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Detailed Analysis of Simultaneous Renal and Liver Allografts in the Presence of DSA

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Background. Liver allografts protect renal allografts from the same donor from some, but not all, preformed donor specific alloantibodies (DSA). However, the precise mechanisms of protection and the potential for more subtle alterations/injuries within the grafts resulting from DSA interactions require further study. **Methods.** We reevaluated allograft biopsies from simultaneous liver-kidney transplant recipients who had both allografts biopsied within 60 d of one another and within 30 d of DSA being positive in serum (positive: mean florescence intensity ≥5000). Routine histology, C4d staining, and specialized immunohistochemistry for Kupffer cells (KCs; CD163) and a C4d receptor immunoglobulin-like transcript-4 were carried out in 4 patients with 6 paired biopsies. **Results.** Overt antibody-mediated rejection was found in 3 of 4 renal and liver allografts. One patient had biopsy-confirmed renal and liver allograft antibody-mediated rejection despite serum clearance of DSA. All biopsies showed KC hypertrophy (minimal: 1; mild: 2; moderate: 1; severe: 2) and cytoplasmic C4d KC staining was easily detected in 2 biopsies from 2 patients; minimal and negative in 2 biopsies each. Implications of which are discussed. Control 1-y protocol liver allograft biopsies from DSA– recipients showed neither KC hypertrophy nor KC C4d staining (n = 6). **Conclusions.** Partial renal allograft protection by a liver allograft from the same donor may be partially mediated by phagocytosis/elimination of antibody and complement split products by KCs, as shown decades ago in controlled sensitized experimental animal experiments.

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Liver allografts, when transplanted in combination with other allografts from the same donor, may protect the extrahepatic organs from some but not all preformed donor specific alloantibodies (DSA).[1-7] However, the liver is less likely to protect the kidney from the injurious effects of de novo DSA.[8] These phenomena, although known for several decades, have (a) never been granularly studied at a microscopic level in paired liver-kidney biopsies from sensitized humans, as determined by single antigen bead serum DSA assays and (b) with recognized criteria for acute liver antibody-mediated rejection (AMR).

Therefore, we sought a more in depth understanding of (a) liver allograft AMR because similar events are more thoroughly characterized in companion renal allografts and (b) "hepato-protective" qualities or interactions among the liver and kidney allografts and circulating DSA by comparing histopathologic findings in each organ in the context of serological and clinical findings. For example, if features of acute AMR in liver/kidney allograft recipients are observed in the kidney and similar changes are present in the liver, our understanding and recognition of the spectrum of hepatic acute and/or chronic AMR changes would improve.

Current evidence suggests that several mechanisms contribute to the hepatoprotection afforded the kidney including (1) secretion of soluble class I HLA (sHLA); sHLA levels in humans correlate with the risk of liver allograft rejection.[9] In experimental animal models, class I sHLA molecule injections or liposomal plasmid donor soluble major histocompatibility

complex (MHC) class I DNA transfection of hepatocytes delayed rejection and improved heart allograft survival,[10] even in sensitized recipients.[11] (2) Controlled experimental animal studies in the 1990s[12-14] provided convincing data showing that nonparenchymal cells (Kupffer cells [KCs] and liver sinusoidal endothelial cells [LSEC]) play a significant role in hepatoprotection. (3) The dual afferent vasculature and large microcirculatory bed distributes the sensitized serum over a larger surface area. (4) The liver can regenerate after an insult.[15]

In contrast to all other solid organ allografts, members of the monocyte/macrophage system, KC, normally line most of the liver microvasculature, partially covering the unique LSEC. LSEC differ from conventional capillaries and more closely resemble lymphatic endothelium.[16] This is an important microanatomical difference between the liver and all other solid organ allografts because KC and LSEC express Fcγ receptors and actively scavenge a variety of AMR-related byproducts such as activated complement components, platelet aggregates, and coagulation proteins, whereas intra-microvascular monocytes/macrophages in all other solid organ allografts are a diagnostic feature of acute AMR in sensitized recipients. KC and LSEC have been shown to remove soluble immune complexes in rodents.[17,18] Although not directly documented to be HLA-DSA complexes, immune complexes are detected with significantly increased frequency in conventional assays in sensitized humans who experience persistent DSA and acute AMR but not in sensitized persons who have cleared serum DSA.[19]

This is particularly important in liver allograft recipients because it has been documented that increased immunosuppression can result in either a decrease in DSA mean florescence intensity (MFI) or DSA elimination.[20] Additionally, fibrosis regression has also been documented in liver allografts unlike kidney allografts. Therefore, to further understand the mechanisms and limitations of liver allograft protection of renal allografts we evaluated histopathologic changes in paired biopsies (liver and kidney) from sensitized simultaneous liver-kidney transplant (SLKT) recipients.

MATERIALS AND METHODS

All SLKT recipients who underwent double organ replacement from the same donor at the Baylor Simmons Transplant Institute from June 1985 to July 2011 and had at least a single pretransplant sample saved in the Simmons Transplant Institute biorepository (88% of the total SLKT transplants during the time frame) were tested for DSA in serum as part of a previously published study.[4] Institutional review board approval was obtained before this study was undertaken. Three of 4 patients received both a renal and liver allograft biopsy within 3 d of one another and the fourth within 60 d of one another. Detection of an MHC class I or II DSA in serum with a MFI of ≥5000 occurred in 5 of 6 serum samples on the same day as the biopsies, 1 occurred 11 d before and case 4 did not have follow-up serum for the second paired biopsies that all happened between 1994 and 2002. All renal allograft biopsies were indication biopsies, documenting graft dysfunction because the Baylor Simmons Transplant Institute does not perform protocol renal allograft biopsies. In addition to indication liver biopsies, all liver transplant patients receive yearly protocol liver biopsies for HCV if viremic, and protocol liver biopsies at years 1, 5, 10, 15, and 20 if not viremic. Protocol liver biopsy is also performed 7 d after the diagnosis of rejection.

All formalin-fixed, paraffin-embedded tissues were sectioned at 4 microns for liver and 3 microns for kidney and routinely stained with hematoxylin and eosin, trichrome, MCH class II, and C4d.[21,22] No frozen tissue was available. All staining was performed at the University of Pittsburgh Medical Center and the histology and immunohistochemistry were evaluated by a single pathologist blinded to the class and MFI of the serum DSA, timing of the biopsies, and which organs were transplanted together (A.J.D.).

