



# HLA incompatible combined liver–kidney transplantation: Dynamics of antibody modulation revealed by a novel approach to HLA antibody characterisation



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## ABSTRACT

This case report confirms the utility of simultaneous liver transplantation in allowing successful kidney transplantation in the face of preformed, high levels of DSA, which would under normal circumstances be associated with hyperacute rejection and kidney graft failure. Antibody characterisation in terms of epitope specificity is more accurate and informative than antibodies described as “antigen-specific” and we suggest a method for identifying and tracking these antibodies; i.e. follow the epitope reaction not the antigen reactions. We consider that this will give a better insight into the behaviour and pathogenicity of HLA-specific sera. In the case presented here this approach has revealed some novel features of the post transplant antibody response in a sensitised recipient. These illustrate three phenomena which challenge current dogmas; an early resynthesis of DSA does not necessarily cause AMR, high levels of DSA can spontaneously modulate, and measurement of antibodies in terms of antigen specificity can give misleading results.

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## 1. Introduction

Combined liver–kidney transplantation is a well-described and relatively successful approach to transplant highly sensitised patients in the absence of an antibody compatible donor [1,2]. Pre-implantation of the liver protects the subsequent kidney transplant by adsorption of donor HLA-specific antibodies while the liver is resistant to immediate antibody mediated rejection. The fact that donor specific antibodies (DSA) can be depleted within minutes has been described in the literature [3–6], but what happens to DSA levels beyond the immediate post-transplant period remains largely unknown. With the advantages of contemporary bead assays and a novel approach to antibody characterisation we have investigated the dynamics of the antibody reduction and early resynthesis in a patient transplanted with combined liver–kidney against high levels of multiple Class I HLA specific antibodies.

## 2. Objectives and hypothesis

We have analysed specificities in terms of epitopes rather than antigens and provide a method for doing so. We hypothesised that this will reveal some unexpected and counter-intuitive aspects of an early donor-specific humoral response which may be generalisable in antibody incompatible transplantation.

## 3. Materials and methods

A 43-year-old lady with autosomal dominant polycystic kidney disease presented for combined liver and kidney transplantation. The recipient and donor HLA types were A23,30; B44,53; Cw6; DR11, 15; DQ 6,7; and A2,3; B7,60; Cw7,10; DR 8,15; DQ4,6; respectively. Immunosuppression consisted of CD25 monoclonal antibody induction, tacrolimus (0.3 mg/kg/day), mycophenolate mofetil (2 g/day) and methylprednisolone 500 mg at operation followed by oral prednisolone 20 mg enterally then tapering. Intravenous immunoglobulin (IVIG) was administered (0.5 g/kg/day) for the first five post operative days.

Pretransplant, antibody screening by single antigen bead assay (LSA104, One Lambda Inc, Canoga Park, CA) showed strong reactivity

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against multiple class I HLAs including subsequent donor mismatches with a calculated reaction frequency of 99%. There was weaker reactivity against a limited group of Class II HLA (DR7, DR9, DR52) but not against the donor's mismatches. The recipient had not received blood products or a previous transplant, and the assumed source of sensitisation was four pregnancies. Epitope specificities of the recipient's antibodies were deduced from the antigen specificities using the Matchmaker programme [7–10]. Epitopes are described in accordance with previously published notations [11,12] and our estimate of epitope reactivity in the recipient's sera is given in Table 1, showing which donor antigens carry the respective epitopes. All the Class I HLA-specific reactions of this serum, against both donor and non-donor antigens could be accounted for by this set of epitopes. One epitope, 80N is present on four of the mismatched antigens (B7, B60, Cw7, Cw10), and epitopes 253Q and 163E + 166E are each carried by two separate mismatched antigens (A2, Cw7 and B7, B60, respectively). Conversely, HLA A2 and B60 reactivities are likely due to two different epitope-specific antibodies in each case, and both B7 and Cw7 reactivities are due to three epitope-specific antibodies. Only Cw10 reactivity seems to be due to a single epitope-specific antibody. Monitoring antibody levels using HLA against which there are multiple antibodies (for example, B7 in this case, against which there are three epitope-specific antibodies) will measure a net effect, potentially obscuring changes in the individual antibodies. To track epitope-specific antibodies, we selected specific beads from the bead assay that carry non-donor HLA types and the minimum number of each of the epitopes, as shown in Table 1, and analysed these rather than the beads matching the donor HLA. We selected three beads that each carry a single epitope recognised by the patient's sera, although there were no beads to resolve 70Q from 80N, 253Q from 267Q or 142T from 253Q.

