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THE HYPERIMMUNIZED RECIPIENT

Hyperacute Rejection of the Kidney in Patients With a Negative Crossmatch

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HYPERACUTE rejection (HAR) of the kidney was first recognized 20 years ago in cases of donor-recipient incompatibility for the major blood group and tissue antigen systems.[1-3] Recently, three patients at the University of Pittsburgh developed immediate graft dysfunction after receiving a kidney from an ABO-compatible cadaveric donor with a negative warm lymphocytotoxic crossmatch. None of the patients had prior transplants or transfusion therapy, and the ischemia times were comparable. One patients had a high panel reactive antibody (PRA) (100% remote, 76% at transplant). The immunopathologic and histopathologic features of these cases were compared with those of a control group without HAR. Our findings suggest that the cascade involved in HAR may be triggered by mechanisms other than those classically described.

MATERIALS AND METHODS

Tissue Processing

The tissue specimens from the three kidneys with HAR and a control group including seven unused donor kidneys, two baseline biopsy specimens after reperfusion, one preanastomosis biopsy sample, and one allograft resected at three days for renal vein thrombosis were processed routinely for light microscopy.

For immunofluorescence (IF), snap-frozen sections were cut at 4 μm and reacted with fluorescein isothiocyanate–labeled primary antisera to IgG (1:20), IgM (1:15), IgA (1:15), C1q (1:20), C3 (1:20), C4 (1:8), and fibrinogen (1:30) from Calbiochem-Behring Corp, LaJolla, CA; α_2-macroglobulin (1:20) and transferrin (1:20) from Cappel Laboratories, West Chester, PA: properdin (1:5) from Atlantic Antibodies through Rupp and Bowman; and Leu 4 (1:60) and Leu 14 (1:25) from Becton Dickinson, Mountain View, CA.

Immunoperoxidase (IP) staining was performed on the paraffin blocks by using a Vectastain ABC kit (Vector Laboratories Burlingame, CA), with primary antibodies to IgG (1:1,000) and IgM (1:1,000) from Dako (Santa Barbara, CA), and C1q (1:40) from Behring Diagnostics (La Jolla, CA). The chromogen was 3'3-diaminobenzidine (Polysciences, Inc, Warrington, PA).

RESULTS

Case 1

A 61-year-old black male, blood type A, with long-standing ulcerative colitis and sclerosing cholangitis was referred for liver transplantation because of increasing jaundice. During the workup he was found to be in renal failure attributed to drug-related interstitial nephritis and liver failure. He underwent cadaveric liver transplantation, which was followed immediately by kidney transplantation. The donor was a 28-year-old white male, blood type A, who died of subarachnoid hemorrhage. The PRA was 0%. The ischemia time was 24 hours. The lymphocytotoxic crossmatch was doubtfully positive just before sur-

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gery and negative just after. The kidney became cyanotic immediately after unclamping. Papaverine and prostaglandins were administered. The kidney was removed after eight hours. RBC-platelet thrombi with rare polymorphonuclear leukocytes (PMNs) were present in the vascular poles of less than 10% of the glomeruli (Fig 1A). There was positive immunostaining for IgM and C1q in vessel walls (Figs 1B and C); IgG was negative.

On the following day the level of liver enzymes rose markedly. The patient received