Liver and kidney histology was semi-quantitatively scored for Banff components, including those commonly associated with serum DSA and AMR in liver and kidney allografts.[22-25] The 2016 Banff criteria for T-cell mediated rejection (TCMR) and AMR was used to score the liver histology.[24]

Single and Multiplex Immunohistochemistry

Paraffin sections were deparaffinized, and single and multiplex immunostains were performed on a Ventana Discovery Ultra (Ventana Medical Systems, Oro Valley, AZ). C4d and MHC II immunohistochemistry was performed as previously described.[22,23]

For multiplex labeling, following pH9 antigen retrieval (S2367; Agilent Technologies/Dako) the following antibodies were added in the following order: (1) CD31 (1:50; Dako, Carpenteria, CA 93013, M0823) for 2 h at room temperature and labeled with anti-mouse horseradish peroxidase (HRP)/Rhodamine 6G (Roche), (2) CD163 (1:500; Biorbyt, St Louis, MO 63132; orb13303) at room temperature for 4 h and labeled with anti-rabbit HRP (Roche) Discovery Red610 (Roche), (3) anti-immunoglobulin-like transcript-4 (ILT4) (1:50, ILT-4; EMD Millipore, Temecula CA; abn1023) for 4 h at room temperature and labeled with anti-rabbit HRP Discovery Cy5 (Roche), and (4) C4d (1:100; Abcam, Waltham, MA 02453, USA ad183311) for 4 h at room temperature and labeled with OmniMap anti-rabbit HRP/Discovery FAM (Roche Diagnostics, Indianapolis, IN). Slides were counterstained with 4′,6-diamidino-2-phenylindole and mounted in Anti-fade Mountant (Life Technologies, Carlsbad, CA).

Case Presentations

Case 1

A 66-y-old pacific-islander man with HCV cirrhosis and membranoproliferative glomerulonephritis received a SLKT with a Model for End-stage Liver disease (MELD) of 20 from a 62-y-old donor with 8.9 h of cold ischemia time and no preformed DSA. Postoperatively he underwent an indication liver biopsy on postoperative day (POD) 5, locally evaluated as "preservation injury" when his alanine aminotransferase (ALT) was 559 U/L, alkaline phosphatase was 102 IU/L, total bilirubin was 6.9 mg/dL, and serum creatinine was 2.4 mg/dL. His cyclosporine level was 423 ng/mL and prednisone dose was 40 mg/d. Continued cholestasis resulted in several biliary procedures, which all showed no stenosis. A solumedrol pulse was given empirically on POD 17 for presumed renal allograft rejection when the creatinine increased from 1.6 to 2.1 mg/dL without improvement. Indication liver and kidney allograft biopsies were undertaken on POD 65 and 66, respectively, when the ALT was 211 U/L, alkaline phosphatase was 247 IU/L, total bilirubin was 2.4 mg/dL, the cyclosporine level was 408 ng/mL, and the prednisone dose was 15 mg/d (Table 1 and

TABLE 1.

Correlation of serum DSA with histology (gray shows de novo DSA)

	Preformed DSA		DSA test days after SLKT	Post-DSA		Bx-days after SLKT	C4d kidney	Kidney path summary	C4d liver	Liver path summary[a]	Outcome
No.	Class I	Class II		Class I	Class II						
1	0	0	65	0	9000	65, 66	Focal ptc positivity	Mild acute AMR (g1-2, i1, t0, v0, ptc1); mild to moderate chronic changes (cg0, ci1, ct1, cv2) likely related to donor disease	Diffuse portal microvascular endothelial cell positivity; negative LSEC; focal KC cytoplasmic positivity	Low-grade acute AMR (i3, h2) with prominent KC hypertrophy with KC C4d staining	Renal allograft failure 3. 5 mo; biliary strictures developed; death 9 y post-SLKT
2	6700	0	13	0	0	15, 13	Diffuse ptc positivity	Mild acute AMR (g1-2, i1, t0, v0, ptc2); no chronic changes (cg0, ci0, ct0, cv0) likely related to donor disease	Diffuse portal microvascular endothelial cell positivity; negative LSEC	Low-grade AMR (i3, h2); KC less prominent than case no. 1	Death 8 y post-SLKT from liver allograft failure
3a	23 000[b]	25 000	54			10, 54	Diffuse ptc positivity	Mild AMR (g1, i1, t0, ptc1); no chronic changes (cg0, ci0, ct0, cv0, ah0)	Diffuse portal microvascular and central vein endothelial cell positivity; negative LSEC	Mixed TCMR and AMR (i3, h2) with central perivenular and sclerosing central vein lesions in regions of prominent C4d deposition	Renal allograft failure 2. 5 y posttransplant: HAT and death 11 y post-SLKT
3b			301	15 000	18 000	301 346	Minimal ptc positivity	Suspicious for AMR (g0, I1, t0, v0, ptc1); mild chronic changes (cg0, ci1, ct1, cvo, ah0)	Negative	Negative for TCMR and AMR with intact architecture with periportal-shunt-type vessel	
4a	13 000	0	5	NA	NA	8, 5	Negative	No specific histopathologic changes (go, i0, t0, v0, ptc0); no chronic changes (cg0, ci0, ct0, cv0, ah0)	Minimal microvascular endothelial cell positivity	Minimal changes (i1, h0) with prominent KC hypertrophy	GFR at 3 y 36 mL/min; follow-up liver biopsy showed plasma cell rich TCMR and chronic AMR; death from a GI bleed 4. 5 y post-SLKT
4b						74, 134	Minimal ptc positivity	Minimal histopathologic changes/ suspicious for minimal AMR (g0, i1, t0, vo, ptc1); mild chronic changes (cg0, ci1, ct1, cv0, ah1)	Minimal portal capillary and LSEC positivity	Plasma cell-rich TCMR with interface activity and central perivenulitis/probably chronic AMR (i2, h2)	

[a]Scoring per Banff 2016 criteria.
[b]C1q positive DSA.
AMR, antibody-mediated rejection; Bx, biopsy; DSA, donor specific alloantibodies; GFR, glomerular filtration rate; GI, gastrointestinal; HAT, hepatic artery thrombosis; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; NA, not available; ptc, peritubular capillary; SLKT, simultaneous liver-kidney transplant; TCMR, T-cell mediated rejection.

