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Belatacept Treatment of Recurrent Late-onset T Cell–mediated Rejection/Antibody-mediated Rejection With De Novo Donor-specific Antibodies in a Liver Transplant Patient

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Background. T cell–mediated rejection that appears and persists late after transplantation is often associated with development of de novo donor-specific antibodies. Treatment of this condition often presents a conundrum because of the uncertainty regarding the trade-off between immunosuppression-related toxicities/complications and restoration of allograft function and structure. **Methods.** Herein, we report an illustrative case of a young 20-y-old otherwise healthy woman who underwent liver replacement for Alagille's syndrome from an ABO-compatible, 6 antigen-mismatched crossmatch-negative 24-y-old man. Although triple baseline immunosuppression was used (tacrolimus, mycophenolate mofetil, and prednisone), she developed rejection 3 d after liver replacement. Despite verified continual immunosuppression compliance, 1.5 y after liver replacement she experienced 6 more rejection episodes over the following 18 mo and development of de novo donor-specific antibody. **Results.** Treatment with belatacept began 3.5 y after transplantation, normalizing her liver tests with no further rejections. A biopsy obtained 6 y after transplantation (postoperative day 2221) was normal, appearing without inflammation or residual fibrosis. **Conclusions.** Belatacept may be a useful treatment approach in this setting.

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INTRODUCTION

In contrast to early acute liver allograft T cell–mediated rejection (TCMR), late-onset and persistent TCMR (1) is frequently, but not invariably, observed in patients noncompliant with immunosuppression[1]; (2) represents a more substantial treatment challenge; (3) is frequently associated with de novo donor-specific antibody (DSA) development; and (4) generally results in a higher incidence of adverse outcomes.[2-4] Additionally, young adults (10–24 y old) have a significantly higher incidence of late TCMR than controls.[5]

Current treatment recommendations for late-onset/persistent TCMR generally include an algorithmic stepwise approach: (1) corticosteroids; (2) increased calcineurin inhibitors; (3) calcineurin inhibitor conversion (eg, from cyclosporine to tacrolimus); (4) addition of an antimetabolite (azathioprine or mycophenolate); and (5) antilymphocyte antibody therapy.[2-4] One group even resorted to local allograft irradiation.[6] These suggested approaches, however, may lead to enhanced immunosuppression-related complications such as malignancies and infections, which are the major causes of morbidity and mortality in adult liver allograft recipients.[7] Even with aggressive therapy, outcomes are still suboptimal in such patients. Therefore, alternative treatment approaches are needed to escape the dichotomy of persistent rejection versus toxicity that plagues many long-term organ allograft survivors.[7,8] Other approaches such as intravenous immunoglobulin, apheresis, proteasomes, and complement inhibitors have not proven to permanently change a B-cell/T-cell response.

The patient described here received treatment in August 2013 after diagnosed with a third rejection with 2 doses

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of methylprednisolone and 3 doses of rituximab (Rituxin, Genentech). The same treatment was administered after the forth rejection (November 2014), after B-cell and complement component 4d (C4d) staining had been noted, with no effect. The effectiveness of belatacept in the kidney trials[9] on TCMR and on prevention of de novo antibody formation with 7 y of follow-up prompted a treatment trial in this patient.

CASE REPORT

A 20-y-old woman underwent liver replacement because of biliary-type fibrosis/cirrhosis secondary to Alagille's syndrome. The donor was a blood group–identical 24-y-old male victim of a traffic accident. There was a 6 human leukocyte antigen mismatch, but a flow cytometric crossmatch was negative without preformed anti-class I or class II human leukocyte antigen antibodies (DSA negative by a solid phase assay). Baseline immunosuppression consisted of tacrolimus, mycophenolate mofetil, and prednisone (Figure 1). The first rejection episode, diagnosed by biopsy on postoperative day (POD) 3, showed typical TCMR-related changes (rejection activity index 4/9) (Table 1). An unsatisfactory response to a steroid cycle (1 g, methylprednisolone with a 6 d prednisone taper 200 to 30 mg/d [recycle]) led to a repeat biopsy on POD 8 during the same episode that showed persistent or slightly worsening TCMR (Figure 2A) requiring 6 d antithymocyte globulin (Thymoglobulin, Genzyme) to control the episode. DSA was not tested at this time.

