examination. The exchange of these skin grafts among members of an individual family was designed to measure the immunogenicity of each of the HL-A haplotypes within the family, as well as that of non-HL-A loci that have not yet been defined. Test skin grafts were not placed on any of the potential recipients.

Unidirectional mixed leukocyte cultures were established between each sibling donor and recipient using the previously described technique. Appropriate controls were also used (19).

Clinical management and evaluation after transplantation. Renal transplantation was carried out as soon as possible after the medical and immunological suitability of potential donor-recipient pairs had been established. The surgical details of the transplantation procedure itself have been described previously (20); pretransplantation transurethral resection of the prostate was performed in one male re-

TABLE I

Summary of Histologic Observations

Patient	Host disease*	Allografts		
		Glomeruli‡	Allograft	Interstitial inflam.
Group 1				
W. B. (0002)	CGN, ANS	↑Mesangium; EDD, local, IgG, IgA in mesangium	Normal	Slight, focal, chronic
V. O. (0008)	CGN, ANS	↑Mesangium; EDD (small, rare)	Smudging slight	Chronic, focal, slight
J. D. (0055)	CGN, MNS	↑Mesangium, 7S, C 3–4, GpC-fix	Smudging, slight	Chronic, focal, mild
C. D. (0092)	CGN, ANS	↑Mesangium, EDD (rare)	Normal	Chronic, mild
H. M. (0138)	CGN, ANS, CPN	↑Mesangium, slight 7S, C 3–4	Smudging, slight	Chronic, focal, slight
J. M. (0186)	CGN	Normal	Normal	Chronic, focal, mild
Group 2				
R. C. (0007)	CGN-mixed membranous and proliferative	↑Mesangium; EDD (rare); IgG, IgM,	Thickened intima	Subcapsular, chronic
F. L. (0083)	MNS	↑Mesangium (rare), EDD (small), IgG, IgM, C 3–4	Sclerosis, moderate	Normal
B. L. (0059)	CGN, MNS	↑Mesangium, focal, EDD (moderate), 7S IgG, IgM, IgA, C 3–4, Gp C-fix	Smudging, slight	Chronic, focal, slight
V. H. (0099)	MNS	↑Mesangium, focal	Normal	Subcapsular, chronic marked
		Allograft		
		Glomeruli‡	Arterioles	Interstitial inflam.
D. C. (0089)	CPN	↑Mesangium, rare	Normal	Chronic, slight
C. M. (0117)	CGN, ANS	↑Mesangium, focal, EDD (rare) 7S, IgM, IgG, C 3–4, GpC-fix	Normal	Chronic, moderate
J. W. (0169)	CGN	↑Mesangium, slight	Normal	Chronic, focal, slight
S. P. (0053)	CGN, ANS	Normal	Normal	Chronic, focal, slight
O. M. (0184)	CGN	Multiple capillary thrombi	Multiple thrombi	Multiple infarcts
Group 3				
C. W. (0009)	CLGN	Cellular and sclerotic lobularity, IgM	Normal	Chronic, nodular, severe

* CGN, chronic glomerulonephritis; ANS, arterial and arteriolar nephrosclerosis; CLGN, chronic lobular glomerulonephritis; MNS, malignant nephrosclerosis; CPN, chronic pyelonephritis.

‡ EDD, electron dense deposits; 7S, 7S immunoglobulins; CpC-fix, fixation of guinea pig complement in vitro (immunohistologic complement-fixation test).

cipient because of moderate prostatism with outflow obstruction, and all recipients underwent bilateral nephrectomy at the time of transplantation. Immunosuppression was initiated in each recipient according to a standard protocol: azathioprine was administered orally in total daily doses of 2.5 mg/kg for 2 days before transplantation, 3 mg/kg on the day of transplantation, and 2 mg/kg thereafter until a reduction of dosage was necessitated by the appearance of either leukopenia or hepatic dysfunction. Additional immunosuppression was not utilized unless suspected immunological rejection supervened. In that even, threatened rejection was treated with oral prednisone, 200 mg/day, until the rejection process had been reversed or maximal improvement of renal function was observed. The dosage of prednisone was then reduced to a maintenance level ranging between 0 and 20 mg/day. Antilymphocyte globulin or local irradiation to the allograft were not utilized. A diagnosis of suspected acute or chronic rejection was established by criteria that have also been outlined previously (20). For the most part, this diagnosis was based on the observation of declining renal function, appropriate changes of urine sodium excretion, fever, increase in size and tenderness of the graft, hematuria and/or proteinuria, and the appearance of hypertension in the absence of other discernible causes of renal functional impairment.

Once the immediate post-transplantation period (60–90 days) had passed and the clinical course of the recipient seemed stable, each patient was examined irregularly at 2 to 8-wk intervals. A complete history and physical examination was performed at the time of each visit; in addition, serial laboratory observations were obtained which at least included routine urinalysis, urine culture, and sequential measurements of the endogenous creatinine clearance (Ccr),[2] total urine protein excretion, and the blood or plasma concentrations of creatinine (Pcr), urea nitrogen (BUN), sodium, potassium, chloride, bicarbonate, calcium, phosphorus, and glucose. Intravenous urography, radio-

[2] *Abbreviations used in this paper:* BUN, blood urea nitrogen; Ccr, creatinine clearance; EDD, electron-dense deposits; MLR, mixed leukocyte reaction; Pcr, plasma concentration of creatinine; PTD, post-tranplantation day.

renography, selective angiography, and other diagnostic procedures were performed whenever indicated or at least at frequent intervals. Surgical biopsy of all renal allografts was performed at least 6 months after renal transplantation irrespective of the presence or absence of a clinical diagnosis of suspected rejection. The appearance of adverse clinical episodes necessitated biopsy more immediately in some patients. A second biopsy specimen was obtained in some patients whose clinical course reflected the subsequent deterioration of renal function for the first time. An effort to quantitate the degree of rejection activity in each allograft biopsy was made and these data are summarized in Table I. The histological criteria for the diagnosis of different patterns of immunological rejection have been described previously (21). These include interstitial edema, focal hemorrhage, and inflammatory infiltrates consisting of lymphocytes, macrophages, and plasma cells around tubules, vessels, and sparsely around glomeruli; vascular changes include local thickening, smudging, or vacuolization of the muscular media, and intimal proliferation with fibrinoid necrosis of arterioles; glomerular changes consisting of thickening of mesangial regions and capillary walls, fibrous capsular crescents, immunoglobulin, and minor sites of complement in the glomerular stalk and capillary walls; and tubular changes consisting mainly of hydropic swelling of tubular cells and occasional hyaline casts with areas of tubular necrosis being present.

