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LETTER TO THE EDITORS

Salvage therapy for refractory rejection and persistence of donor-specific antibodies after intestinal transplantation using the proteasome inhibitor bortezomib

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With a high risk for cellular and humoral alloimmune responses and no reliable noninvasive rejection markers, intestinal transplant (ITX) recipients require a close post-transplant monitoring. Graft biopsies are performed regularly, but their restriction to the mucosal component imposes limitations in the evaluation of processes occurring in deeper layers of the intestine [1]. C4d staining is not established and its interpretation varies largely in ITX recipients [2]. As a result of a significant association between the early appearance of anti-HLA antibodies and acute rejection episodes, frequent donor-specific antibody (DSA) screening is crucial and may disclose antibody-mediated rejection (AMR) [3].

In addition, AMR is less responsive to anti-rejection treatment, entailing chronic manifestations of graft rejection and allograft losses [4]. Even though plasmapheresis and rituximab reduce the concentration of circulating HLA antibodies, both methods are ineffective against antibody-producing plasma cells, which belong to the bone marrow $CD138^{+}CD20^{-}$ long-lived plasma cells compartment. Consequently, splenic and bone marrow plasma cells do not respond to typical desensitization regimens [5]. Proteasome inhibitors like bortezomib deplete non-malignant plasma cells in experimental models [6]. The immune-modulating effects include activity against normal plasma cells and suppression of T-cell function [7], providing effective treatment of antibody-mediated and acute cellular rejection (ACR) [8]. Preliminary reports have provided evidence of its efficacy in the reduction and elimination of DSA after renal, pancreas, and multivisceral transplantation representing an alternative treatment strategy for AMR [9–11].

We report the successful salvage treatment with bortezomib in a patient with refractory acute rejection associated with persisting DSA levels after ITX. A 46-year-old patient received an isolated intestinal graft for ultra-short bowel syndrome. Initial immunosuppression consisted of tacrolimus (trough levels 15–20 ng/ml) and steroids (40 mg/day). Induction therapy consisted of thymoglobulin [total dose 7.5 mg/kg bodyweight (BW)] and infliximab (5 mg/kg BW single dose), of which the latter is regularly applied to mitigate ischemia/reperfusion injury and to deplete effector memory $CD8^{+}$ T cells [12,13]. Graft biopsies were performed every 2 days.

The HLA antibody status was assessed using a combination of tests including complement-dependent lymphocytotoxicity test (CDC) (Biotest, Dreieich, Germany) and the LABScreen^{TM} test (One Lambda, Canoga Park, CA, USA). Sera were screened for lymphocytotoxic HLA antibodies by CDC according to the protocol of the National Institute of Health. To differentiate between specific HLA-IgG antibodies and non-HLA-specific IgM antibodies, the CDC test was performed in parallel by adding dithiothreitol (DTT) to reduce the non-HLA-specific IgM antibodies. For the detection and specification of panel reactive HLA antibodies (PRA), a lymphocyte panel of HLA-typed blood donors was used.

On postoperative day (POD) 14, DSA testing revealed high levels of anti-donor HLA DQ7 (2000MFI) and DQ8 (900MFI) antibodies, together with nondonor-specific antibodies (NDSA), entailing immediate plasmapheresis (Fig. 1). DQ4 and DR53 were immunodominant NDSA. On POD 19, the patient experienced a mild ACR and received steroid pulse therapy (5×1000 mg) and rituximab (375 mg/m^2), which decreased histological rejection signs. Five days later, rejection relapsed to grade 1 (Fig. 2 a) and DSA levels continued to rise, so that thymoglobulin (1.5 mg/kg BW for five consecutive days) and intravenous immunoglobulin (i.v. Ig; total: 1 mg/kg/BW) were initiated together with a second dose of rituximab. By POD 42, the patient had received three cycles of plasmapheresis (five applications on five consecutive days each) and two applications of rituximab, but DSA testing still revealed high levels of DSA (DQ7: 4500MFI; DQ8: 1500MFI). Allograft biopsies displayed persistent inflammatory signs, low-grade fibrosis, cryptitis, and an increased rate of apoptoses (up to six apoptotic bodies/10 crypts) defined as indeterminate for rejection (Fig. 2b). C4d staining revealed inconclusive results. The patient complained of diarrhea, abdominal distension, and pain. The persistence of DSA level, histological changes, and clinical signs indicative of an ongoing AMR stimulated discussion about