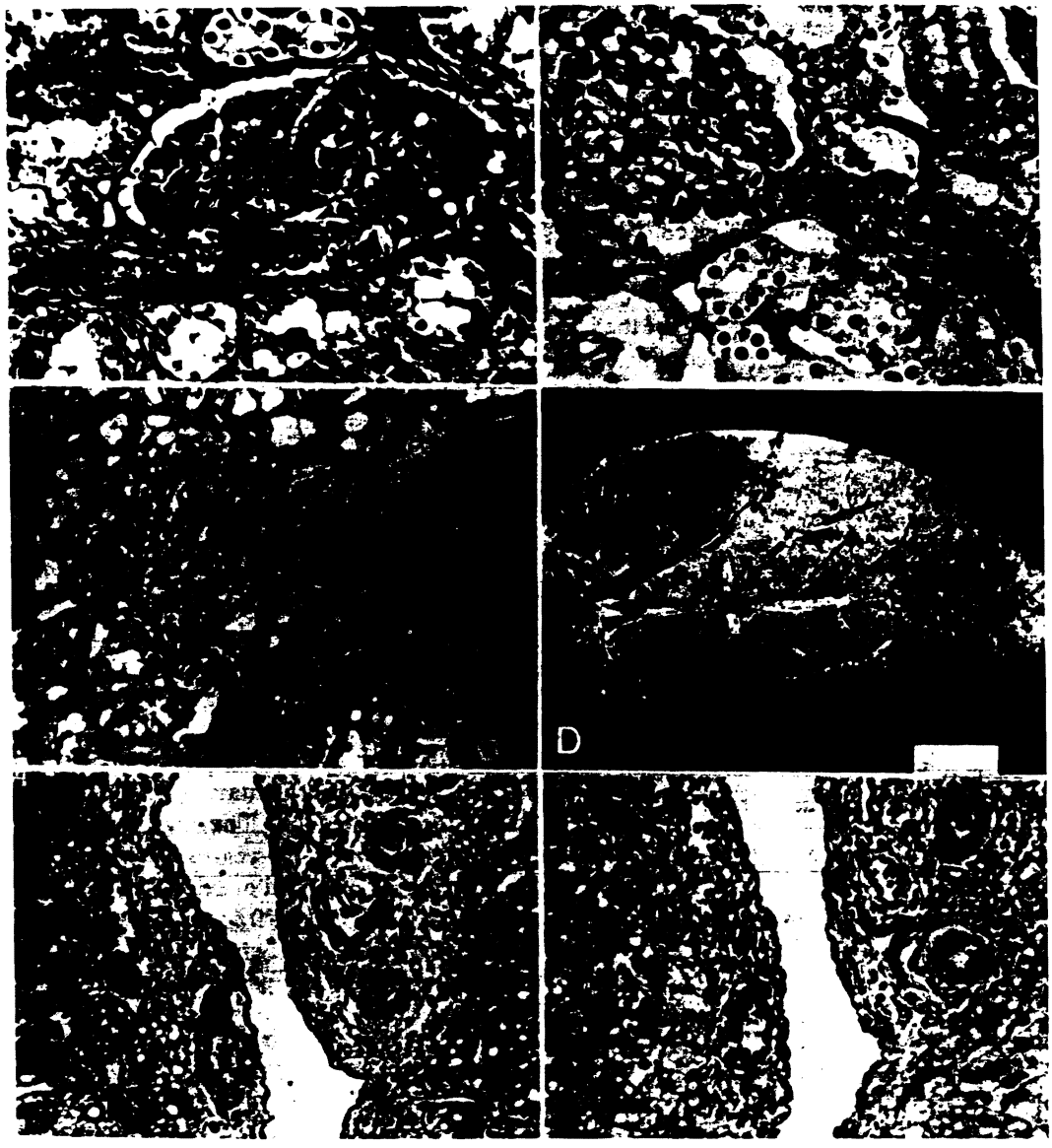

Fig 1. Case 1, resected allograft kidney and liver. (A) Glomerulus with thrombosis at the vascular pole (hematoxylin-eosin [H&E]; original magnification ×200 for all panels except panel D). (B) Positive IP staining for IgM in an arteriole. Staining in glomerular capillary lumina is nonspecific. (C) Positive IP staining for C1Q in the walls of the interlobular artery. (D) Allograft liver showing large areas of infarction. (E) Positive IP staining for IgM in artery walls. (F) Positive IP staining for C1Q in the same artery.

a second liver transplant on the fourth day, but he did poorly and died, without autopsy, on the sixth day. The resected allograft liver showed geographic areas of infarction not limited to the subcapsular regions (Fig 1D). IgM and C1q were present in artery walls (Figs 1E and F). Examination of the native liver revealed a bile duct carcinoma in addition to pericholangitis.