Surgical specimens. The recipient kidneys and the biopsy specimens from the allografts were evaluated by light, electron, and immunofluorescence microscopy. Each surgical specimen was divided into three portions. One portion was fixed in buffered 10% formalin, and processed in standard fashion for routine light microscopy. Sections were stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver-Masson, elastic, or methyl-green-Pyronine techniques. The second portion was divided into 1-mm fragments, quickly immersed in cold 4% glutaraldehyde buffered with 0.1 M sodium cacodylate, postfixed in 1% osmium tetroxide, and embedded in Epon. Sections for examination were then made using a Porter-Blum ultramicrotome. These sections, stained with uranyl magnesium acetate and lead citrate, were examined and photographed using a Hitachi HS-7 or HS-8 electron microscope (Hitachi America, Ltd., Indianapolis, Ind.). The third portion of tissue was rapidly frozen in gelatin and stored at −70°C. Sections cut at 4 μ were stained with fluorescein-labeled antibodies reactive with human 7S immunoglobulins, IgG, IgM, B_1C globulin, albumin, fibrinogen, and with guinea pig serum followed by anti-guinea pig complement labeled with fluorescein isothiocyanate. After staining, cover slips were put in place using 50% glycerine and the slides were examined using a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N. J.) with an HBO-200 W mercury vapor light source.

TABLE II

HL-A Genotypes

	Family	Patient	HL-A genotype of donor and recipient*
Group 1	0002	W. B.	2, Ao 4; [2], 8
	0008	V. O.	2, 7; Ao 62, Ao 54
	0055	J. D.	3, 7; 9, [7]
	0092	C. D.	3, 7; 2, 12
	0138	H. M.	3, 7; 3, Ao 29
	0186	J. M.	[2], W10; 2, 12
Group 2	0007	R. C.	2, [7]; 3, 7
	0083	F. L.	[2], Ao 54; 2, 12
	0059	B. L.	2, 8; 3, 7
	0099	V. H.	2, Ao BH; 2, 12
	0089	D. C.	2, 12; [11], 8
	0117	C. M.	3, 7; 1, Ao 27
	0169	J. W.	Ao BH, W5; Ao BH, Ao 34
	0053	S. P.	2, 7; 9, [7]
	0184	O. M.	9, Ao 21; 1, 12
Group 3	0009	C. W.	3, Ao 21; Ao 6, Ao 64

* HL-A genotypes were determined by haplotype analysis of family data. Semicolons separate the specificities determined by the two haplotypes and brackets indicate uncertainty of antigen assignment. Numbers preceded by W and Ao are workshop and local specificities, respectively.

TABLE III

Survival Time of Skin Grafted from Renal Recipient to HL-A Identical Sibling Donor

Family	Patient	Survival
		days
0005	J. D.	White graft (ABO incompatible)
0089	D. C.	23
0083	F. L.	24
0099	V. H.	37
0007	R. C.	39
0008	V. O.	25
0169	J. W.	19
0053	S. P.	17 (ABO incompatible)

Chemical methods. 24-hr collections of voided urine were utilized for all measurements of endogenous creatinine clearance; samples of peripheral venous blood were obtained at the end of each urine collection period. The concentration of creatinine in plasma and urine was measured on a Technicon AutoAnalyzer (Technicon Corp., Tarrytown, N. Y.) utilizing a modification of the method of Folin and Wu(22). The total protein concentration of urine was determined by the biuret method of Foster, Rick, and Wolfson (23). Standard laboratory methods were utilized for other determinations.

RESULTS

General. Each recipient was shown by serologic testing to be genotypically HL-A identical with his sibling donor. The HL-A genotypes of the 16 donor-recipient pairs are listed in Table II. Skin grafts from renal allograft recipients to renal donors survived for a prolonged period (mean = 26.3 days, SE 3.2) which corresponds with HL-A identity (Table III).

Assessment of mixed leukocyte reactivity between the leukocytes of selected donor-recipient pairs failed to demonstrate stimulation in either direction. Nevertheless, the leukocytes of both the recipient and donor al-

TABLE IV

Mixed Leukocyte Culture Stimulation Indices for HL-A Identical Renal Transplant Donors and Recipients

	A.* Bm	A. Xm	B. Am	B. Xm	X. Am	X. Bm
I.						
W. B. (0002)	1.0	30.0	2.2	88.9	(Did not respond)	
V. O. (0008)	1.0	5.5	1.2	32.0	38.0	52.0
J. D. (0055)	1.2	26.7	1.0	8.0	16.3	16.7
C. D. (0092)	1.0	22.5	1.1	15.8	37.1	25.7
H. M. (0138)	1.2	2.2	0.7	4.0	3.5	5.3
J. M. (0186)	1.0	3.4	0.9	5.5	5.0	6.3
$\bar{x}$	1.07	15.05	1.18	25.70	19.98	21.20
SE	0.04	5.19	0.21	13.32	7.51	8.56
II.						
R. C. (0007)	0.83	26.0	0.80	17.0	8.5	9.2
F. L. (0083)	1.3	4.0	1.4	4.4	3.7	8.3
B. L. (0059)	2.6	83.3	0.9	69.6	23.6	21.6
V. H. (0099)	1.6	78.0	1.0	4.0	9.3	11.9
D. C. (0089)	1.1	5.0	1.1	3.0	7.0	4.7
C. M. (0117)	(No MLC data)					
J. W. (0169)	1.6	8.8	1.2	12.0	10.0	13.3
S. P. (0053)	1.1	4.0	1.0	9.5	5.6	6.3
O. M. (0184)	1.2	15.0	1.0	11.8	8.0	12.5
$\bar{x}$	1.42	28.01	1.05	16.41	9.46	10.98
SE	0.19	11.79	0.06	7.79	2.14	1.86
III.						
C. W. (0009)	(No MLC data)					

* A, recipient leukocytes; B, donor leukocytes; X, unrelated individual leukocytes; m, mitomycin-treated leukocytes; X, sibling sharing no HL-A haplotype with donor and recipient.

ways evidenced an appropriate response to mitomycin-treated cells from an unrelated population and, conversely, they were fully capable of stimulating an appropriate mitotic response in unrelated and untreated leukocytes. These results are summarized in Table IV.

From a clinical point of view, the results of renal transplantation in this group of 16 recipients must be regarded as quite satisfactory. Only one of the allografts has failed to survive; the remaining 15 allografts continue to function (average Ccr: 80±SD 23 ml/min; range 27–113 ml/min) after an average follow-up period of 33 months (range: 10–68 months). Furthermore, detectable episodes of acute rejection were never experienced by 6 of the 16 patients; hence, 27% of the recipients have never required any type of immunosuppression other than azathioprine. In addition, even in those patients who did experience one or more episodes of suspected acute rejection, such episodes were usually delayed in onset (average onset: 134 days after transplantation), mild in nature (maximal Pcr equaled 2.7±SD 1.97 ml/100 ml), and easily controlled by the administration of prednisone. Over-all, for the entire group, the average maintenance dose of prednisone at 6 and 12 months post-transplantation was only 12 and 11 mg/day, respectively, and full-time occupational rehabilitation was achieved in 94% of the patients within 6 months. The functional status of individual allografts at sequential points in time is summarized in Table V. The maximum rise in BUN and Pcr, the minimal 24 hr urine output, and the duration of prednisone administration with the rejection episodes are included in Table VI.