Tables S1 and **S2, SDC**, http://links.lww.com/TXD/A545). De novo class II DSA of MFI 9000 was found in our evaluation on the same day as this liver biopsy but was unknown to clinicians at the time. The patient had progressive renal function decline and was initiated on hemodialysis 3.5 mo posttransplant. The patient developed biliary strictures, was continued on hemodialysis and, died from a subdural hematoma 9 y posttransplant.

Case 2

A 53-y-old Caucasian woman with HCV cirrhosis and diabetic nephropathy with a MELD of 18 received a SLKT from a 52-y-old donor with 14 h of cold ischemia time with preformed isolated class I DSA of MFI 6700. Indication liver biopsy on POD 6 showed "rejection" and a steroid recycle was initiated when the ALT was 276 U/L, alkaline phosphatase was 246 IU/L, and total bilirubin was 3.4 mg/dL. Her cyclosporine level was 317 ng/mL, azathioprine dose was 200 mg/d, and prednisolone dose was 20 mg/d. Protocol liver biopsy on POD 13 showed "residual rejection" (ALT was 81 U/L, alkaline phosphatase was 466 IU/L, and total bilirubin was 2.6 mg/dL), renal biopsy on POD 15 for an increased serum creatinine (1.7–3.1 mg/dL) showed "rejection," and anti-CD3 monoclonal antibody was initiated. Of note, repeat DSA testing on the day of the liver biopsy was negative. The patient's creatinine improved posttreatment to 1.0 mg/dL. At month 2, the patient had recurrent liver allograft "rejection" and was switched to tacrolimus (level 23.7 ng/mL). Rejection recurred 2 wk later, and she was given a steroid recycle. The posttreatment liver biopsy showed persistent duct damage but no overt rejection. She died of liver allograft failure 7.5 y posttransplant while being treated with tacrolimus monotherapy.

Case 3

A 47-y-old African American woman with acute liver failure experienced hepatic artery thrombosis after her primary liver allograft and was retransplanted with an SLKT at a MELD of 21 with a 24-y-old donor with 9 h of cold ischemia time with preformed class I DSA of MFI 23 000 and class II DSA of MFI 25 000. Renal biopsy on POD 10 was locally evaluated as "no rejection" when the serum creatinine increased to 2.8 mg/dL, but a steroid recycle was given anyway. Indication liver biopsy on POD 54 when the ALT was 46 U/L, alkaline phosphatase was 196 IU/L, and total bilirubin was 0.6 mg/dL was locally diagnosed as TCMR that was treated with a steroid recycle, and a protocol liver biopsy at the end of the treatment showed persistent rejection treated with more steroids (ALT was 39 U/L, alkaline phosphatase was 140 IU/L, and total bilirubin was 0.4 mg/dL). The patient was maintained on cyclosporine (level 409 ng/mL), mycophenolate 1000/d, and prednisone 10 mg/d. At year-1, DSA testing showed persistent class I DSA with MFI of 15 000 and a de novo class II DSA with MFI of 18 000. Protocol 1-y liver biopsy when the ALT was 35 U/L, alkaline phosphatase was 62 IU/L, and total bilirubin was 0.4 mg/dL showed regression of fibrosis from stage 1 periportal fibrosis and stage 2 pericentral fibrosis to no fibrosis. Despite improvement in liver histology, acute renal failure at 2 y was locally thought to be secondary to acute tubular necrosis and a protocol liver biopsy when the ALT was 14 U/L, alkaline phosphatase was 136 IU/L, and total bilirubin was 0.5 mg/dL showed "rejection." Renal dysfunction resulted in a switch to rapamycin 5 mg/d, mycophenolate 1000 mg/d, and prednisone 5 mg/d, and shortly thereafter the patient developed ascites (2.5 y posttransplant). The patient died with renal failure on hemodialysis with recurrent hepatic artery thrombosis 11 y and 2 mo posttransplant.

Case 4

A 38-y-old Caucasian man with alcohol induced cirrhosis and interstitial nephritis underwent SLKT with a MELD of 37 from a 45-y-old donor with 5.2 h of cold ischemia time with preformed class I DSA of MFI 13 000. He underwent indication liver biopsy on POD 6 showing "rejection" with ALT of 123 U/L, alkaline phosphatase of 193 IU/L, and total bilirubin of 4.1 mg/dL while taking tacrolimus (level 23 ng/mL) and Prednisone 20 mg/d. He was treated with a steroid recycle and the addition of mycophenolate at 2000 mg/d. A renal biopsy "without rejection" was performed on POD 8 when the serum creatinine was 1.8 mg/dL. One-y protocol liver biopsy showed incidental rejection when the ALT was 44 U/L, alkaline phosphatase was 299 IU/L, and total bilirubin was 0.7 mg/dL while taking tacrolimus (level 5.4 ng/mL), mycophenolate 500 mg/d, and prednisone 10 mg/d that was treated with a steroid recycle. Rejection recurred 6 mo later while on tacrolimus (level 8.6 ng/mL), mycophenolate 1500 mg/d, and prednisone 5 mg/d (ALT was 17 U/L, alkaline phosphatase was 160 IU/L, and total bilirubin was 0.7 mg/dL). Glomerular filtration rate at year 3 had declined to 36 mL/min. No follow-up DSA testing was available. The patient died from a gastrointestinal bleed 4.5 y posttransplant at an outside hospital.

Other Results

The above mini-case reports describe the details of each individual case, but some elements, such as laboratory values and histology, were more consistent among the cases and therefore described in aggregate.