Case 2

A 49-year-old white female, blood type A, with chronic glomerulonephritis and a history of Grave's disease received a cadaveric kidney from a 51-year-old white female, blood type A, who died of a cerebrovascular accident. The warm lymphocytotoxic crossmatch was negative. The patient had a high PRA (99% remote and 76% at the time of kidney transplantation). The ischemia time was 20 hours. After unclamping, the transplanted kidney became cyanotic. Papaverine and prostaglandins were administered, but the kidney had to be removed after five hours. Microscopically, there were nuclear fragments and inflammatory cells in 40% of the glomeruli (Fig 2A). Only rare thrombi were present in glomerular capillaries. There was only trace-positive IF immunostaining for IgM in the mesangium and C1q, C3, and properdin at the vascular pole of some glomeruli. Leu 4 (T cell)-positive cells were present in 30% of the glomeruli (Fig 2B).

Case 3

A 60-year-old white male, blood group O, with end-stage polycystic renal disease received a kidney from a 30-year-old white male, blood group O, who died in a motor vehicle accident. The PRA was 2%, and the warm lymphocytotoxic crossmatch was negative. The ischemia time was 32 hours. It was learned that gram-negative rods had been cultured from the donor's trachea and significant *Pseudomonas* organisms from his urine. The kidney became soft and dusky 15 to 20 minutes after unclamping, and the patient became hypotensive. Papaverine was administered without effect. The kidney was removed after five hours. The mate kidney was not used for transplantation, and so both kidneys were available for pathological examination.

The resected allograft contained RBC-platelet thrombi with neutrophils in 66% of the glomeruli, about 10% of which were necrotic as well (Fig 3A). The tubules showed acute tubular necrosis (ATN), and one small arterial thrombus was present. The unused mate kidney was normal by light microscopy (Fig 4A). By IF staining, there was 2+ IgM and trace C3 and properdin in the mesangium of the allograft (Figs 3B C, and D). The mate

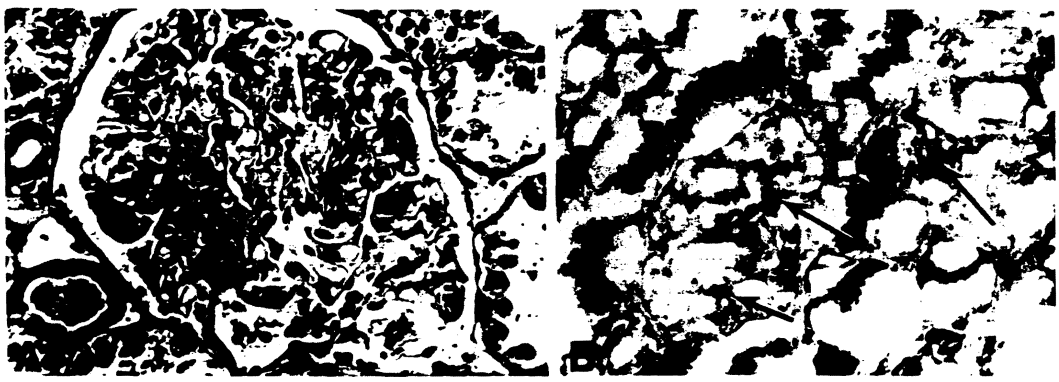

Fig 2. Case 2, resected allograft kidney. (A) PMNs and karyorrhectic nuclear debris in a glomerulus (H&E; original magnification ×200 for both panels). (B) Positive IP staining for Leu 4 (T cells) at arrows.