Despite the satisfactory nature of these overall results, evaluation of individual patients revealed considerable heterogeneity in terms of the post-transplantation clinical course, the functional response of the allograft, and the attendant morphologic alterations in the biopsy specimens of the allografts.

Each of the 16 recipients was placed in one of three distinct clinical groups; each of these groups represents

TABLE V

Summary of Renal Function

Family		Time post-transplant							
	Days.....30 Months.....1	185 6	360 12	730 24	1100 36	1460 48	1826 60	2191 72	
Group 1									
W. B. (0002)	BUN	21	14	12	14	14	18	16	18
	Pcr	1.0	1.2	1.2	1.3	1.5	1.4	1.4	1.5
	Ccr	98	110	84	92	78	99	100	96
	U. Prot.	<0.2	0.3	0.2	0.8	<0.2	<0.2	0.67	1.86
V. O. (0008)	BUN	28	18	14	22	16	13	18	
	Pcr	0.9	0.8	0.8	0.8	1.1	1.0	1.2	
	Ccr	82	108	103	114	112		75	
	U. Prot.	<0.18	0	0	0		0.07	0.07	
J. D. (0055)	BUN	19	19	19	18	19	16		
	Pcr	1.5	1.3	1.1	1.4	1.3	1.3		
	Ccr	68	75	144	97	93	96		
	U. Prot.	0.35	0	0	0.42	0.05	0.03		
C. D. (0092)	BUN	30	15	13	17	15			
	Pcr	1.6	1.3	1.3	1.4	1.2			
	Ccr	89	92	68	80	96			
	U. Prot.	<0.23	<0.17	1.15	0	0.05			
H. M. (0138)	BUN	16	16	15	17				
	Pcr	1.0	0.9	1.1	1.2				
	Ccr	90	155	132	113				
	U Prot.	<0.19	<0.15	<0.17	<0.19				
J. M. (0186)	BUN	12	13	20					
	Pcr	0.8	1.0	1.0					
	Ccr	95	86	86					
	U. Prot.	<0.12	<0.9	<0.2					
Group 2									
R. C. (0007)	BUN	27	14	12	10	12	11	12	
	Pcr	1.3	1.3	1.2	1.3	1.2	1.4	1.5	
	Ccr	86	84	94	74	92	82	84	
	U. Prot.	<0.2	0	<0.27	0	0.13	0.2	0.11	
F. L. (0083)	BUN	18	13	17	15	15			
	Pcr	0.9	0.8	1.3	1.2	1.2			
	Ccr	54	82	70	72	66			
	U. Prot.	1.7	<0.38	3.2	<0.43	<0.56			
B. L. (0059)	BUN	28	20	14	15	16			
	Pcr	0.9	1.2	1.1	1.2	1.4			
	Ccr	88	82	126	116	110			
	U. Prot.	1.0	0	<0.29	<0.18	<0.21			
V. H. (0099)	BUN	7	12	13	14	35			
	Pcr	0.5	1.0	0.7	0.9	1.9			
	Ccr		62	52	36	27			
	U. Prot.		3.28	0.72		0.28			

BUN in mg/100 ml; Pcr, (plasma creatinine) in mg/100 ml; Ccr, (creatinine clearance) in ml/min; U. Prot., (urinary protein) in g/day.

TABLE V—(*Continued*)

Family		Time post-transplant: Days 30 / Months 1	185 / 6	360 / 12	730 / 24	1100 / 36	1460 / 48	1826 / 60	2191 / 72
Group 2									
D. C. (0089)	BUN	16	9	12	11				
	Pcr	1.1	0.8	0.9	0.9				
	Ccr	39	70	76	86				
	U. Prot.	<0.19	0	0	<0.13				
C. M. (0117)	BUN	21	24	22	20				
	Pcr	1.8	1.5	1.5	1.6				
	Ccr	45	62	39	48				
	U. Prot.	<0.23	0.55	<0.21	<0.13				
J. W. (0169)	BUN	25	19	15					
	Pcr	1.1	1.5	1.4					
	Ccr	103	105	70					
	U. Prot.	1.0	<0.17	<1.2					
S. P. (0053)	BUN	21	12	12	19				
	Pcr	1.1	1.4	1.2	1.6				
	Ccr	99	70	122	84				
	U. Prot.	0.39	0	0.29	0.41				
O. M. (0184)	BUN	36	22	20					
	Pcr	1.3	1.2	1.4					
	Ccr	51	60	68					
	U. Prot.	0.69	0	0.29					
Group 3									
C. W. (0009)	BUN	44	32	39	60				
	Pcr	2.0	2.1	2.3	8.6				
	Ccr	52	34	50	6				
	U. Prot.		5.6	2.5	3.5				

a separate and important clinical event that may accompany transplantation among HL-A identical donor-recipient pairs.

TABLE VI

Summary of Renal Function with Rejection Episode

Patient	PTD rejection	Maximum Pcr	Maximum BUN	Minimal Ccr	Lowest 24 hr urine output	PTD prednisone discontinued
	days				*ml*	*days*
Group 2						
R. C.	27	1.9	34	66	1500	913
F. L.	8	2.7	37	11	1457	—
B. L.	44	1.7	34	49	1060	—
V. H.	7	1.6	27	6	513	59
D. C.	37	1.9	22	15	740	188
C. M.	34	8.1	110	17	1600	-
J. W.	8	1.6	29	52	725	—
S. P.	4	4.6	89	9	1960	—
O. M.	7	1.6	43	38	1502	—

— indicates prednisone therapy continuing.

Group 1; no discernible episode of acute rejection

Six patients were placed in this group; none of them exhibited clinical evidence of acute rejection and steroid therapy was not required. For the most part, the allografts in these patients have maintained excellent function for prolonged periods and associated histological alterations have been mild and minimal in degree. Clinical events observed in patient C. D. were fairly typical of those seen in this group as a whole.