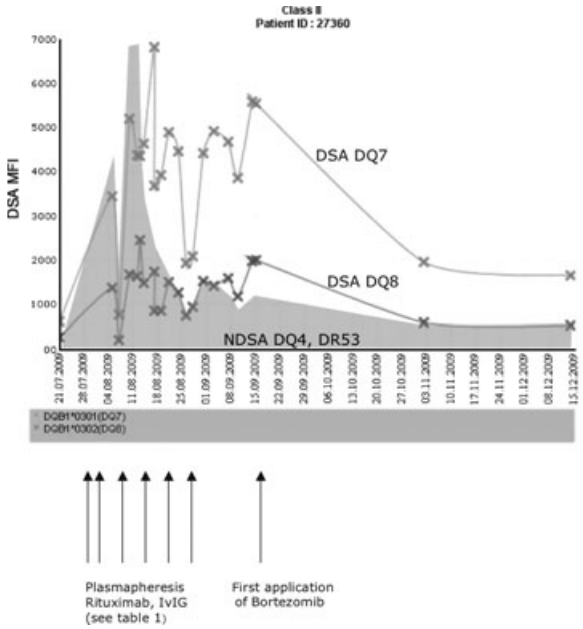

Figure 1 Post-transplant course of donor-specific (DSA) and immunodominant nondonor-specific antibodies (NDSA). The DSA specificities DQ7 and DQ8 are represented over time. NDSA specificities, i.e. DQ4 and DR53 are represented in the background curve.

therapeutic alternatives. Because of suspected AMR, we initiated bortezomib on POD 62 in standard labeled dosage (1.3 mg/m^2/POD 62, 65, 69, 72).

The HLA antibodies were screened before the first bortezomib application and thereafter on a weekly basis. In addition, we performed graft biopsies twice a week and laboratory tests including platelet count. One week after the last application of bortezomib, biopsy specimen disclosed resolution of antibody-mediated graft injury and there was a significant reduction in DSA. There was no documented infection, nausea or peripheral neuropathy, and platelet count remained stable upon bortezomib treatment. Today, anti-HLA antibodies are almost entirely eliminated and there is a complete resolution of inflammatory graft alteration under tacrolimus (trough levels 4–5 ng/ml) and mycophenolate mofetil (2 × 1000 mg).

As a result of persisting DSA levels despite exhaustive treatment, histological signs of chronic allograft alterations (i.e. fibrosis) impended to result in chronic allograft rejection.

Antibody-mediated rejection after ITX is inadequately characterized. There is evidence for a significant association between vascular injury and a significant peak of panel reactive antibodies early after transplantation, which may represent a form of acute vascular rejection and is accompanied by poor graft survival [2]. Bortezomib has been reported to successfully reduce or eliminate DSA after transplantation [5,11,12] and showed the same effect in our patient. One study described ineffectiveness of bortezomib as a sole desensitization agent in patients with subclinical and subacute AMR more than 1 year after kidney transplantation [14]. Among other suggestions for its ineffectiveness, authors point out the late timing of application. Recent priming of B cells or recall stimulation of memory B cells shortly after transplantation may generate short-lived plasma cells with a high DSA production, which might be more susceptible to proteasome inhibition-induced apoptosis. This is further supported by recently published results, reporting success with a proteasome inhibitor-based combination therapy as primary treatment in early acute AMR [15]. Bortezomib targets antibody-producing plasma cells, but not circulating antibodies, which have already caused graft injury; circulating antibodies still need to be eliminated by plasmapheresis and rituximab.