The patient recovered from the transplant and the TCMR with normal liver chemistries. A 1-y protocol biopsy (POD 366) was essentially normal with mild nodular regenerative hyperplasia changes while she was maintained on tacrolimus, mycophenolate, and steroids. Thereafter, the timing of subsequent rejection episodes and the immunosuppression used are summarized in Table 1.

Seventeen months after transplant (POD 523), the second episode of mild TCMR, was diagnosed on a biopsy that showed focal portal stromal and minimal C4d microvascular endothelial cell positivity. DSA was not determined at this time. Treatment with a steroid recycle resulted in rapid normalization of her liver chemistries, but 2 mo later, increasing liver chemistries again occurred. A liver allograft biopsy (POD 597) showed a third episode of mild TCMR with occasional portal-based CD20+ B cells, no increased plasma cells (CD-138 negative), minimal C4d staining in portal microvascular endothelial cells, and stromal positivity. Treatment included methylprednisolone boluses 1 g/d for 2 d. De novo DSA was reported (DQ4, 5000 mean fluorescence intensity [MFI]; DQ8, 7000 MFI), prompting treatment with 3 doses of rituximab.[9]

Three and half months later (POD 698), a fourth episode of mild-to-moderate TCMR occurred. The biopsy also showed occasional (<5%) portal-based plasma cells and minimal portal microvascular endothelial cell C4d positivity (Figure 2B). It was treated with a steroid recycle as described above. No rituximab was given; a follow-up biopsy (POD 706; Table 1) was clean.

An additional 4 mo later (POD 829), a fifth episode of mild TCMR occurred. The biopsy showed an infiltrate dominated by T cells (CD3) and occasional B cells and plasma cells. Equivocal biliary epithelial cell senescence suggestive of early chronic rejection appeared, with a negative C4d stain, but this inadequate biopsy was only 4 mm long. No fibrosis was seen in spite of the recurrent/persistent TCMR, but DQ4 and DQ8 DSA persisted with unchanged MFI. The combination of persistent DSA and suboptimal/inadequate biopsy raised the possibility of coexistent antibody-mediated rejection (AMR). Intravenous steroid therapy followed by oral steroids was repeated. With the failure of our standard immunosuppression to control the recurrent/persistent TCMR, the maintenance immunosuppressive protocol was changed to sirolimus (Rapamune, Pfizer), which we use with cyclosporine and steroids. She was maintained on a high dose of steroids, 20 mg daily, which was very slowly tapered.

Seven months later (POD 1051) in November 2014, when the steroid dosage was 7.5 mg, the sixth episode of mild TCMR developed. The liver allograft biopsy also showed focal biliary epithelial cell senescence-related changes (Figure 2C), consistent with the elevated gamma glutamyl transpeptidase, and one portal tract showed increased CD20+ B cells. The rejection progressed despite a steroid cycle. A follow-up biopsy of the same episode (POD 1056) showed persistent mild TCMR with increasing B cells but no plasma cells and negative C4d staining. Repeat DSA testing showed a DQ4 of 6560 MFI and DQ8 of 5800 MFI. Five days of antithymocyte globulin, and steroids followed by hydrocortisone 1 g daily for 6 d was administered together with 2 doses of rituximab.

After the steroid course, 6 d later, another follow-up biopsy (POD 1062) showed persistent indeterminate to mild ACR with minimal portal stromal C4d positivity. This was treated with hydrocortisone boluses, 1 g daily for 6 d, and