Patient C. D. (family 0092). This 42-yr old male was admitted in June 1968, with a 2 yr history of decreasing renal function and hypertension. In July 1968, the patient underwent bilateral nephrectomy and renal transplantation. The recipient's kidneys displayed severely sclerotic glomeruli in which the basement membranes were collapsed, thickened, while wrinkled and occasional glomeruli exhibited limited, local, and granular capillary deposits of IgM, IgG, and IgA. The ar-

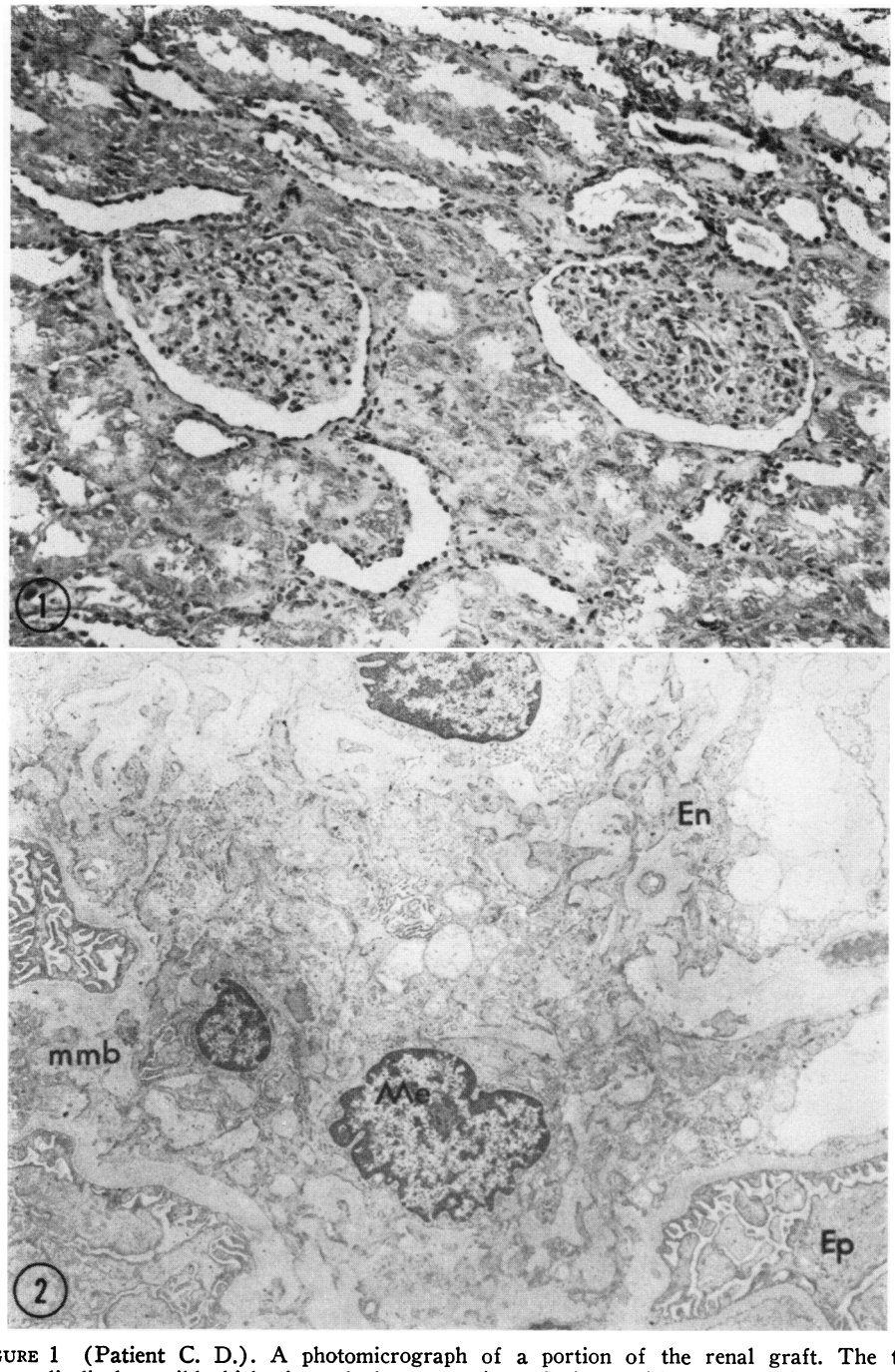

FIGURE 1 (Patient C. D.). A photomicrograph of a portion of the renal graft. The two glomeruli display mild thickening of the mesangium. Stained with hematoxylin and eosin, × 140.

FIGURE 2 (Patient C. D.). An enlarged mesangial region with a greatly increased amount of membrane matrix (mmb) and portions of several capillary loops of the renal graft; mesangial cell (Me), epithelial cell (Ep), and endothelial cell (En). Electron micrograph, × 3700.

terioles were sclerotic and the interstitium contained a large number of chronic inflammatory cells. The histologic diagnosis was chronic glomerulonephritis with severe arterial and arteriolar sclerosis. The patient has been maintained on azathioprine alone postoperatively and prednisone therapy has never been required.

