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CASE REPORT

Four-year allograft survival in a highly sensitized combined liver–kidney transplant patient despite unsuccessful anti-HLA antibody reduction with rituximab, splenectomy, and bortezomib

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The authors have no conflict of interest.

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Summary

Although donor-specific lymphocytotoxic antibodies are regarded as a contraindication for kidney transplantation (KTx), the data available for liver or combined liver or kidney transplantation (cLKTx) are scarce. Here, we report a case of a highly sensitized young man receiving his sixth liver and second kidney graft. Multiple anti-HLA antibodies were present at the time of transplantation. As a result of suspected antibody-mediated graft damage, the patient was treated with rituximab, plasmapheresis, intravenous immunoglobulins, splenectomy, and bortezomib to decrease the antibody production. So far, patient and allograft survival has reached 4 years despite failure to achieve a permanent reduction of anti-HLA antibodies, and particularly nondonor directed antibodies.

Introduction

Anti-HLA antibodies (Abs) are associated with an inferior graft survival after kidney transplantation (KTx) [1,2]. Although a positive complement-dependent cytotoxicity (CDC) crossmatch is considered as a contraindication for KTx, HLA matching or preoperative crossmatching is not routinely performed before liver transplantation (LTx). In case of combined liver and kidney transplantation (cLKTx), a crossmatch is not performed on a regular base. It is hypothesized that a kidney allograft is protected from antibody-mediated graft damage by rapid adsorption or neutralization of alloantibodies by the liver allograft [3], and hence a positive CDC crossmatch does not represent a contraindication [4]. Nevertheless, there is rising evidence of a negative impact on long-term graft survival of such antibodies on both, the kidney and liver graft [5–8].

Here, we report the case of a highly sensitized 28-year-old man who received his sixth liver and second kidney allograft 4 years ago and our futile efforts to reduce anti-HLA Abs applying all immunosuppressive options (anti-CD20 Ab rituximab, plasmapheresis, splenectomy, intravenous immunoglobulins (IVIg), and proteasome inhibitor bortezomib) impacting the B-cell system.

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Patient and methods

The patient received a liver graft for acute liver failure in 1991 at the age of 10 years. The graft functioned well until 2003. Following retransplantation, the second graft was lost with arterial and bile duct complications in 2005 as was the third graft in 2006, a fourth graft from a living donor was lost in 2007. A fifth graft transplanted in 2007 combined with a first KTx because of calcineurin inhibitor (CNI) induced chronic renal failure failed in 2009. The histology of the explanted organs revealed chronic rejection with focal fibrosis and cholestasis of the liver and interstitial fibrosis and tubular atrophy of the kidney. The patient received a sixth LTx and second KTx without prior crossmatch, with complete HLA mismatch and multiple repeat mismatches (Table 1). The transplantation was a surgical challenge resulting in an operation time of more than 8 h and a laparostoma requiring several revisions. The initial immunosuppression (IS) consisted of ATG (Thymoglobulin®, Genzyme, Germany) on day 0 and 2, Tacrolimus (Prograf®, Astellas, Germany) and steroids.

Serum of the patient was collected in 2009 pre- and post-operatively around the cLTX to evaluate the presence of anti-HLA Abs by single antigen assays (One Lambda Inc., LABScreen® Single Antigen assays, Canoga Park, CA, USA). Potentially donor directed Abs (pDDA) were defined positive with raw mean fluorescence intensity (MFI) >1500. A high resolution HLA-typing of the current donor was performed retrospectively to verify donor specificity.

B-lymphocyte counts in the peripheral blood were monitored by flow cytometry on a FACS Canto (BD Bioscience, San Jose, California, USA) using monoclonal antibodies against CD19 and CD20 (4G7 and L27, both BD Bioscience) as previously described [9].

Results

Antibody screening was performed immediately after transplantation and revealed retrospectively a positive CDC crossmatch and multiple preformed pDDA. Although HLA-I pDDA B60 decreased rapidly after transplantation, HLA-DQ5 Abs and antibodies against previous grafts (e.g. HLA-A24) were still detectable with MFI > 1500 (Fig. 1, week 1). Therefore, a single dose of rituximab (375 mg/m^2, MabThera®, Roche, Germany) was administered at day 5 post-transplant and plasmapheresis (plasma exchanged 2.5–3 times the estimated plasma volume during each treatment session) was initiated at day 9 after cLKTx for nearly 4 weeks. Figure 1 (week 2 and 3) demonstrates that neither Rituximab nor plasmapheresis had an effect on nondonor directed HLA-Abs (e.g. HLA-A24), but successfully depleted B-cells to <0.5% of peripheral blood lymphocytes (PBL) (not shown).