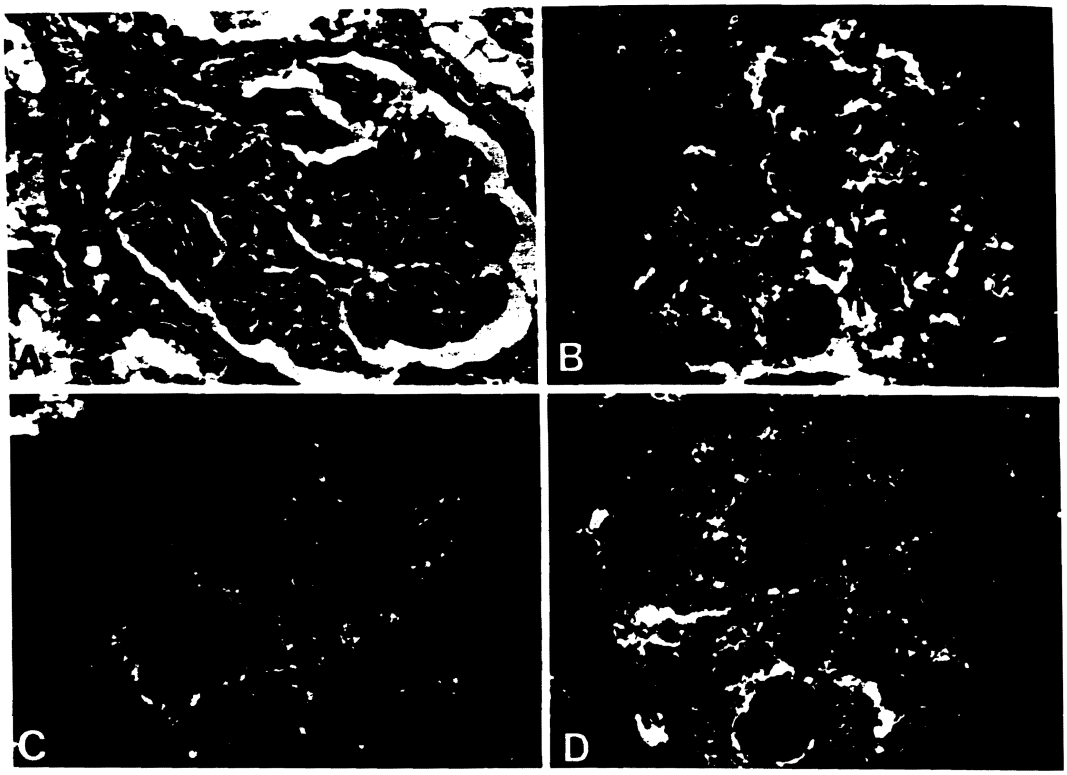

Fig 3. Case 3, resected allograft kidney. (A) Glomerulus with thrombosis, congestion, and PMNs (H&E: original magnification ×200 in all panels). (B, C, and D) Positive IF immunostaining for IgM, C3, and properdin, respectively.

kidney contained C3 and properdin in similar distribution (Figs 4B and C).

Control Cases

Light microscopic and immunostaining results of the non-HAR kidneys are in Table 1 along with those of the three cases described. Four cases with extensive glomerular thrombosis due to disseminated intravascular coagulation (DIC) (three cases) and malignant hypertension (one case) exhibited light microscopic findings similar to those in classic HAR, ie, glomerular thrombosis and cortical necrosis. Immunoglobulins and complement were demonstrable in the thrombi but not in the walls of the arteries and arterioles. The cases with normal histology or simple ATN did not exhibit significant immunostaining. The patient who developed renal vein thrombosis for technical reasons had an ABO-compatible donor, negative PRA, and negative crossmatch. The kidney that was removed at three days exhibited renal vein thrombosis, acute interstitial hemorrhages, and severe ATN. The glomeruli were filled with RBCs and PMNs. IF staining showed only trace C1Q in a few artery walls.