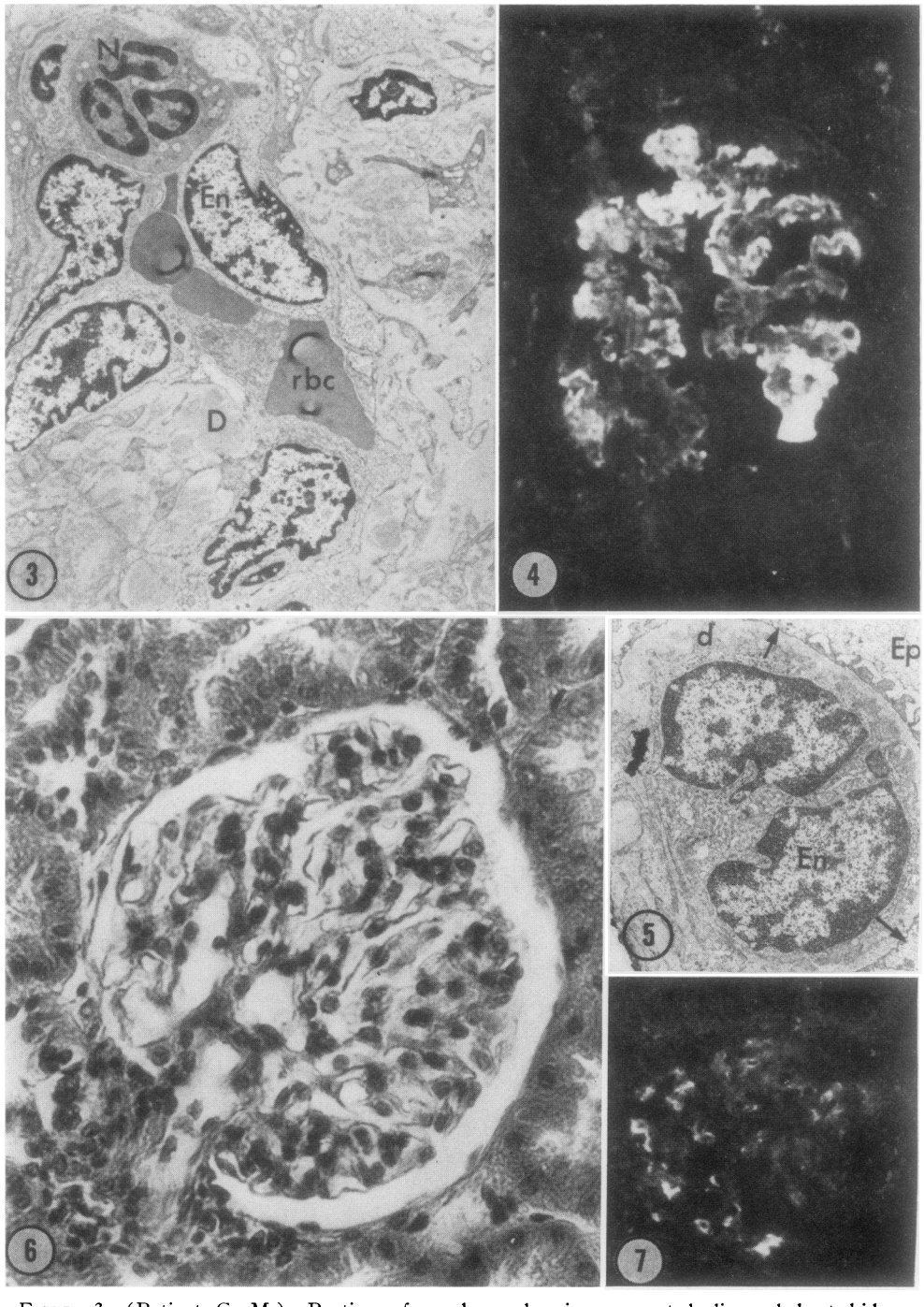

FIGURE 3 (Patient C. M.). Portion of a glomerulus in a resected diseased host kidney showing greatly expanded mesangium containing extensive electron-dense deposits (D): endothelial cells are increased in number and a neutrophil (N) is present in a narrowed capillary lumen; red blood cell (rbc). Electron micrograph, × 3400.

FIGURE 4 (Patient C. M.). A glomerulus from the same kidney illustrated in the previous photomicrograph, shows rather extensive course granular and globular deposits of IgM along capillary walls and in mesangium. Immunofluorescence micrograph, × 200.

FIGURE 5 (Patient C. M.). Photomicrograph of glomerulus from a renal biopsy of allograft showing thickened mesangium and glomerular membranes. Stained with hematoxylin and eosin, × 400.

Allograft morphology. The allograft was biopsied in April 1969. Glomerular alterations were mild or infrequent and consisted of rare, tiny electron-dense subendothelial deposits and local expansion of the mesangial matrix (Figs. 1 and 2). Rare, tiny local capillary deposits of IgM and C'3 were observed by immunofluorescent microscopy. Perivascular and periglomerular chronic inflammatory infiltration was noted. These changes were interpreted as those of very mild graft rejection (Table I).

Clinical course. Proteinuria has not been observed since the immediate post-transplant period and the BUN has remained within normal limits. After the 1st month post-transplantation, the Pcr has ranged from 1.1 to 1.3 mg/100 ml.

Group 2; suspected episodes of acute rejection

Each of the nine patients in this group exhibited one or more episodes of suspected acute rejection; steroid therapy was required for its control and a varying improvement of allograft function was obtained in each instance. A definite reduction of Ccr was observed during each acute episode, and histological evidence of immunological rejection was obtained in each instance.

Seven of the nine patients showed no signs of acute rejection in the 1st wk post-transplantation. Acute rejection was observed 8–42 days post-transplantation (mean = 24 post-transplantation days (PTD) in this group. Patient C. M. (family 0117) had a postoperative course representative of some of this patient group.

Patient C. M. (family 0117). This 38-yr old male was admitted in October 1968, with a 2 month history of progressive renal failure complicated by severe diastolic hypertension. In November 1968, the patient underwent bilateral nephrectomy and renal transplantation. Histological examination of the recipient's kidneys demonstrated chronic glomerulonephritis and malignant arteriolar nephrosclerosis with extensive intramesangial and intramembranous electron-dense deposits (EDD) and globular deposits of IgG, IgM, IgA, and C'3 within many glomeruli (Figs. 3 and 4).

Allograft morphology. Follow-up biopsies of the allograft were performed on two occasions: the first in January 1969, and the second in April 1970. The observed abnormalities in the initial biopsy were judged to have progressed at the time of the second biopsy. The glomerular basement membrane at the time of the first biopsy was slightly thickened, and rare, small, and local EDD were seen (Figs. 5 and 6). Immunoglobulin and complement localization were not observed; however, the interstitium contained a minor mononuclear cell exudate which was localized between an artery and a vein. The second renal biopsy specimen displayed glomerular changes of approximately the same intensity as those noted at first; nevertheless, limited, local, and granular deposits of IgG, 7S immunoglobulins, IgM (Fig. 7), human complement, and fixation of guinea pig complement could be identified within the glomeruli at this time.

Clinical course. Initial immunosuppression consisted of azathioprine alone; however, on the 34th PTD the patient exhibited a deterioration in renal function that was interpreted as acute rejection. The reduction of Ccr responded only moderately to the institution of prednisone therapy. 3 months postoperatively, follow-up intravenous urography demonstrated a moderate degree of urinary obstruction, a ureteral pyeloplasty was carried out with satisfactory technical results. Despite early difficulties with rejection, urinary tract obstruction, and a persistent urinary tract infection, the BUN has remained stable between 18 and 24 mg/100 ml. Most recently, the Pcr has been 1.5 mg/100 ml, and the Ccr has equaled 77 ml/min without significant quantitative proteinuria (Table V).

Two of the nine recipients developed an apparent episode of acute rejection very early after transplantation; the clinical events suggested the presence of performed recipient antibodies which reacted with donor antigens. High-dose steroid therapy was required, a marked and sustained loss of allograft function occurred, and histological examination of the allografts revealed the presence of several features which suggested that humoral immunity was involved in the rejection process. One of the two patients demonstrated cytotoxic antibody in his pretransplant serum sample. His clinical history follows.

Patient O. M. (family 0184). This 42-yr old male was admitted with an 18 month history of progressive renal insufficiency. Important complications included severe hypertension and recurrent pericarditis with effusion; the latter required pericardiectomy 6 months before transplantation. The patient underwent bilateral nephrectomy and renal transplantation in March 1970. Histological examination of the recipient's kidneys revealed glomerulonephritis. Intramembranous EDD, glomerular deposits of IgG, IgM, and C'3 were identified

FIGURE 6 (Patient C. M.). Electron micrograph of capillary loop in a glomerulus from the renal graft with swollen endothelial cells (EN), tiny subendothelial electron-dense deposits (d), and local loss of foot processes (arrows) of overlying epithelial cell (Ep). Electron micrograph, × 3600.

FIGURE 7 (Patient C. M.). A glomerulus from the allograft showing local deposits of IgM in segments of capillary walls. Immunofluorescence photomicrograph, × 160.