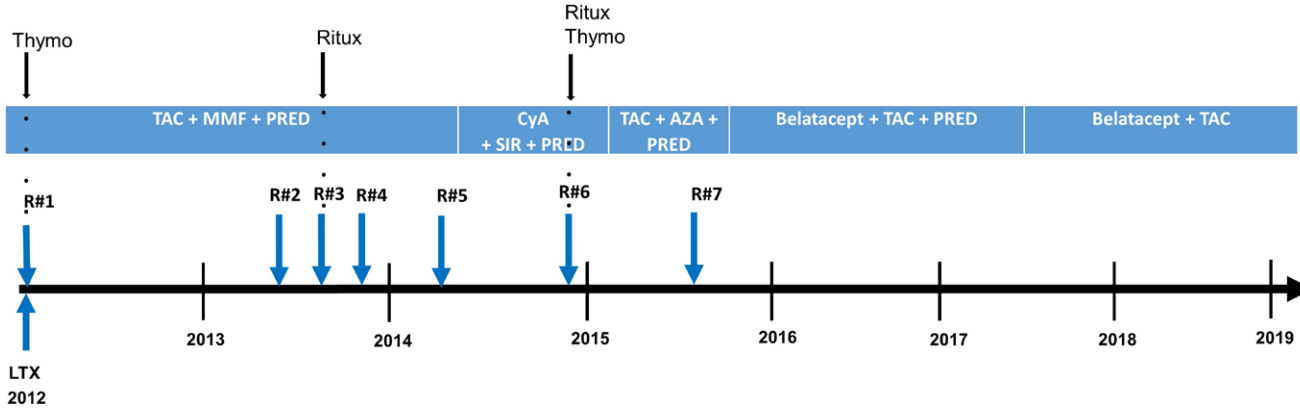

FIGURE 1. Immunosuppression at T cell–mediated rejection (TCMR) episodes 1–7. AZA, azathioprine; CyA, cyclosporine; LTX, liver transplantation; MMF, mycophenolate mofetil; PRED, prednisone; R#, rejection number; Ritux, rituximab; SIR, sirolimus; TAC, tacrolimus; Thymo, thymoglobulin.

TABLE 1.

Summary of liver biopsy findings

Days posttransplant	Biopsy findings	C4d results
3 (TCMR #1)	Mild TCMR with focal neutrophilia; possibly a component of AMR (RAI 4/9)	NA
8	Mild to moderate TCMR (RAI 5/9) with portal capillaritis and CPV	NA
15	Partially treated TCMR (RAI 1/9) with residual CPV	NA
85	Spotty hepatocyte apoptosis with minimal residual CPV (RAI 1/9); CMV ruled out	NA
137	Mild NRH changes	NA
366	Mild NRH changes	NA
523 (TCMR #2)	Mild TCMR (RAI 4/9) with active CPV containing occasional plasma cells	Focal portal stromal and minimal sinusoidal and portal microvascular
530	Treated TCMR	NA
597 (TCMR #3)	Mild TCMR (RAI 3-4/9) with persistent CPV	Minimal portal microvascular
698 (TCMR #4)	Mild to moderate TCMR (RAI 5/9) with focal CPV and occasional plasma cells	Minimal portal microvascular
706	Treated TCMR and mild NRH changes	NA
829 (TCMR #5)	Mild TCMR (RAI 3/9) with focal CPV and possible early BEC senescence changes; rare B cells	Neg[a]
1051 (TCMR #6)	Mild TCMR (RAI 3/9) with persistent CPV and focal BEC senescence-related changes	NA
1056	Mild TCMR (RAI 3/9); increased B cells	Neg
1062	Indeterminate to mild TCMR (RAI 3/9)	Minimal portal stromal
1247 (TCMR #7)	Mild NRH changes; indeterminate for TCMR (RAI 2/9); minimal residual CPV	NA
2221	Normal or minimal histopathological changes	Neg

[a]Very tiny tissue fragment remaining for staining.

AMR, antibody-mediated rejection; BEC, biliary epithelial cell; C4d, complement component 4d; CMV, cytomegalovirus; CPV, central perivenulitis; NA, not available; NRH, nodular regenerative hyperplasia; RAI, rejection activity index; TCMR, T cell–mediated rejection.

maintenance was changed to tacrolimus, azathioprine, and steroids. Azathioprine was chosen, because it is an antimetabolite suppressing both T and B cells in the bone marrow. Mycophenolate does not affect the B-cell activity much. The prednisone was administered at 20 mg per day and tapered to 15 mg daily 4 mo later and to 12.5 mg daily after an additional 2 mo. The biopsies obtained in November and December 2014 (POD 1051, 1056, 1062) showed mild persistent TCMR. Questions of a biliary issue arose because of focal mild ductular metaplasia of periportal hepatocytes on CK7 staining. Ultimately, these changes were attributed to low-grade ductopenia and rejection-related biliary epithelial senescence-related changes (Table 1).