DISCUSSION

HAR of the kidney was recognized 20 years ago in transplants across major blood group and tissue antigen systems,[1-4] and in some patients with negative crossmatches.[3] Classic pathological changes described include early accumulation of PMNs in glomeruli and peritubular capillaries, progressive glomerular thrombosis, tubular necrosis, and eventual

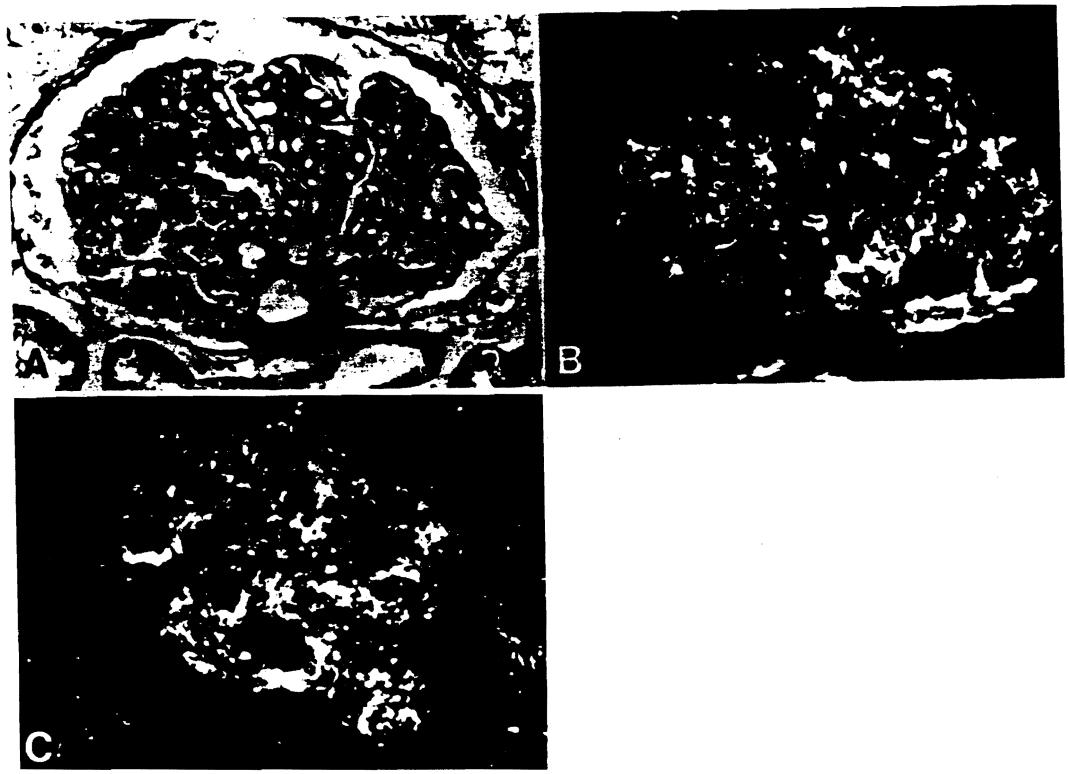

Fig 4. Case 3, unused donor kidney. (A) Glomerulus (H&E; original magnification ×200 in all panels). (B and C) Positive IF immunostaining for C3 and properdin, respectively.

cortical necrosis. Reaction of host humoral antibodies with antigens on donor cells serves as one trigger of the clotting mechanism, which then proceeds in a nonspecific fashion.[2-5]

Antigen systems other than the ABO groups that contribute to HAR reactions are leukocyte antigens,[6] endothelial and monocyte antigens,[7] and B cell antigens.[8] It is also documented that glomerular thrombosis identical to HAR may occur secondary to endothelial damage after pulsatile perfusion.[9] However, in such cases no specific deposition of immunoglobulins and complement is detected.[9] Our cases did not fit the established pathogenetic concepts. Although all three had a classic HAR rejection clinically, the pathological findings vary and suggest that still other factors of sufficient magnitude to incite a HAR reaction, yet be undetectable in standard tests, may also be effective triggers of the cascade.