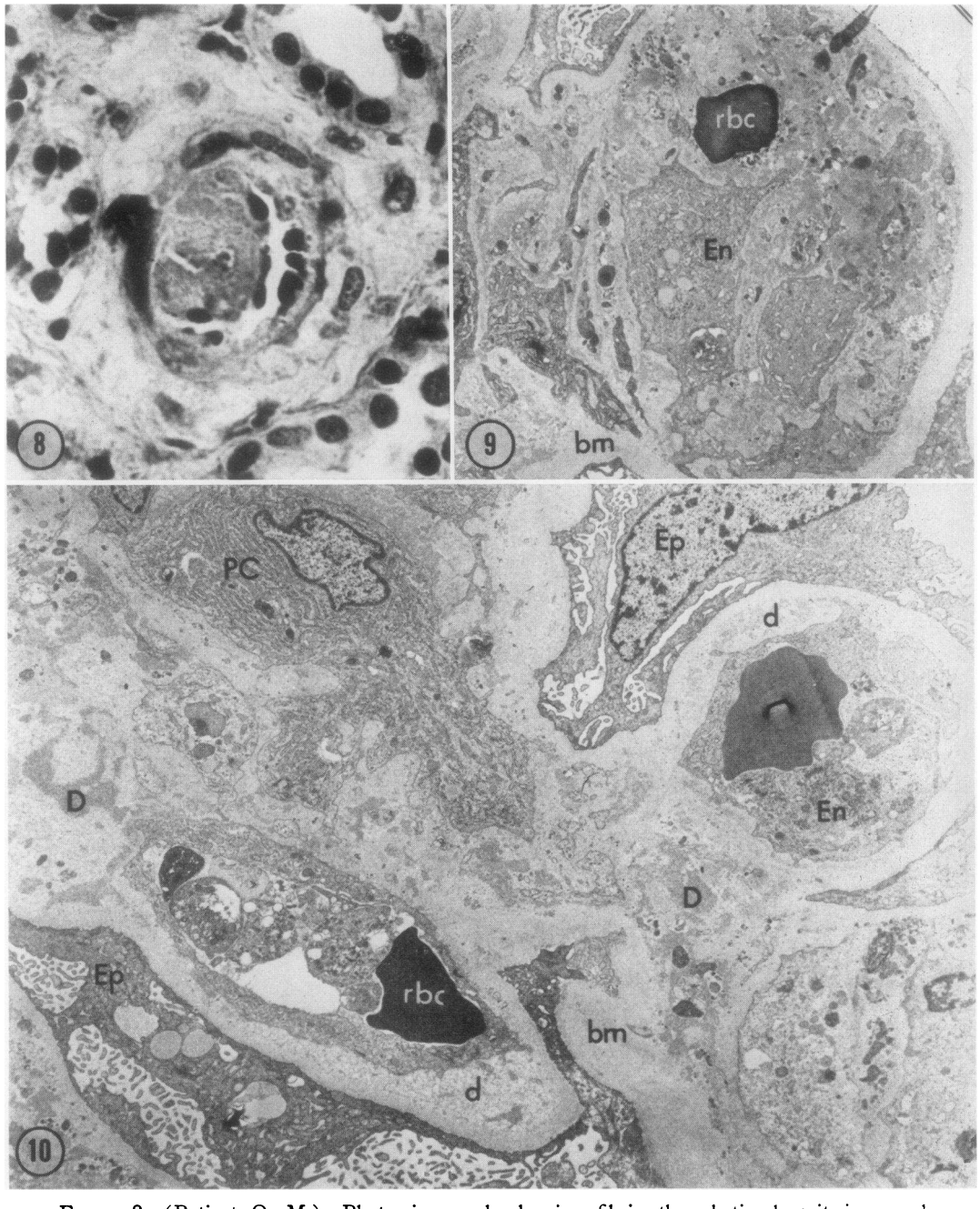

FIGURE 8 (Patient O. M.). Photomicrograph showing fibrin thrombotic deposit in vessel wall of renal allograft. Stained with hematoxylin and eosin, × 720.

FIGURE 9 (Patient O. M.). Portion of a capillary loop of glomerulus in renal allograft showing plugging of its lumen by electron-dense material including strands of fibrin; red blood cell (rbc), endothelial cell (En), and capillary basement membrane (bm). Electron micrograph, × 2040.

FIGURE 10 (Patient O. M.). Several capillary loops with subendothelial, loosely granular deposits (d) and mesangial region containing electron-dense deposits (D) in the same glomerulus as the previous photomicrograph; probable plasma cell in mesangium (PC), capillary basement membrane (bm), red blood cell (rbc), endothelial cell (En), and epithelial cell (Ep). Electron micrograph, × 3750.