Table 1. Upper panel: HLA typing of recipient and donors. Lower panel: raw MFI of pDDA and non-DDA pretransplant, 1, 2 and 3 years after the sixth liver and second kidney transplant. Antibodies against HLA-C and DP were absent.

	A	B	DR	DQ
Recipient	**1**	**8**	**3**	**2**
1. LTX	26,33	35	4,11	7,8
2. LTX	24	35,62	4,13	6,8
3. LTX	3	56	1,15	5,6
4. LTX	2,31	7,60	9,13	6,9
5. LTX, 1. RTX	1	8,57	3,4	2,3
6. LTX, 2. RTX	**2,3**	**18,60**	**1,4**	**5,8**

Mismatched HLA-antigen *	Pre Tx **	+1 year	+2 years	+3 years
A2(0201)	234	79	205	159
A2(0206)	**5937**	249	307	182
A3	1058	652	1569	807
A24	**7381**	**6299**	**13648**	**7632**
A26	**4166**	132	195	110
A31	**7280**	871	*1552*	644
A33	**4537**	112	175	94
B7	**7875**	868	*1641*	963
B18	205	120	241	87
B35	**4003**	540	911	413
B56	**3888**	1088	*1884*	1318
B57	68	64	115	102
B60	**5827**	104	122	85
B62	*1550*	284	662	387
DR1	**2141**	207	433	852
DR4	166	244	278	281
DR9	**5496**	358	332	238
DR11	624	193	214	207
DR13	197	138	95	79
DR15	*2229*	499	**2646**	891
DQ5	*2281*	**4502**	**7840**	**8736**
DQ6	**9595**	**7348**	**9296**	**7731**
DQ7	*1881*	**2763**	**7811**	**7477**
DQ8	**10352**	435	1104	*1518*
DQ9	**7606**	461	1198	*1601*

*Mismatched HLA-antigens of the current organs in bold, repeated mismatches underlined.

**Positive MFI > 2500 in bold, weakly positive 1500–2500 in italics, negative <1500.

Two weeks after cLKTx, the patient developed cholestasis and bile duct necrosis requiring surgical revision. No acute cellular rejection (ACR) was observed in previous liver biopsies, and arterial perfusion was uneventful throughout the postoperative period. Therefore, an antibody related, i.e. humoral rejection was suspected. Apart from continuing plasmapheresis no change in the immunosuppression was done, based on the medically critical situation of the patient. Because of the increasing titers of HLA-DQ5 pDDA and lack of response to treatment, a splenectomy was performed 4 weeks after cLKTx under the hypothesis to effectively reduce antibody production and stopping the

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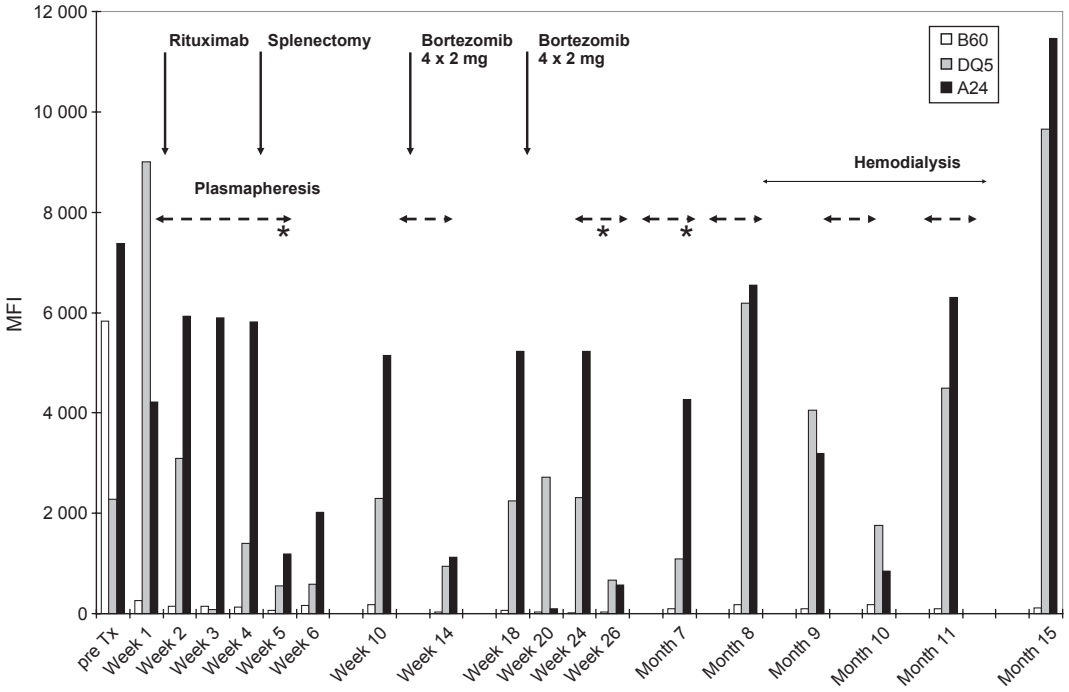