High levels of antibody can both saturate and inhibit these beads assays, exhibiting such phenomena as the high dose hook effect [13]. We therefore tested samples at neat and diluted (1:10) and observed the same patterns of antibody changes at each serum dilution. The results of testing at neat are presented.

#### 4. Results

The surgical procedures were uneventful and immediate liver and kidney graft function established. By post-operative day five the serum creatinine had fallen to 108 µmol/L, but then between days seven and ten this rose progressively to 197 µmol/L. A kidney biopsy undertaken on day nine displayed acute tubular injury with vacuolation, diffuse C4d staining, but no inflammatory infiltrate in interstitium, large vessels, peri-tubular capillaries or glomerulus (Fig. 1b,c). The tacrolimus level on the day of biopsy was 15 ng/ml (high performance liquid chromatography/tandem mass spectrometry). Considering this information a diagnosis of tacrolimus toxicity was made despite the biopsy data in isolation suggesting AMR. This was vindicated following tacrolimus dose reduction to give a level of 8 ng/ml three days later with subsequent improvement in renal function (day 13

**Table 1**

Summary of donor specific reactivity. Reactive epitopes are shown alongside the HLA mismatched donor antigen and the selected 'tracker' antigens on which they are expressed. \*The full description of this epitope is given as [41A + 46E + 67Y / 43P + 46E + 67Y / 43P + 46E + 70Q] / 43P + 69A + 70Q / 43P + 70Q + 76E / [46E + 65Q + 67Y / 46E + 65Q + 70Q] in El-Awar et al. [11]; we have abbreviated this to 70Q for ease of use.

HLA mismatch	Epitope identified	Tracker antigen
A2	142T	A69
	253Q	A25, A69
A3	None	None
B7	80N	B8, B42
	70Q*	B42
	163E + 166E	B47
	80N	B8 B42
B60	163E + 166E	B47
	80N	B8 B42
Cw7	253Q	A25, A69
	80N	B8 B42
	267Q	Cw17
Cw10	80N	B8 B42

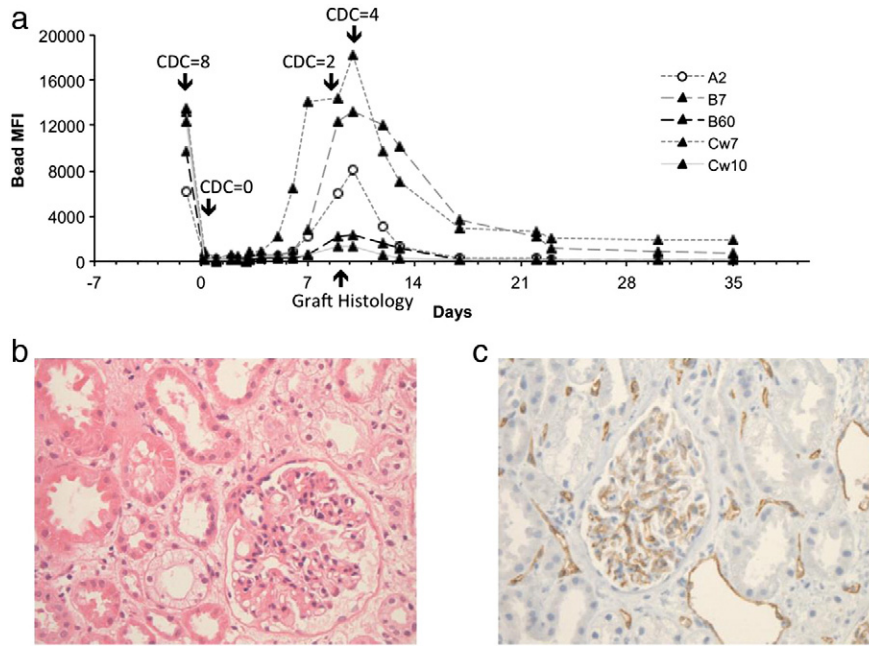
creatinine: 145 µmol/L; day 16 creatinine: 120 µmol/L). Her subsequent clinical course was uneventful with stable renal function 40 months post-transplantation (serum creatinine: 126 µmol/L; eGFR: 47 ml/min; urine albumin:creatinine ratio: 14.2 mg/mmol).