In the first case, the deposition of IgM and C1Q in arterial walls in both the kidney and liver supports the clinical impression that a HAR reaction occurred in both organs, even though thrombi were demonstrable only in a few glomeruli. The doubtful positive crossmatch converting to normal suggests that antibodies were consumed. It is possible that the drug therapy altered or reversed the coagulopathy. The trigger for the coagulation cascade is not known. Possibly the patient had antibodies to some tissue antigens as the result of his autoimmune disorders or his bile duct carcinoma. The second patient also had an episode of HAR that was typical clinically but not histologically. There were no glomerular thrombi; instead, there was karyorrhectic nuclear debris and increased numbers of T

Table 1. Pathological Findings in Cases of Hyperacute Rejection, Unused Donor Kidneys, Baseline Biopsies, and Renal Vein Thrombosis

Cases	Light Microscopy	IgG	IgM	C1Q	C3	Properdine
Hyperacute rejection						
1	Glomerular thrombosis	0	Art Gloms	Art Gloms	NA	NA
2	Nuclear fragments	0	Mes	Art	Art	Art
3	Glomerular thrombosis					
	used:	0	Mes	Mes	Mes	Mes
	unused:	0	0	Mes	Mes	Mes
Unused donor kidney						
4, GSW	DIC	0	Thr	Thr	NA	NA
5, GSW	DIC,	0	Thr	Thr	Thr	Thr
6, stroke	Malignant nephrosclerosis	Thr	Thr	Thr	Thr	Thr
7, sepsis	DIC,	Thr	Thr	Thr	Thr	NA
8, lac, renal vein	Normal	0	0	0	NA	NA
9, sepsis	ATN	0	0	0	NA	NA
10, viremia	Normal	0	0	Mes	NA	NA
Baseline biopsies						
11, postperfusion	Normal	0	0	0	NA	NA
12, postperfusion	Normal	0	0	0	NA	NA
13, preanastamosis	Normal	0	0	0	NA	NA
Renal vein thrombosis						
14	Hemorrhage	0	0	Art	0	0

Abbreviations: NA, not applicable; Mes, mesangial; Thr, thrombus; Art, arterial wall; GSW, gunshot wound.

cells. We found T cells only rarely in glomeruli from other cases. The patient's high PRA implicates an initial reaction with some HLA antigens on endothelial cells despite a close HLA match between donor and recipient. The pathological findings suggest a mechanism by which T cells either mediate cytotoxicity within the glomerulus or are recruited very early in the process. The third patient had a typical HAR, both clinically and histologically. The presence of C3 and properdin in both used and unused kidneys and the history of gram-negative infection in the donor suggest that the sequence of events in this case started with endotoxin activation of complement in the donor. Upon reperfusion, this Schwartzman reaction continued in the new host.

Another unusual feature of our cases is that IgM and not IgG, as previously reported,[3] was found consistently along with C1Q in the tissues. IgM antibodies may appear earlier than IgG antibodies and thus be more readily detectable in tissue during the first few hours. We found deposition of IgM and complement in arterial walls only in HAR. Other kidneys with widespread thrombosis and necrosis due to DIC exhibited either immunoglobulin and complement deposition in thrombi, but not in arterial walls, or deposition of only complement in vessel walls. This suggests that IgM-C′ in vessel walls may be a specific indicator of vascular rejection.

In summary, three patients with ABO-compatible donors and negative crossmatches had clinically typical episodes of hyperacute rejection. They demonstrated varied histological and immunopathologic findings, which suggests that HAR may occur when clinical and laboratory predictors are negative and that different pathogenetic mechanisms, including nonimmunologic ones, may lead to activation of the clotting sequence. IgM and complement deposition may be a specific marker for HAR of the kidney.

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