Figure 1 pDDA, mean fluorescence intensity (MFI) during the first 15 month post-transplantation. B60 and DQ5: representative anti-HLA class I and II Abs potentially directed against the current grafts. A24: representative anti-HLA class I Abs against previous grafts. * = Marks the application of 3 × 500 mg steroids because of biopsy proven acute rejection.

antibody-mediated rejection (AMR). Despite the success of splenectomy regarding reduction in HLA-Abs (Fig. 1, weeks 5 and 6), ACR of the liver (Banff 5/9) was diagnosed in a biopsy 1 week later. As a consequence, high-dose steroids and IVIg were given. Plasmapheresis was restarted and everolimus (Certican®, Novartis, Switzerland) was added to the dual IS regimen with low-everolimus trough levels (3–5 ng/ml). Despite long-term treatment on the intensive care unit and multiple operations, kidney function normalized to a s-creatinine <1 mg/dl. Three month after transplantation, liver enzymes and s-creatinine increased. As shown in Figure 1, week 10 HLA-DQ5 pDDA reappeared. A CDC crossmatch was positive at this time point. Because of suspected AMR of both liver and kidney, a first cycle of the proteasome inhibitor bortezomib (4 × 2 mg Velcade®, Janssen Cilag, Germany) was started and plasmapheresis was performed for 2 weeks. Renal function stabilized, liver function tests normalized and antibodies decreased (Fig. 1, week 14).

The patient was discharged 4 months after transplantation with a s-creatinine of 2.1 mg/dl. He was readmitted 3 days later with rising γGT and s-creatinine (2.4 mg/dl). Kidney biopsy revealed again ACR. IS was adjusted to everolimus and tacrolimus trough levels of 5–8 ng/ml and a second cycle of bortezomib was administered because of the reappearance of Abs (Fig. 1, week 18). Plasmapheresis was restarted. In addition, the patient received high-dose steroids two times within the next month without significant improvement of renal function.

A kidney biopsy performed 8 months post-transplantation as a result of a second rise in s-creatinine (>4 mg/dl) showed thrombotic glomerulopathy and preglomerular vasculopathy and was interpreted as CNI induced HUS. CNI was discontinued and MPA added (Myfortic®, Novartis, Switzerland) to everolimus. Unfortunately, the biopsy was complicated by bleeding with haematuria and urinary bladder tamponade resulting in kidney failure. The patient had to be discharged on dialysis, at that time with adequate liver function. HLA-DQ5 pDDA and nondonor directed Abs were both present in the patient's sera with MFI > 6000 (Fig. 1, month 8). B-cell counts were still below 0.5% of PBL (not shown).

The patient refused chronic plasmapheresis and continued outpatient treatment at a different dialysis center. Dialysis could be stopped after 3 months with acceptable kidney function (GFR MDRD 37 ml/min) and normal liver values despite high-MFI pDDA (Fig. 1, month 15).

Within the next year, the patient was hospitalized two times because of gastrointestinal bleeding and gastroenteritis with everolimus overexposure, but without a rejection episode. Four years after transplantation, the patient is alive with normal liver function and a stable GFR of 23 (3 years) and 28 (4 years) ml/min (MDRD) on a CNI-free immunosuppressive regimen (MPA, everolimus, and steroids).