The patient's immediate pretransplant serum was reactive against all class I HLA mismatches except A3, giving a cumulative donor-specific reactivity corresponding to an MFI of 55,000 (sum of all DSA bead values). Forty minutes following liver arterial anastomosis (just prior to kidney implantation) a further serum sample was taken followed by daily samples for two weeks, then at weekly intervals. We noted the following changes in specific bead MFI values and these are shown graphically in Fig. 1a. The sample taken 40 min following liver transplantation showed a dramatic reduction of all detectable Class I HLA antibody specificities, including those against non-donor antigens. Thus all the patient's Class I HLA-specific antibodies were absorbed by HLA epitopes on the donor liver. The combined MFI values of the DSAs at 40 min is 1618, a 97% reduction of the pretransplant level, with all donor-specific reactions below the negative threshold. As serum dilution due to blood volume changes and perioperative transfusions also effect changes in antibody levels we used MFI values of the non-donor specific Class II HLA antibodies (DR7, DR9 and DR52) to estimate the magnitude of these factors. The combined MFI values against the DR7, DR9 and DRB52 beads in the pre-transplant serum are 3093. In the 40 minute sample the reactivity against these antigens is 1310, 42% of the pretransplant level. Thus around 58% of the immediate reduction in HLA specific antibodies can be seen to be due to non-specific factors. This is in very close agreement with that reported by Key et al. [3]. On day five reactivity against HLA Cw7 returned (MFI: 2150), and the remaining DSAs and non-DSAs reappeared on day six. Between days nine and ten all prior antibodies reached peak post-transplant levels after which they declined spontaneously, and all except those for Cw7 return to baseline 35 days post-transplantation. Retrospective cytotoxic crossmatches (complement-dependent cytotoxicity (CDC), without AHG augmentation) showed a strong reaction (8 score) with the pretransplant sample, and a negative reaction with the 40 minute sample. Finally, the specificities of the pre- and post-transplant antibodies are the same and differ only quantitatively.

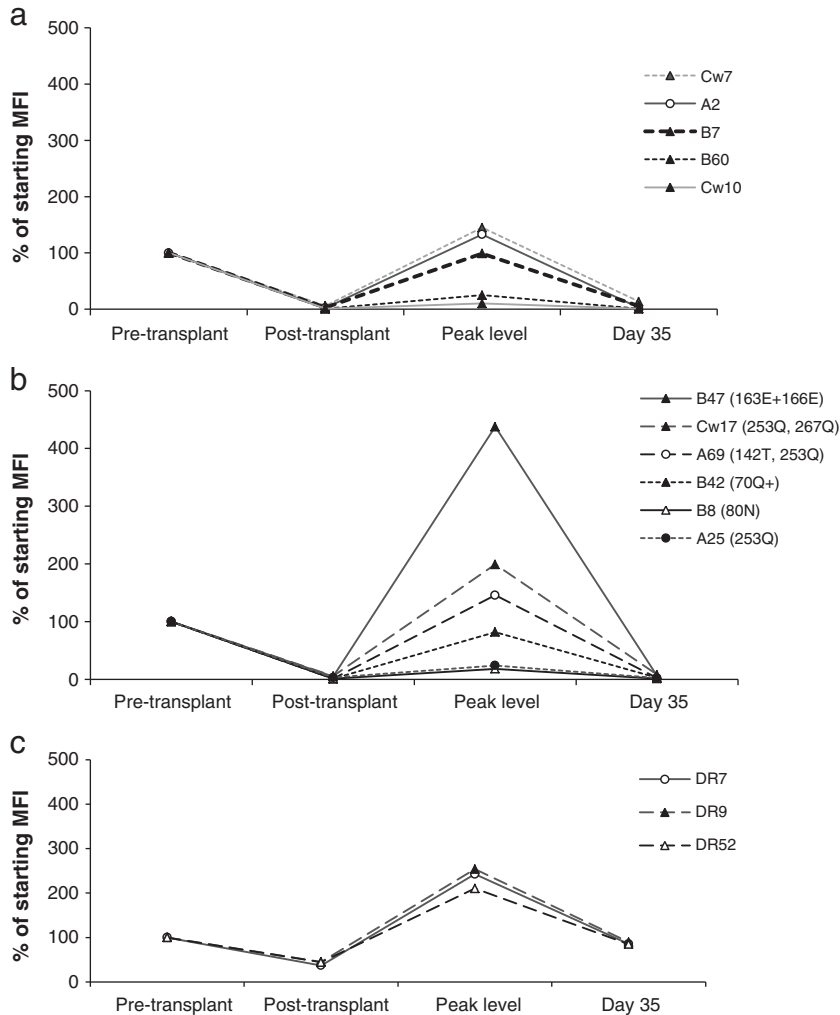
Overall there does not appear to be an early antibody response against any of the mismatched antigens as the levels do not significantly exceed pre-transplant levels (Figs. 1a and 2a). A different picture emerges if epitope reactivities are considered through the measurement of surrogate tracker antigens (Fig. 2b). It can be seen that the post-transplant response is marked for those reactions representative of donor-specific responses, with third party antibody levels (DR7, DR9 and DR52) showing markedly reduced increases post-transplant and lacking the dramatic fall from peak levels which typifies the donor-specific response (Fig. 2c). Fig. 2c also shows that the third party-specific antibodies return to pre-transplant levels by day 35 while all donor-specific antibodies fall to <10% of pre-transplant levels. We were able to resolve single epitope binding for three antibodies (Table 1). Most notably, the peak level of 163E + 166E-specific antibody was 440% of the pretransplant level (Fig. 2b). In contrast to this, the lowest proportionate antibody reappearances were seen for reactions against epitopes 253Q and 80N, at 10% and 20% of their pretransplant levels, respectively. Analysis of the remaining antibodies was confounded by only having access to beads carrying more than one of the relevant epitopes. Although not able to directly assess the response against 267Q, by using a Cw17 bead (253Q and 267Q) we found a 180% change from pretransplant to peak level and, as we know that the antibody against 253Q was greatly reduced, it seems clear that the increase against this Cw17 bead is due to increased reactivity against 267Q. Similarly the response against 142T is likely to be greater than the 150% when tracked with the A69 bead (142T and 253Q epitopes). The level of antibody against 70Q appears to be similar for pre-transplant and peak (80%) although this is confounded by 80N. As the peak, post-transplant reactivity against 80N was shown to be 20% of the pre-transplant level, 70Q reactivity also seems to be underestimated in the day ten sample.