Despite the relatively unimpressive serum ALT values at the time of liver biopsies, portal inflammation was present in all cases that varied from minimal to moderate with portal capillaritis/inlet venulitis, except for 2 biopsies (second biopsy for case number 3 and the first for case number 4); neither of these liver biopsies showed diagnostic evidence of acute AMR per current criteria (Table 2). Additionally, KC hypertrophy, indicative of material uptake (https://ntp.niehs.nih.gov/nnl/hepatobiliary/liver/kchyper/index.htm) was seen in all cases and varied (minimal [n = 1]; mild [n = 2]; moderate [n = 1] and severe [n = 2]) (Figures 1–4 and **Figures S1–S7, SDC**, http://links.lww.com/TXD/A544). An example of severe KC hypertrophy is shown in Figure 2. On closer examination, it was apparent that KCs contained intra-cytoplasmic debris and occasionally entire cells. Of note, C4d staining was also found in the cytoplasm of KCs that also stained for class II MHC by immunohistochemistry in 2 biopsies. As a control, 1-y protocol liver biopsies selected on the basis of negative DSA testing conducted within 60 d after the biopsy (days 1, 2, 2, 3, 18, and 46) showed that all were negative for KC C4d staining; 1 showed minimal KC hypertrophy, all remaining biopsies were negative for KC hypertrophy (Figure 2, bottom panels). None of the control biopsies showed evidence of AMR; 2 showed indeterminate (n = 1) or mild (n = 1) TCMR, 2 showed minimal nonspecific changes, and 2 showed mild cholangiopathic changes.

The primary cellular infiltrate for the SLKT cases was lymphocytic with 2 cases having significant portal eosinophils present and 1 having a significant number of plasma cells.[21]

TABLE 2.

Median values with (interquartile ranges) for liver histology and C4d staining are presented (scale for individual values 0–3)

Histology	
Inflammation	
Portal inflammation	2 (1–2)
Interface severity	1 (0–1)
Lobular disarray	2 (1–2)
Lobular inflammation	2 (1–2)
KC hypertrophy	2 (2–3)
Central perivenulitis	0 (0–0)
Sinusoidal capillaritis	2 (2–3)
Hepatocellular cholestasis	0 (0–2)
Fibrosis	
Portal tract collagenization	0 (0–0)
Portal venopathy	0 (0–0)
Portal fibrosis	0 (0–0)
Central fibrosis	0 (0–0)
C4d staining	
Portal vein	2 (1–2)
Portal capillaries	2 (1–3)
Sinusoids	2 (1–3)
Portal stroma	2 (1–3)
Central vein	0 (0–2)
Total	7.5 (4–13)

KC, Kupffer cell.

Similarly, the lobular infiltrate was predominantly lymphocytic with 2 cases having a significant number of plasma cells present. Four of the 6 liver biopsies met criteria for acute AMR, and 1 met criteria for chronic AMR (Table 1).[24]

C4d staining in the liver was present in the portal veins, portal capillaries, portal stroma, and sinusoids but was less frequently found around the central veins with a median total score of 7.5 (Table 2).

Renal histology showed a median glomerulitis (g) score of 1, interstitial inflammation (i) score of 1, peritubular capillaritis (ptc) score of 1, and C4d was present in 5 of 6 biopsies. The primary interstitial inflammatory infiltrate was plasmacytic in 4 of 6 cases. As a result, 3 cases were diagnostic of renal allograft AMR, 2 were suspicious for renal allograft AMR, and 1 was normal.

Multiplex immunohistochemistry for CD31/CD163/ILT4/C4d was attempted on 5 of the 6 liver allograft biopsies (Figure 3) and in normal native human livers as a control. Colocalization of ILT4 and CD163 in KCs was clearly seen in all normal human livers, similar to what we previously reported in liver allografts.[26] Using single immunostains, C4d clearly localized to MHC II+ KCs, as shown in the biopsy from case 1. Multiplex immunolabeling yielded suboptimal results, likely related to the age of the tissue specimens, which led to high background "edge effect" staining. However, in interpretable areas of case 1, similar to the single immunolabeling results, C4d immunolabeling appeared to be present in KCs that also labeled with ILT-4+/CD163+ (Figure 3). However, more studies are needed to confirm this finding because of suboptimal multiplex staining.

DISCUSSION

Although it is well established that a liver allograft has the capacity to protect a renal allograft from the same donor

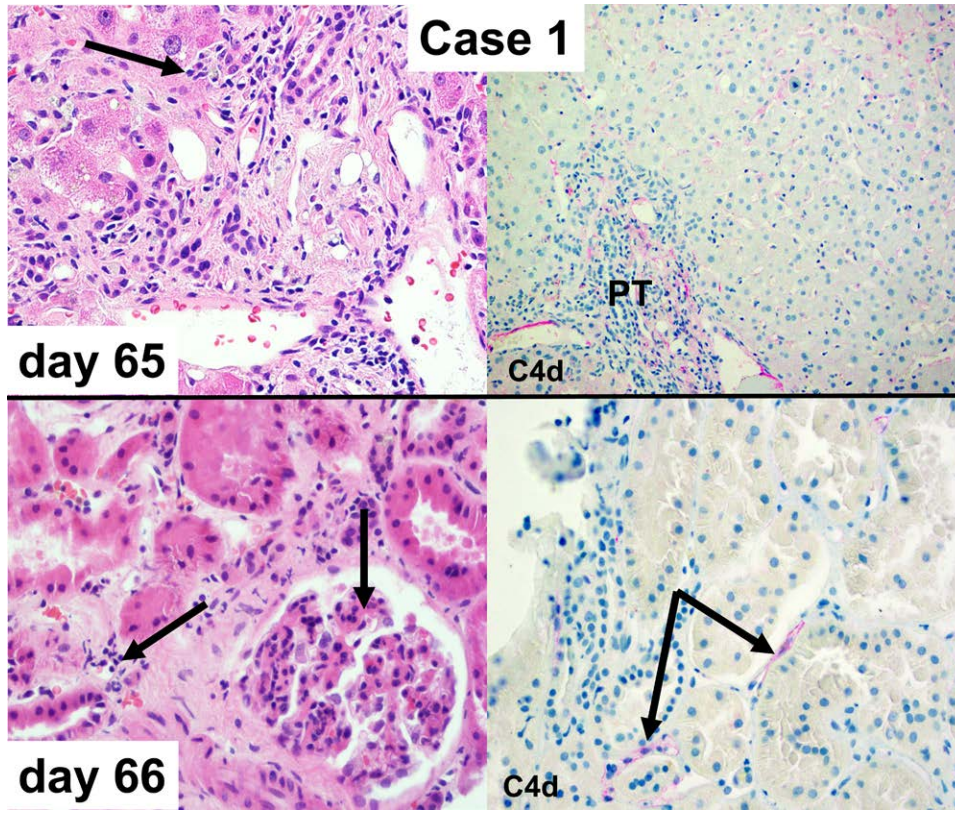

FIGURE 1. Liver allograft obtained on day 65 after OLTx (upper row). Note the low-grade lymphoplasmacytic portal (left panel), mild inlet venulitis (arrow), and strong portal microvascular endothelial C4d positivity. A kidney allograft biopsy obtained 1 d later (day 66) showed low-grade glomerulitis and ptc (arrows) and focal peritubular capillary C4d deposition on FFPE tissue. FFPE, formalin fixed paraffin embedded; OLTx, orthotopic liver transplant; PT, portal tract; ptc, peritubular capillaritis.