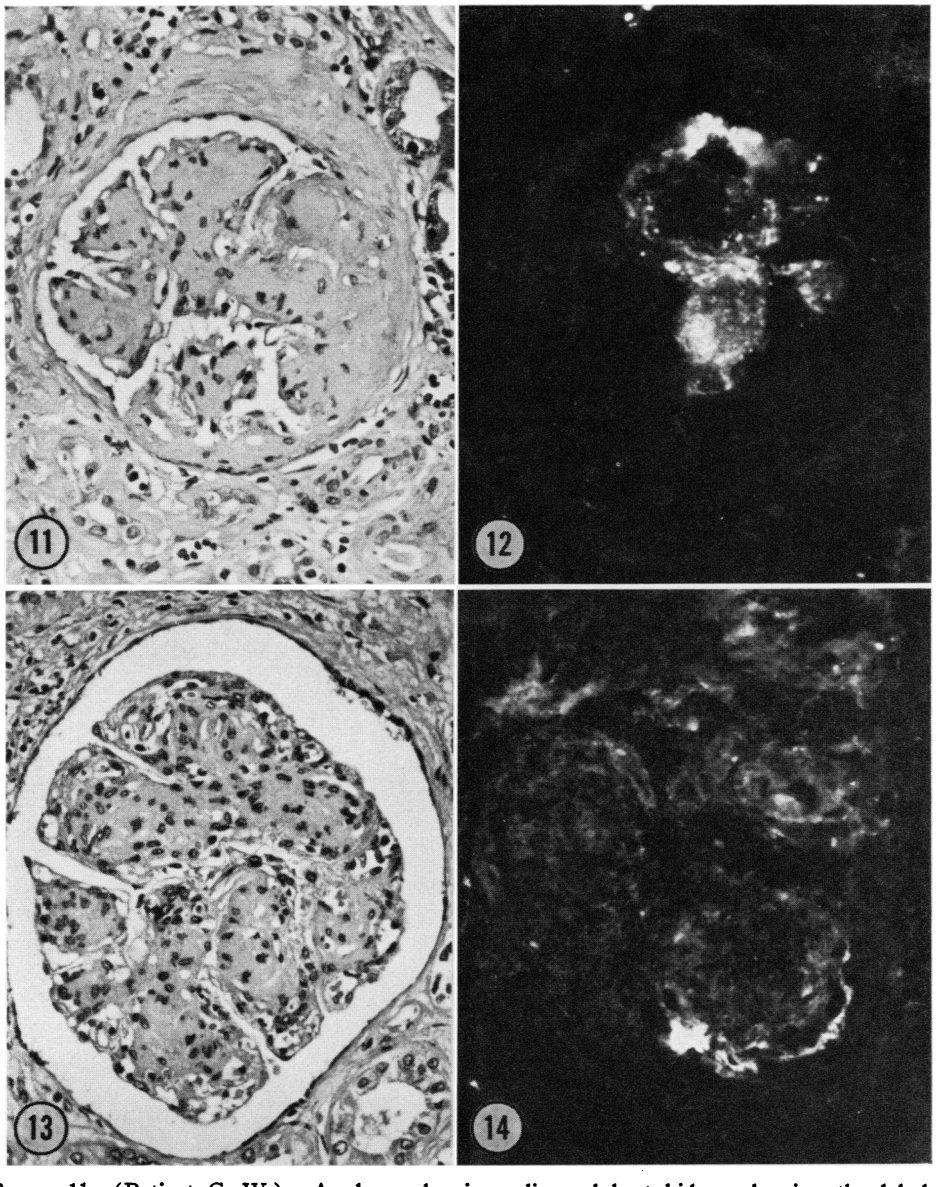

FIGURE 11 (Patient C. W.). A glomerulus in a diseased host kidney showing the lobular mesangial sclerosis characteristic of chronic lobular glomerulonephritis; there is also considerable glomerular capsular sclerosis and local glomerular-capsular adhesion. H & E × 180.

FIGURE 12 (Patient C. W.). Several loops of another glomerulus in the host kidney showing granular sites of fixation in vitro of guinea pig complement along peripheral portions of capillary walls. Immunofluorescence micrograph of immunohistologic complement-fixation test, × 265.

FIGURE 13 (Patient C. W.). A glomerulus in the first graft biopsied about 18 months after transplantation showing lobular mesangial sclerosis and cellularity as manifestation of recurrence in the renal allograft of original host renal disease. Stained with hematoxylin and eosin, × 170.

FIGURE 14 (Patient C. W.). Another glomerulus in the renal allograft showing limited local granular distribution of IgM in peripheral portion of one capillary wall; the mesangial nodules are essentially free of immunoglobulin. Immunofluorescence micrograph, × 230.

as were local granular glomerular capillary deposits. Azathioprine alone was administered during the early postoperative course. However, on the 7th PTD the Pcr rose from 1.1 to 1.6 mg/100 ml and the Ccr fell from 65 to 52 ml/min. At this point high dose prednisone therapy was instituted which gave a reduction in the Pcr, however, the Ccr remained in the range of 40–60 ml/min. Proteinuria was approximately 1 g/day. An arteriogram on the 14th PTD revealed irregular cortical margins compatible with diffuse small vessel occlusion. The patient was placed on heparin therapy without response.

Allograft morphology. The first biopsy of the allograft was performed in April 1970, approximately 1 month after transplantation. The tissue showed areas of acute cortical necrosis presumably secondary to homogenous eosinophilic masses of fibrin occluding many small renal vessels (Fig. 8). Similar deposits were observed within the tufts of many renal glomeruli (Figs. 9, 10). IgM, fibrinogen, and less intense staining for IgG and C'3 could be identified in isolated "thrombotic" glomerular capillary lesions. These changes were felt to reflect thrombonecrotic changes suggestive of a humoral element in the rejection episode of the allograft. Because of pathological observations which suggested the presence of pre-existing humoral immunity, the patient's pretransplant serum was screened against a cell panel derived from 60 unrelated normal volunteers. This panel is known to demonstrate all defined HL-A antigens as well as numerous antigens that have been identified but not yet officially named. The patient exhibited positive cytotoxic antibody for lymphocytes from 23 of the 60 individuals. Several cross-match studies utilizing pretransplant serum from the recipient and lymphocytes from the donor were negative. The specificity of the detected antibody could not be ascertained. A repeat biopsy in September 1970, revealed hyalinized vessels and glomeruli which had presumably derived from resolution of the process that was observed in the first biopsy specimen. However, the predominant picture was that of normal renal tissue (Table I).

Clinical course. At the present time, 1 yr after transplantation, the Ccr is 75 ml/min, the BUN is 16 mg/100 ml, and the Pcr is 1.5 mg/100 ml. There is no significant proteinuria (Table V).

Group 3; recurrence of original disease

One patient (C. W.) exhibited a gradual progression of declining renal function with heavy proteinuria. Steroid therapy did not prove beneficial and histological examination of the allograft revealed a morphological pattern that was almost indistinguishable from that observed in the recipient kidneys. Histological features of immunological rejection were not observed.

Patient C. W. (family 0009). This 27-yr old male was admitted in April 1966, with a 2 yr history of rapidly progressive glomerulonephritis. In May 1966, the patient underwent bilateral nephrectomy and renal transplantation. The recipient's kidneys revealed characteristic chronic lobular glomerulonephritis. The lobules in practically all glomerular tufts possessed solid acellular central hyaline areas with nuclei at the periphery. IgM and IgG immunoglobulins were exhibited as short linear deposits in some peripheral glomerular capillary loops around sclerotic mesangial nodules (Figs. 11, 12). At operation, after removal of the vascular clamps, the kidney remained soft and with evidence of poor flow and demonstrated persistent oliguria. The Pcr leveled off in the range of 2 mg/100 ml and the Ccr was in the range of 50 ml/min with proteinuria (3–5 g/day). The clinical impression at the time was that the impaired renal function was secondary to acute tubular and cortical necrosis. The patient was treated with azathioprine alone until the 363rd PTD at which time a reduction in Ccr from 50 to 40 ml/min, and a rise in Pcr from 1.9 to 2.3 mg/100 ml were noted. Prednisone was instituted in high doses and subsequently tapered until PTD 560 at which time the patient was receiving 20 mg daily.

Allograft morphology. The allograft was biopsied in January 1968 (616 PTD) and revealed distinctive lobular proliferative glomerulonephritis involving all glomeruli in a pattern identical with that observed originally in the host kidneys (Figs. 13 and 14). Round cell infiltrates were evident in focal areas of the interstitium all with interstitial fibrosis which could be interpreted as representing acute rejection. Arterial and arteriolar changes of the type associated with chronic rejection were not evident, however. Local globular deposits of IgM were found in isolated glomerular capillary loops (Table I).

Clinical course. The patient's renal function diminished progressively despite treatment with steroids and azathioprine, the Ccr was 10 ml/min on PTD 596 Table V). The patient eventually required repetitive hemodialysis and transplantation of a cadaver kidney was performed on 11 April 1968.