In June 2015 (POD 1247), liver function again deteriorated. A biopsy was indeterminate for the seventh episode of TCMR but showed focal portal inflammation and minimal interface activity, perhaps related to the presence of DSA (DQ4, 4900 MFI; DQ8, 3900 MFI). Liver-related autoantibodies (eg, antimitochondrial antibodies, smooth muscle antibody, liver kidney microsomal antigen, and antinuclear antibody) were negative before transplant and remained so. Pretransplant cytomegalovirus testing was immunoglobulin G (IgG) positive, immunoglobulin M (IgM) negative, and polymerase chain reaction negative. Subsequent testing 1 and 2 y later showed the same results. Epstein-Barr virus testing before starting belatacept was IgG positive and IgM negative.

Because of the persistence of recurrent TCMR together with development of DSA and clinical course as described above, belatacept (Nulojix, Bristol-Myers Squibb) was started 3 y and 5 mo after transplantation at a dosage of 5 mg/kg once monthly. The patient is still on the same dose monthly. Her insurance approved the drug from the beginning. Before starting the new treatment, her white blood cell count, which had been steady at 4000 to 5000×10^9/L, decreased to $<2000 \times 10^9$/L, so azathioprine was held and later discontinued. Prednisone was decreased to 5 mg daily, and the tacrolimus level was maintained at 4 to 6 ng/mL, our standard long-term maintenance tacrolimus level. The patient's liver function tests normalized within 1 mo of starting belatacept and has remained entirely normal since then.

The patient has not experienced any more rejection episodes and is still receiving belatacept 5 mg/kg monthly. Additionally, she was changed to slow-release tacrolimus (our current standard protocol) (Envarsus, Veloxis) 2 mg daily. The tacrolimus level has been kept <2.0 to 2.5 ng/mL. Corticosteroids were discontinued 2.5 y after starting belatacept. Her current (8.25 y after transplant) liver function results are as follows: total bilirubin of 0.7 μmol/L; alkaline phosphatase, 64 IU/L; aspartate aminotransferase, 28 IU/L; alanine aminotransferase, 33 IU/L; gamma glutamyl transpeptidase, 34 IU/L; and albumin, 3.9 g/dL. Her renal function is normal, with a creatinine of 0.6 mg/dL and blood urea nitrogen of 15 mg/dL. However, the DSA persists at a slightly lower MFI (DQ4, 4000 MFI; DQ8, 3000 MFI). The only biopsy done after starting belatacept, which occurred 6 y after transplantation (POD 2221) and 2.5 y after introduction of belatacept, showed no pathology and negative C4d staining (Figure 2D).

DISCUSSION

Late-onset and persistent TCMR is often associated with[10-12] and perhaps facilitated by[13] circulating DSA; it remains a therapeutic challenge as exemplified in this reliable, verified highly compliant, and otherwise young and healthy female recipient. Monitoring her tacrolimus levels for years, they were very consistent with taking her medication as ordered. Although we found no evidence of noncompliance, without daily blood tests or some other verifiable monitoring tool, we cannot exclude noncompliance. Her youth and otherwise healthy status likely enabled her to weather all the rejection-related treatments and high maintenance immunosuppression doses. Yet, despite the heavy immunosuppression treatment including treatment with rituximab twice, she