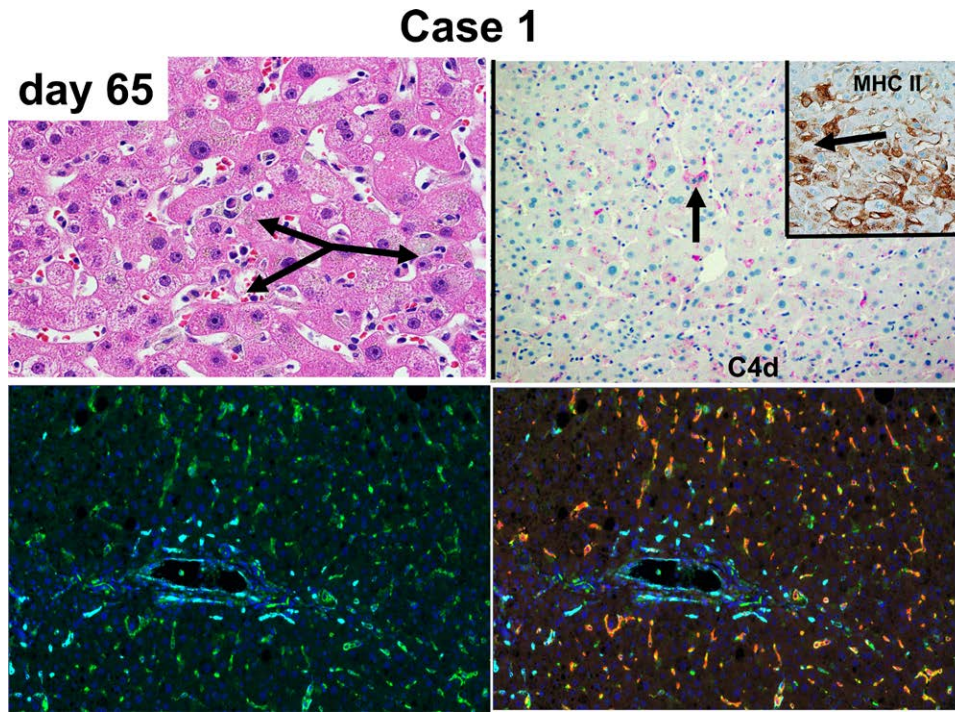

FIGURE 2. Higher magnification of the lobule from case 1. Note the marked KC hypertrophy (upper left panel; arrows), some of which contained intra-cytoplasmic debris and occasional cells. C4d staining (right panel) showed cytoplasmic labeling for C4d in enlarged KC (pink staining), which also stained strongly for MHCII, as expected (inset; brown staining). This observation led us to conclude that some C4d is deposited or contained within KC. The bottom left panel shows a normal human liver stained for CD34 (cyan) that highlights the portal microvascular and ILT4 (green) that highlights KCs within the lobules. The bottom right panel adds CD163 (red) that highlights KCs, which overlaps with the green ILT4 stain resulting in a yellow-orange color within the sinusoids indicating that CD163+ KC also express ILT4. ILT4, immunoglobulin-like transcript-4; KC, Kupffer cell; MHCII, major histocompatibility complex II.

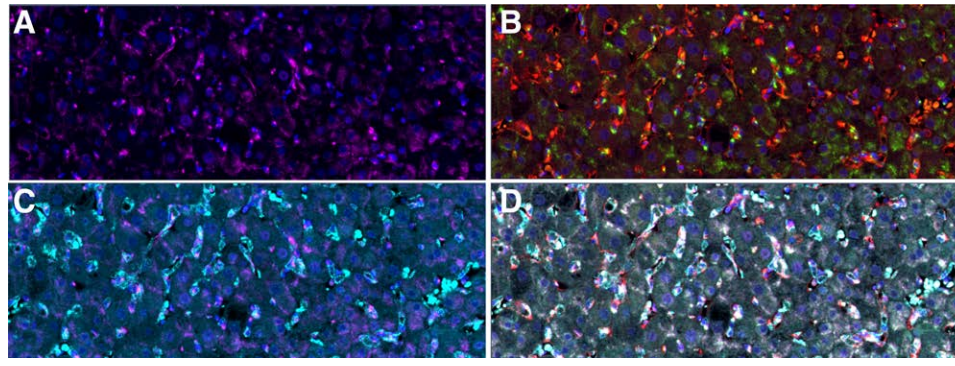

FIGURE 3. Case 1 in area of low-background (see text). A, Only 1 label (ILT4; pink) is shown in this image that highlights KCs within the lobules of case 1 that showed marked KC hypertrophy. B, This panel simultaneously shows 2 labels, CD31 highlights the sinusoidal endothelium (red) and C4d (fluorescent green); note the lack of yellow staining that would indicate C4d deposition on the CD31+ sinusoidal endothelium. Instead, the C4d is highlighting sinusoidal cells. C, This panel simultaneously shows 2 labels, ILT4 (pink) and CD163 (turquoise). The staining shows overlap within the KCs, but not necessarily in the same subcellular location. D, This panel simultaneously shows all 4 labels, CD31 (red), ILT4 (pink), C4d (fluorescent green), and CD163 (turquoise). The overlapping staining of C4d, CD163, and ILT4 appears white in this combined image, which localizes to KCs. ILT4, immunoglobulin-like transcript-4; KC, Kupffer cell.