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Table 1 demonstrates MFI of anti-HLA Abs against previous grafts and the current graft 1, 2 and 3 years after cLKTx. It is remarkable, that besides donor-directed Abs, several other antibodies directed against previous grafts (e.g. HLA-A2 (0206), HLA-A26) are cleared, while donor-directed HLA-DQ5 Abs increased and HLA-DQ8 Abs are diminished.

Discussion

Reports on the impact of donor directed anti-HLA Abs in cLKTx are controversial [5,6,8,10,11]. Because of growing evidence of AMR in KTx, we regard the Abs detected in our patient by CDC crossmatch and single antigen assays as clinically relevant. We assume that anti-HLA class I Abs against the current grafts were rapidly absorbed by the liver [11,12] as they disappeared in patients sera immediately after cLKTx. This is in accordance with a report of Dar *et al.* [13] demonstrating a clearing of anti-HLA class I Abs by the graft(s).

HLA class I is expressed on all cell types and is upregulated on hepatocytes after transplantation, whereas class II is expressed on Kupffer cells, interstitial cells, and endothelia only and is upregulated on bile ducts after transplantation [14]. This might explain the preferential absorbance of class I Abs by liver grafts.

In our patient, HLA-DQ5 pDDA were not cleared. HLA-DQ antigens seem to be weakly expressed on bile ducts [15], nevertheless HLA-DQ Abs are reported to be associated with inferior outcome after KTx [16] and Dar *et al.* report a high rate of AMR of the kidney in patients with HLA class II Abs after cLKTx. Furthermore, the prevalence of cytotoxic donor-specific Abs was confirmed by a positive CDC crossmatch prior and post-cLKTx in our patient. It is therefore assumed that the patient needs continuous treatment, especially as there is no clear cut-off level using single antigen tests to define the absence of harmful donor-directed Abs [17–19].

Reports on successful treatment of AMR are available for all applied therapies, i.e. rituximab [20–22], splenectomy [23], and bortezomib [24–26]. The mechanism of action of rituximab does not involve immediate antibody reduction as plasma cells are CD20 negative [27]. Consequently, rituximab has no effect on nondonor directed Abs (e.g. A24) in our patient while splenectomy was highly effective reducing all Abs for about 4 weeks. Proteasome inhibition is reported to cause plasma cell apoptosis [28], therefore our hope was that this treatment would lead to a long lasting reduction in Ab production. Unfortunately this was not the case, although bortezomib reduced Abs partially and liver enzymes and kidney function improved.

It could be discussed that an ongoing treatment with bortezomib and/or plasmapheresis may have caused a longer lasting effect on Ab production, but this approach was refused by the patient.

The initial decrease in antibodies in this patient is most likely contributed to two effects: first the blood loss during the reoperation and second an antibody absorption by the liver graft. Although the liver graft permanently cleared several donor directed Abs especially against HLA class I antibodies against HLA class II (e.g. HLA-DQ5) were only transiently reduced, which might be explained by the different expression and/or secretion of HLA class I and class II molecules by the grafted organs.

Antibodies against previous grafts were resistant to all our therapeutic efforts. Despite successful depletion of B-cells in the peripheral blood by rituximab, splenectomy, and bortezomib succeeded only for a limited time space in reducing anti-HLA Abs. The CNI-free immunosuppressive regimen consisting of MPA and everolimus did not contribute to a discontinuation of anti-HLA Ab production. Nevertheless, it might be owed to our aggressive but specific immunosuppressive regimen that both organs survived for at least 4 years.

From the case presented here, we learned that (i) the relevance of potentially donor-directed Abs detected by single antigen assays needs to be better understood; (ii) in highly sensitized LTx and cLKTx patients immunological risk stratification should be taken more seriously (iii) none of the currently available therapeutic approaches succeeded in achieving long-lasting down regulation of preformed anti-HLA Abs.

Neither crossmatch nor Ab monitoring is yet part of the evaluation process in LTx. Unfortunately, given the current allocation procedure and cold ischemia time challenges, such an endeavor might face unsurmountable impediments.

Authorship

MK: wrote article, collected data, analyzed data, performed study, designed study. CG: wrote article, collected data. LA: wrote article, analyzed data. JMP, MV: performed study. NK: analyzed data, designed study. FT, TE, BN: analyzed data, designed study, performed study.

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