DISCUSSION

The importance of the HL-A system to transplantation biology has been established clearly by Dausset, Rapaport, Ivanyi, and Colombani (24), Amos (3), Ceppellini, et al. (2), Terasaki and Mickey (6), and numerous other investigators. The validity of this statement was first established by the observation that the survival of test skin grafts was prolonged greatly when they were exchanged among HL-A identical, ABO-compatible subjects (8). This finding provided a necessary and im-

portant in vivo documentation of serological predictions. Nevertheless, despite prolonged survival, skin graft rejection still occurred over widely varying intervals of time. The variability of survival, even among HL-A identical pairs, suggested the possibility that minor incompatibilities might still exist, and it pointed to the need for identifying these loci and testing their immunogenicity. The observation of similar survival times when skin grafts from several individuals with the same HL-A haplotypes were placed on a single recipient with a known haplotype allowed one to conclude that the HL-A loci controlled the most important antigens in human transplantation. There are, however, exceptions and the exchange of skin grafts between family pairs with known haplotypes permits productive study of the HL-A locus and assessment of the importance of unidentified non-HL-A loci. Cross-immunization between HL-A identical individuals is extremely important if antibodies against non-HL-A antigens are to be obtained. Planned immunizations such as these should provide investigators with serological reagents for the identification and definition of minor non-HL-A loci.

The fact that none of the present donor-recipient pairs exhibited lymphocyte stimulation during mixed leukocyte culture provides collaborative evidence for the validity of serotyping, and suggests identity or close linkage between the loci which control the HL-A system and the mixed leukocyte reaction. The failure of non-HL-A differences between the family pairs to cause lymphocyte stimulation may be interpreted as supportive evidence, as presented by Plate, Ward, and Amos (12), Seigler, Ward, Amos, Phaup, and Stickel (19), that differences at a "mixed leukocyte reaction (MLR) locus" are necessary to activate responder cells in vitro. Data accumulated in the mouse by Dutton (25) and Häyry and Defendi (26) suggests that the summation effect of minor loci will indeed cause stimulation in MLR between congenic strains that are identical for the H-2 locus. The failure of MLR to reflect and correlate with skin graft survival time, and immediate and long-term renal function in familial human transplantation as reported by Seigler et al. (19) suggests that, in its present form, the MLR is not the most sensitive or reliable method of predicting genetic compatibility for human transplants, or that linkage equilibrium exists between the HL-A and MLR loci.

The present experience indicates that transplantation between HL-A identical subjects is not always heralded by uniformly good results. The fact that the six patients in group 1 did not exhibit discernible episodes of rejection or require steroid therapy clearly reflects the close degree of histocompatibility between these particular donor-recipient pairs. Three additional patients (in group 2) who exhibited acute rejection and required steroid therapy maintained near normal renal function after complete discontinuance of steroids. They demonstrated clinical changes suggestive of a mild immune response similar to that one might suspect in the presence of major loci compatibility and no more than a few minor incompatibilities. Two additional patients in group 2 have had similar clinical courses and are being maintained on 7 and 3 mg/day of steroids, respectively. The relatively normal histology of the allografts in these five patients, as well as the six patients in group 1 reflects the close genetic compatibility. The relatively early and more severe rejection episodes seen in two other recipients from group 2 suggests the presence of synergism or summation between numerous incompatibilites at non-HL-A loci. These two individuals demonstrated moderate to marked histologic changes of rejection in their allografts. The sustained loss of renal function correlated with the severity of the observed histologic findings.

The very early and severe rejection episodes in the two remaining patients in group 2 strongly points to the probability of previous sensitization leading to pre-existing immunity in the recipient to donor antigens. The latter case indeed demonstrated a cytotoxic antibody in his pretransplant serum that reacted with lymphocytes from 23 of 60 unrelated donors. The significance of a positive lymphocytotoxicity cross match in transplantation has been reported by Patel and Terasaki (27) as well as others, however, a hyperacute type response can be observed with a negative lymphocytotoxicity cross-match between recipient serum and donor cells (28). A negative lymphocyte lysis is interpretable only within the limitations of this complement-dependent serological test and immune adherance utilizing kidney cells, or mixed agglutination, or leuko-agglutination may indicate a positive cross-match in the same donor-recipient pair and prospectively predict a hyperacute rejection. The diffuse thrombosis in the allograft as noted in this case (Fig. 8) was in keeping with a humoral immune response as has been reported by others (29, 30). However, the fact that the lesions resolved with resultant vascular damage and little evidence of diminished renal function indicates that this case was not typical of classical hyperacute rejection.

The variability of the severity of the histological manifestations of rejection suggests further that other non-HL-A loci are important determinants of histocompatibility. In no instance were the morphologic changes in these patients as severe as those reported by others. Although repeat biopsy examination in one patient (patient 0117) suggested that histological damage had progressed, it was not felt that the observed changes were sufficient to lead to loss of function in this allograft.

The continued maintenance of function with histological evidence of rejection in patient 0099 (after 2 yr without immunosuppression) is of particular interest; this event suggests the occurrence of some form of drug-induced tolerance or enhancement. Currently, all of the recipients in this report are undergoing complete re-evaluation in an effort to detect the presence of a "blocking" or enhancing type antibody in their sera. A drug (or drug plus antigen)-induced tolerance should be much more easily accomplished between subjects who are HL-A identical.

Glomerular alterations, presumably unrelated to graft rejection, were generally of a minor degree. The allograft of the patient in group 3 revealed changes in all glomeruli that are typical in lobular, proliferative glomerulonephritis (Figs. 13 and 14) 18 months after transplanation. The patient had the same changes in his own kidneys. Even though there was a round cell infiltrate in the interstitium as is seen in rejection, there was absence of vascular changes associated with chronic rejection. An eluate of the allograft was not studied for antiglomerular basement membrane antibody, however, the histologic findings indicated a recurrence of the original disease.

It is indeed difficult to differentiate rejection from recurrence of glomerulonephritis on the basis of histology alone. Many of the morphologic changes are similar. More sensitive and precise serologic techniques to detect and differentiate antibodies specific for histocompatibility antigens from glomerular basement membrane antibodies are needed to resolve this problem.

Until recently only ABO and HL-A typing and MLR were evaluated for correlations with the long-term results in kidney transplantation. The question of cellular immune competence of allograft recipients was little studied because of the inadequacy of in vitro tests to measure cellular immunity. Recent data concerning lymphocyte transformation, inhibition of macrophage migration, and cell-mediated cytotoxicity indicate that it may be possible to begin to evaluate cell mediated immunity. Some of the observed variability of rejection activity in the patients in this report could certainly be secondary to different degrees of cellular immune competence. At the present time our recipients are being evaluated before immunosuppression and transplantation, at the time of rejection episodes, and chronically in an effort to correlate cellular immunity with immediate and long-term results.

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