from some but not all preformed DSA,[1-6,27] our small study offers some intriguing insights worthy of further investigation. Specifically, (1) even though DSA had been "cleared" from the serum, evidence of antibody interaction (diffusely positive portal microvascular C4d deposition and microvasculitis) was seen in both the liver and kidney allografts with negative serum DSA in 1 of our cases (case number 2); (2) routine histopathologic features of acute AMR were quite similar in both organs; (3) unique hepatic features of AMR include (a) C4d staining in diffusely positive cases abruptly stopped at the level of sinusoidal endothelium and (b) KCs became hypertrophied, which was not seen in control liver allografts, and in 2 cases showed cytoplasmic C4d staining likely indicative of KC phagocytosis/elimination of activated complement and antibody/antigen complexes similar to experimental animal models[13]; (4) in contrast to all other solid organ allografts, the normal liver microvascular is lined by intravascular macrophages (KC), which strongly express ILT4, a known receptor of soluble C4d split products, which can be immunomodulatory;[28] and (5) after an acute AMR injury with resultant fibrosis, hepatic regeneration with fibrosis regression can occur (case number 3), in contrast to renal

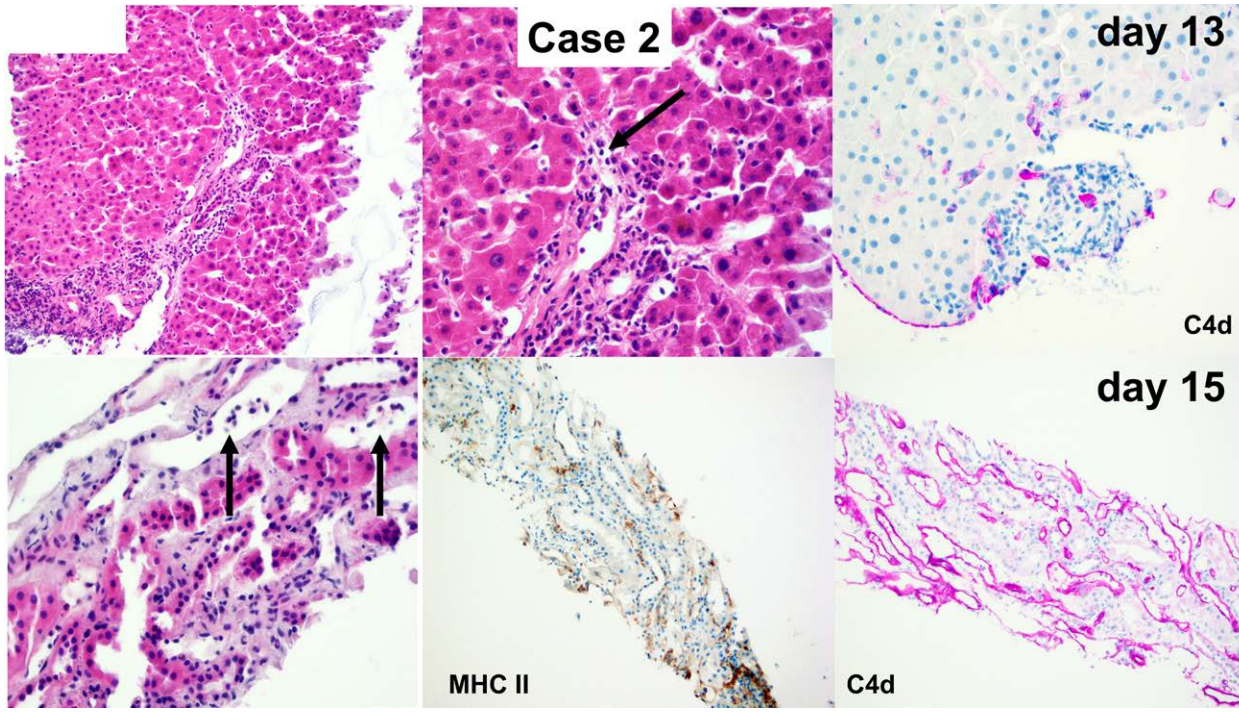

FIGURE 4. Case 2 showing the liver allograft biopsy (top row) obtained on day 13 and kidney allograft biopsy (bottom row) obtained on day 15. Note the mild portal inflammation and mild microvascular inflammation (arrow; middle panel). Liver C4d deposition (upper right panel) was largely limited to the portal microvascular endothelium, whereas only minimal sinusoidal and KC staining was seen (Table 1). The kidney allograft (lower row) showed changes diagnostic of acute AMR with ptc and diffuse peritubular capillary C4d deposition (bottom right panel). AMR, antibody-mediated rejection; KC, Kupffer cell; MHCII, major histocompatibility complex II; ptc, peritubular capillaritis.

allograft injury that is not known to regress. Interestingly, recent studies show that neuropilin-1 is a novel receptor for covalently bound C4d, C3d, and iC3b,[29] which is expressed on macrophages/KCs and hepatic stellate cells.[30,31]

It was surprising that one of our patients (case number 2) had isolated class I DSA pretransplant but developed unequivocal evidence of both liver and renal allograft AMR within a couple of weeks after transplantation but without detectable DSA in serum. In general, the liver is able to better protect the kidney from preformed class I DSA than class II because of its large size, soluble secretion of HLA class I and ubiquitous expression of HLA class I;[4,32,33] however, in this case, that did not prevent overt acute AMR from occurring. We cannot exclude the possibility that a complement fixing non-HLA donor specific autoantibody or alloantibody was also present and acted alone or synergized with the preformed HLA DSA to injure the allografts or that immunoglobulinG3 HLA-DSA subtype dominated the profile. However, we believe this is less likely since only minimal sinusoidal C4d staining was present and previously we reported that strong C4d sinusoidal staining is often found in patients with de novo Angiotensin II type 1 receptor autoantibodies.[34]

Although we cannot exclude the possibility of another alloantibody or autoantibody injury, clinically this patient was originally diagnosed with steroid-refractory rejection necessitating anti-CD3 monoclonal antibody treatment with recurrent TCMR diagnosed 4 and 6 wk later. After a switch of calcineurin inhibitor from cyclosporine to tacrolimus, only bile duct injury and progressive fibrosis were found on repeat biopsy. This led to eventual graft failure from recurrent HCV and death, a previously reported complication that can occur in patients with DSA.[35,36] As a result, this may represent intra-graft biding of the HLA DSA resulting in ongoing injury despite its absence in serum. Alternatively, DSA may have been bound in the allograft, subsequently cleared, and the patient had progressive fibrosis from HCV alone. Further study with graft elution of antibodies from fresh tissue will be required to determine if DSA can be present in liver allografts, while negative in serum, and if so for how long.