#### 5. Discussion

Rapid HLA specific antibody depletion by an allogeneic liver is a well described phenomenon but the post-transplant serological changes during the following few days has not to our knowledge been investigated previously. We have exploited this setting to describe the dynamics of antibody resynthesis and when approached from the perspective of epitope-reactivity dramatic responses are evident which are not apparent when looking at whole antigen-specific responses. While monitoring an anti-donor response against donor antigens has the benefit of potentially measuring the total load of antibody against each antigen, this can hide the magnitude of an individual antibody response. In this case there was no evidence of an early anti-donor humoral response when conventional, donor HLA-specific antibodies were considered. Measuring antibody levels on a bead carrying non-donor HLA (B47) revealed nearly a five fold increase in antibodies against the epitope [163E + 166E] carried by donor antigens B7 and B60. This rise in antibody, significantly above pre-transplant levels and proportionately greater than that of a third party antibody (DR9) is evidence of a specific anti-donor immune response, similar to that described previously in antibody incompatible kidney transplantation [14].



**Fig. 1.** a). Changes in donor reactive bead MFI values through the early pre- and post-transplant periods; transplant on day 0. b,c) Kidney graft histology at day 9 post transplantation. b) H&E staining showing vacuolation of occasional proximal tubules without cellular infiltrate; c) C4d immunohistochemistry showed diffuse linear staining of glomerular and peritubular capillaries.



**Fig. 2.** Changes in donor HLA antigen reactivity (a), donor HLA epitope reactivity (b), third-party HLA reactivity (c) at key peri-transplant times. In each case reactivity is given relative to the immediate pre-transplant MFI levels (100%).