We applied bortezomib as a rescue therapy in a highly endangered patient with persistent DSA levels and ongo-

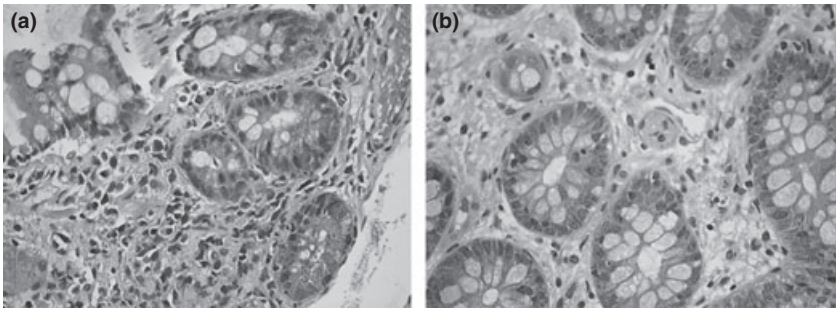

Figure 2 (a) Relapse of mild acute rejection after steroid pulse therapy with shortening of villi, mild to moderate inflammatory infiltrate, cryptitis, accumulation of activated intraepithelial lymphocytes, decreased epithelial cell height, nuclear enlargement and hyperchromasia, and up to 12 apoptoses/10 crypts. (b) Persistent alterations consistent with indeterminate for rejection after three cycles of plasmapheresis and two applications of rituximab; localized infiltrates of activated and blastic lymphocytes and some eosinophilic granulocytes, occasionally infiltration of lymphocytes into crypts, and degenerative alterations to the epithelium with shifted nucleus–plasma relation as well as elevated mitotic activity. Less than six apoptotic bodies/10 crypts.

ing graft injury in the early phase after ITX. The treatment was well tolerated. In this setting, the use of bortezomib as an adjunct agent to plasmapheresis, i.v. Ig, and rituximab might be a new treatment option for AMR after ITX.

Disclosure

We declare that there are no conflicts of interest.

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References

1. Ruiz P, Garcia M, Pappas P, *et al.* Mucosal vascular alterations in isolated small-bowel allografts: relationship to humoral sensitization. *Am J Transplant* 2003; **3**: 43.
2. Troxell ML, Higgins JP, Kambham N, *et al.* Evaluation of C4d staining in liver and small intestine allografts. *Arch Pathol Lab Med* 2006; **130**: 1489.
3. Lee PC, Zhu L, Terasaki PI, Everly MJ. HLA-specific antibodies developed in the first year posttransplant are predictive of chronic rejection and renal graft loss. *Transplantation* 2009; **88**: 568.
4. Celik A, Saglam F, Cavdar C, *et al.* Successful therapy with rituximab of refractory acute humoral renal transplant rejection: a case report. *Transpl Proc* 2008; **40**: 302.
5. Perry DK, Pollinger HS, Burns JM, *et al.* Two novel assays of alloantibody-secreting cells demonstrating resistance to desensitization with ivIG and rATG. *Am J Transplant* 2008; **8**: 133.
6. Everly JJ, Walsh RC, Alloway RR, *et al.* Proteasome inhibition for antibody-mediated rejection. *Curr Opin Organ Transplant* 2009; **14**: 662.
7. Wang X, Luo H, Cehn H, *et al.* Role of proteasomes in T-cell activation and proliferation. *J Immunol* 1998; **160**: 788.
8. Perry DK, Burns JM, Pollinger HS, *et al.* Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. *Am J Transplant* 2009; **9**: 201.
9. Govil A, Walsh RC, Tevar A, *et al.* Bortezomib-based treatment of antibody mediated rejection in pancreas allograft recipients. *Clin Transpl* 2009; 443.
10. Island ER, Gonzalez-Pinto IM, Tsai HL, *et al.* Successful treatment with bortezomib of a refractory humoral rejection of the intestine after multivisceral transplantation. *Clin Transpl* 2009; 465.
11. Everly MJ, Everly JJ, Susskind B, *et al.* Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 2008; **86**: 1754.
12. Reutzel-Selke A, Hartmann J, Pratschke J, *et al.* Cold ischemia does not interfere with tolerance induction. *Transplantation* 2009; **87**: 1116.
13. Miller EA, Ernst JD. Anti-TNF immunotherapy and tuberculosis reactivation: another mechanism revealed. *J Clin Invest* 2009; **119**: 1079.
14. Sberro-Soussan R, Zuber J, Suberbielle-Boissel C, *et al.* Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. *Am J Transplant* 2010; **10**: 681.
15. Walsh RC, Everly JJ, Brailey P, *et al.* Proteasome-inhibitor based primary therapy for antibody-mediated renal allograft rejection. *Transplantation* 2010; **89**: 277.