In light of the above case, it is essential to highlight that MFI is not strictly quantitative, and as a result SLKT patients with DSA pretransplant require follow-up testing posttransplant to ensure clearance in the absence of overt graft dysfunction, regardless of the MFI. Also most DSAs have Fc binding regions, depending on their immunoglobulinG subclass, and therefore may increase the probability of TCMR posttransplant, as previously described and seen here.[37] Therefore, even with resolution of serum DSA, DSA-positive patients have a higher risk for TCMR necessitating closer short-term follow-up and a low threshold for biopsy to diagnose and treat this early.[38]

Turning from injury to hepatoprotection, a primary and important mechanism of hepatic protection appears to reside in the KCs ability to phagocytose injurious products (eg, platelet aggregates, coagulation proteins, activated complement components) as previously described and proven in experimental models (12, 13). It has long been known that C4d staining is less prominent in liver than renal allografts even when complement-biding DSA is present in serum.[39] This was previously thought to solely result from the large vascular bed distributing DSA over a greater surface area within the liver. Although this extensive area of distribution and variable expression of MHC II[40] certainly diminishes the intensity of injury and staining, this study confirms that KCs express one soluble C4d receptor (ILT4)[28] and likely another (neuropilin-1),[29] which is expressed on macrophages/KCs and

hepatic stellate cells.[30] A critical role for KC is supported histologically by marked enlargement of these cells, prominent C4d staining of their cytoplasm in a couple of cases and ILT4 expression. However, given our small case series and suboptimal multiplex staining this will require further confirmation.

Only 1 patient (case 3) had follow-up DSA testing that documented positivity and had a second set of combined biopsies. The liver histology in the second set of biopsies was relatively unremarkable except for periportal shunt vessel development. Of note, the presence of strong MHC class II staining in the liver on the first biopsy was no longer seen on the second, and fibrosis regression was seen on the follow-up liver biopsy. Despite liver allograft fibrosis improvement, the serum creatinine had increased from 1.6 to 2.0 mg/dL and prompted the renal allograft biopsy. Unlike the liver histology, the renal allograft demonstrated low grade AMR that was not originally recognized. Because no immunosuppression alterations were made the serum creatinine continued to slowly climb until dialysis was initiated. Although the follow-up liver biopsy was relatively benign, the shunt vessel formation may have been an indicator of DSA-induced microvascular injury because this patient went on to die from hepatic artery thrombosis and renal failure.

This study is somewhat provocative, so it must be reiterated that it is a small cohort of 4 patients with 6 paired biopsies. Although all of our patients in this study died, they are not a representative sample of all SLKTs in the program, since renal allograft biopsy is only undertaken for cause at our center. A further weakness is our inability to rule out non-HLA DSA and more granularly characterize the DSA as a possible cause of injury in the cases. We were not able to assess for immunosuppression compliance because the database does not collect this data. One patient did not have follow-up DSA testing at the time of repeat biopsies, which is another weakness of our study. As a result of these weaknesses, further study will be needed to confirm our findings and determine their incidence, prevalence and better define their natural history.

In conclusion, unique hepatic features of AMR we describe herein include endothelial cell C4d staining that abruptly stopped at the sinusoids in formalin-fixed, paraffin-embedded tissue, and markedly hypertrophied KCs. We also saw AMR with DSA in serum that had recently decreased to <1000 MFI and improvement in liver allograft fibrosis after AMR resolution. Therefore, it is essential to remember that chronic AMR of the liver can occur despite normal liver injury tests,[22-24,39] and in this cohort mild acute AMR occurred with only very mild increases in liver injury tests.