Pre-transplantation there is likely to be an equilibrium between antibody synthesis and catabolism. Post-transplantation the equilibrium will also involve adsorption on to the donor HLA and the effects of immunosuppression. The 5–6 day lag before DSAs became detectable suggests a time taken to saturate donor HLA. During this lag phase it is uncertain whether antibody synthesis was attenuated or maintained at pre-transplant rates. The third party, Class II HLA specific antibodies had recovered to pre-transplant levels by day two which suggests the latter: the difference between DSA and non-DSA levels being specific adsorption. There may be differential rates of adsorption of the DSAs and this might be best explained if epitope specificities are considered because the relative number of each epitope on the donor can be determined. There are four antigens (B7, B60, Cw7, and Cw10) carrying the 80N epitope (as shown in Table 1) which would enhance adsorption and therefore cause the low peak level of the corresponding antibody. Two donor antigens carry 253Q (A2 and Cw7) and the peak post-transplant level of this antibody was also low but this was also helped by the pre-transplant level being only about 25% that of the 80N-specific antibody. Epitopes 142T, 70Q, and 267Q are each represented on a single donor HLA and the peak levels of their corresponding antibodies were similar to the pre-transplant levels implying less effective adsorption although their measurements were confounded. The exception is the high peak level of the [163E + 166E]-specific antibody, represented on two donor HLA (B7 and B60), but it is clear that this is due to an increase in the rate of synthesis.

Thus, although overall the pre-transplant and peak post-transplant levels were broadly similar, the pattern of the epitope-specific antibodies changed dramatically. These changes also coincided with changes in serum cytotoxicity in a complement-dependent assay. The pre-transplant serum was strongly cytotoxic (killing score 8), but at post-transplant days 9 and 10, the cytotoxicity was noticeably lower with killing scores of 2 and 4 respectively (shown in Fig. 1a). At this point serological reactivity was dominated by antibodies against [163E + 166E] together with reactivity against 267Q and 142T, and significantly depleted levels against 80N and 253Q. Perhaps the change in cytotoxicity reflects the change in antibody composition of the sera rather than a change in antibody level. Alternatively each antibody might differ intrinsically in their pathogenicity. We did investigate IgG subclass composition of the A2, B7, and Cw7 specific antibodies but found differences neither between the specificities nor between samples at pre-transplant or post-transplant antibody peak (data not shown). Serum creatinine did rise with the post-transplant rise in donor-specific antibodies but tacrolimus levels were above target, and tacrolimus nephrotoxicity was implicated, with an improvement in graft function following tacrolimus dose reduction and subsequent stability over considerable follow-up. High levels of serum DSA following early resynthesis were therefore seemingly insufficient to cause rejection of the kidney. Although it is tempting to speculate that this is a protective effect of the liver, this phenomenon has also been described in more conventional HLA antibody incompatible kidney transplantation [15].

The final phenomenon revealed by daily sampling and antibody testing during the first few post-transplant weeks is the rapid rise followed by a seemingly spontaneous fall in antibody levels. IVIg was administered daily for the first five post-operative days, with the rationale being to effectively 'mop up' any remaining antibody that may not have been absorbed by the liver, and as an extra precautionary measure given that Olausson et al. reported the loss of 2/7 kidney transplants in an earlier study [2]. It is possible that IVIg treatment at this time may have interfered with the bead assay readout, but a recent study on the effects on IVIg on single antigen bead assays concluded that any reduction in MFI is moderate, with a median MFI reduction not greater than 1100 [16]. In addition, given that previous studies have reported the same falls in DSA to baseline levels we feel it is justified to conclude that the disappearance of antibody is due to genuine adsorption by HLA expressed on the liver and kidney [3–5]. Crucially, no treatment such as IVIG, rituximab or bortezomib, was used to modulate the

resynthesis of the DSA following its reappearance at around day 5, yet by post-operative day 35 most of the antibodies had all but disappeared. The cause of this modulation is unknown but again this is not unique to combined liver–kidney transplantation, having been described in antibody-incompatible transplantation following extracorporeal antibody removal [17].

In summary, this case provided an opportunity to investigate a novel approach to characterise and monitor HLA-specific antibodies. Antibody characterisation in terms of epitope specificity is potentially more accurate and informative than antibodies described as "antigen-specific" and we suggest a method for identifying and tracking these antibodies—i.e. follow the epitope reactions and not the antigen reactions. We contend that this will give insight into the behaviour and pathogenicity of HLA-specific sera. In the case presented here this approach has revealed some novel features of the post transplant antibody response in a sensitised recipient. These illustrate three phenomena that challenge current dogmas—an early resynthesis of DSA does not necessarily cause antibody-mediated rejection, high levels of DSA can spontaneously modulate, and measurement of antibodies in terms of solely antigen specificity can give misleading results.

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