REFERENCES

1. Starzl TE, Demetris AJ, Todo S, et al. Evidence for hyperacute rejection of human liver grafts: the case of the canary kidneys. *Clin Transplant*. 1989;3:37–45.
2. Saidman SL, Duquesnoy RJ, Demetris AJ, et al. Combined liver-kidney transplantation and the effect of preformed lymphocytotoxic antibodies. *Transpl Immunol*. 1994;2:61–67.
3. Dar W, Agarwal A, Watkins C, et al. Donor-directed MHC class I antibody is preferentially cleared from sensitized recipients of combined liver/kidney transplants. *Am J Transplant*. 2011;11:841–847.
4. O'Leary JG, Gebel HM, Ruiz R, et al. Class II alloantibody and mortality in simultaneous liver-kidney transplantation. *Am J Transplant*. 2013;13:954–960.
5. Olausson M, Mjornstedt L, Norden G, et al. Successful combined partial auxiliary liver and kidney transplantation in highly sensitized cross-match positive recipients. *Am J Transplant*. 2007;7:130–136.
6. Askar M, Schold JD, Eghtesad B, et al. Combined liver-kidney transplants: allosensitization and recipient outcomes. *Transplantation*. 2011;91:1286–1292.
7. Yazawa M, Cseprekal O, Helmick RA, et al. Association between post-transplant donor-specific antibodies and recipient outcomes in simultaneous liver-kidney transplant recipients: single-center, cohort study. *Transpl Int*. 2020;33:202–215.
8. Parajuli S, Aziz F, Blazel J, et al. The utility of donor-specific antibody monitoring and the role of kidney biopsy in simultaneous liver and kidney recipients with de novo donor-specific antibodies. *Transplantation*. 2021;105:1548–1555.
9. Minguela A, Torio A, Marin L, et al. Implication of soluble and membrane HLA class I and serum IL-10 in liver graft acceptance. *Hum Immunol*. 1999;60:500–509.
10. Sumimoto R, Kamada N. Specific suppression of allograft rejection by soluble class I antigen and complexes with monoclonal antibody. *Transplantation*. 1990;50:678–682.
11. Geissler EK, Graeb C, Tange S, et al. Effective use of donor MHC class I gene therapy in organ transplantation: prevention of antibody-mediated hyperacute heart allograft rejection in highly sensitized rat recipients. *Hum Gene Ther*. 2000;11:459–469.
12. Gugenheim J, Charpentier B, Gigou M, et al. Delayed rejection of heart allografts after extracorporeal donor-specific liver hemoperfusion. Role of Kupffer cells. *Transplantation*. 1988;45:628–632.
13. Gugenheim J, Le Thai B, Rouger P, et al. Relationship between the liver and lymphocytotoxic alloantibodies in inbred rats. Specific absorption by nonparenchymal liver cells. *Transplantation*. 1988;45:474–478.
14. Gugenheim J, Amorosa L, Gigou M, et al. Specific absorption of lymphocytotoxic alloantibodies by the liver in inbred rats. *Transplantation*. 1990;50:309–313.
15. O'Leary JG, Demetris AJ, Friedman LS, et al. The role of donor-specific HLA alloantibodies in liver transplantation. *Am J Transplant*. 2014;14:779–787.
16. Demetris AJ, Bellamy CO, Gandhi CR, et al. Functional immune anatomy of the liver-as an allograft. *Am J Transplant*. 2016;16:1653–1680.
17. Johansson AG, Lovdal T, Magnusson KE, et al. Liver cell uptake and degradation of soluble immunoglobulin G immune complexes in vivo and in vitro in rats. *Hepatology*. 1996;24:169–175.
18. Lovdal T, Andersen E, Brech A, et al. Fc receptor mediated endocytosis of small soluble immunoglobulin G immune complexes in Kupffer and endothelial cells from rat liver. *J Cell Sci*. 2000;113(Pt 18):3255–3266.
19. Manez R, Kelly RH, Kobayashi M, et al. Immunoglobulin G lymphocytotoxic antibodies in clinical liver transplantation: studies toward further defining their significance. *Hepatology*. 1995;21:1345–1352.
20. Tokodai K, Miyagi S, Nakanishi W, et al. Effects of re-augmenting maintenance immunosuppression on post-transplant donor-specific HLA antibodies in liver transplantation. *Transpl Immunol*. 2020;63:101334.
21. O'Leary JG, Michelle Shiller S, Bellamy C, et al. Acute liver allograft antibody-mediated rejection: an inter-institutional study of significant histopathological features. *Liver Transpl*. 2014;20:1244–1255.
22. O'Leary JG, Cai J, Freeman R, et al. Proposed diagnostic criteria for chronic antibody-mediated rejection in liver allografts. *Am J Transplant*. 2016;16:603–614.
23. O'Leary JG, Smith C, Cai J, et al. Chronic AMR in liver transplant: validation of the 1-year cAMR score's ability to determine long-term outcome. *Transplantation*. 2017;101:2062–2070.
24. Demetris AJ, Bellamy C, Hubscher SG, et al. 2016 Comprehensive update of the Banff working group on liver allograft pathology: introduction of antibody-mediated rejection. *Am J Transplant*. 2016;16:2816–2835.
25. Venturi C, Sempoux C, Bueno J, et al. Novel histologic scoring system for long-term allograft fibrosis after liver transplantation in children. *Am J Transplant*. 2012;12:2986–2996.
26. Levitsky J, Burrell BE, Kanaparthi S, et al. Immunosuppression withdrawal in liver transplant recipients on sirolimus. *Hepatology*. 2020;72:569–583.
27. Taner T, Gandhi MJ, Sanderson SO, et al. Prevalence, course and impact of HLA donor-specific antibodies in liver transplantation in the first year. *Am J Transplant*. 2012;12:1504–1510.
28. Hofer J, Forster F, Isenman DE, et al. Ig-like transcript 4 as a cellular receptor for soluble complement fragment C4d. *FASEB J*. 2016;30:1492–1503.
29. Battin C, De Sousa Linhares A, Paster W, et al. Neuropilin-1 acts as a receptor for complement split products. *Front Immunol*. 2019;10:2209.

30. Wang S, Zhao L, Zhang X, et al. Neuropilin-1, a myeloid cell-specific protein, is an inhibitor of HIV-1 infectivity. *Proc Natl Acad Sci U S A*. 2022;119:e2114884119.
31. Available at https://www.proteinatlas.org/ENSG00000099250-NRP1/single+cell+type/liver.
32. Wierzbicki AS, Pendleton S, McMahon Z, et al. Rimonabant improves cholesterol, insulin resistance and markers of non-alcoholic fatty liver in morbidly obese patients: a retrospective cohort study. *Int J Clin Pract*. 2011;65:713–715.
33. O'Leary JG, Kaneku H, Demetris AJ, et al. Antibody-mediated rejection as a contributor to previously unexplained early liver allograft loss. *Liver Transpl*. 2014;20:218–227.
34. O'Leary JG, Demetris AJ, Philippe A, et al. Non-HLA antibodies impact on C4d staining, stellate cell activation and fibrosis in liver allografts. *Transplantation*. 2017;101:2399–2409.
35. U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med*. 1994;331:1110–1115.
36. O'Leary JG, Kaneku H, Jennings L, et al. Donor-specific alloantibodies are associated with fibrosis progression after liver transplantation in hepatitis C virus-infected patients. *Liver Transpl*. 2014;20:655–663.
37. O'Leary JG, Kaneku H, Banuelos N, et al. Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. *Am J Transplant*. 2015;15:1003–1013.
38. O'Leary JG, Kaneku H, Jennings LW, et al. Preformed class II donor-specific antibodies are associated with an increased risk of early rejection after liver transplantation. *Liver Transpl*. 2013;19:973–980.
39. Neil DAH, Bellamy CO, Smith M, et al. Global quality assessment of liver allograft C4d staining during acute antibody-mediated rejection in formalin-fixed, paraffin-embedded tissue. *Hum Pathol*. 2018;73:144–155.
40. Kim PT, Demetris AJ, O'Leary JG. Prevention and treatment of liver allograft antibody-mediated rejection and the role of the 'two-hit hypothesis.' *Curr Opin Organ Transplant*. 2016;21:209–218.