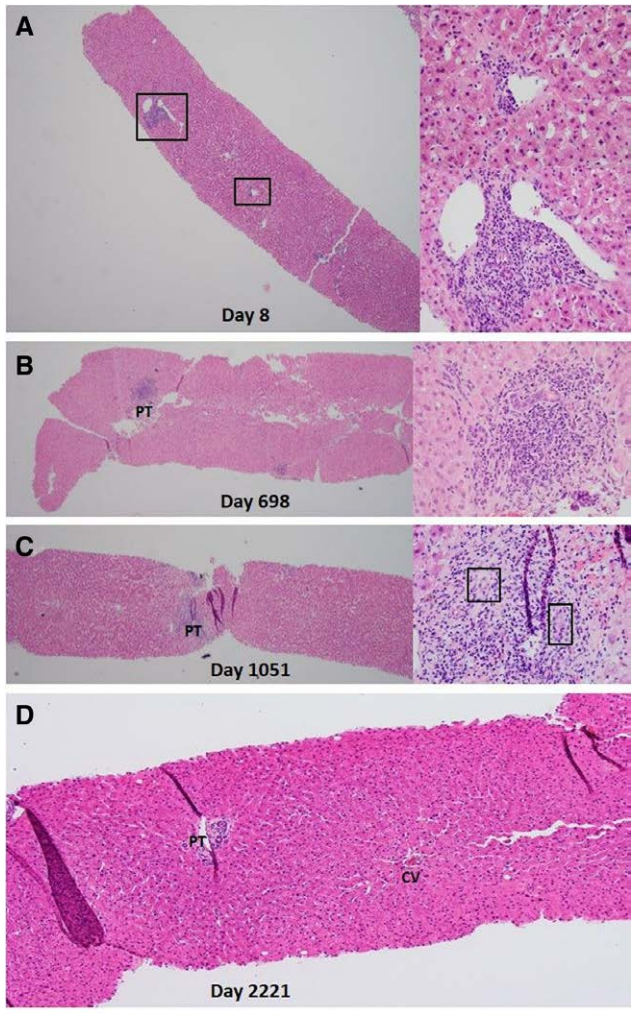

FIGURE 2. Biopsy development before and after treatment for AMR. Biopsy images for (A) day 8 (40× and 400×), (B) day 698 (40× and 400×), (C) day 1051 (40× and 400×), and (D) day 2221 (100×). The day 8 biopsy obtained during T cell–mediated rejection (TCMR) episode 1 shows a low magnification overview with a representative portal tract (large rectangle) and central vein (small rectangle) shown at higher magnification in the lower and upper right insets, respectively. The day 698 biopsy (TCMR #4) shows a representative portal tract with noticeable lymphocytic inflammation. The right panel inset shows that same portal tract at higher magnification. The day 1051 biopsy (TCMR #6) shows persistent mononuclear portal tract inflammation; rectangles in the right lower panel show bile ducts with early biliary epithelial cell senescence-related changes, consistent with the elevated gamma glutamyl transpeptidase levels and suggestive of early chronic rejection (Table 1). The day 2221 biopsy, after treatment with belatacept, shows normal architecture and lack of inflammation or fibrosis. AMR, antibody-mediated rejection; CV, central vein; PT, portal tract.

continued to develop late-onset and persistent TCMR and de novo DSA when first tested at 2 y after transplantation. Plasmapheresis and intravenou immunoglobulin were not used at the time because they only neutralize and remove the antibodies but do not stop their production. The underlying cause of the persistent rejection is likely related to its mixed chronic TCMR/AMR nature, perhaps with the T-cell response being facilitated by DSA.[13] Multiple recent studies show an association between DSA and late-onset TCMR[4] perhaps mediated via indirect alloantigen presentation mechanisms for DSA production and target opsonization for augmented TCMR responses.[4,10,13,14]

The above speculations are based on the observation that belatacept treatment significantly changed the clinical and histopathological course of events in a patient with minimal Envarsus doses and tacrolimus levels. However, we cannot assume that these low levels do not have an effect so many years from the transplantation and especially in combination with costimulatory blockade. The reduction of immunosuppressive medications other than belatacept has been done very slowly in an attempt to avoid recurrence of the high-frequency TCMR and development of AMR. As in all our patients, we strive to keep maintenance immunosuppression low to minimize the risk for malignancies and infection. Importantly, the regimen used has had no noticeable side effects. The ITN 030ST Immune Tolerance Network study of gradual withdrawal of immunosuppression in liver transplant patients (AWISH) study showed that 67.5% of patients could be safely lowered to <50% of baseline dose.[15]

One question that needs to be raised is whether the patient has developed tolerance. This is possible but is a question that cannot be answered at this time. To simply discontinue her immunosuppression at this time, when she had such massive rejection history, does not seem proper now. A failure could be catastrophic. Note, in the ITN 030ST AWISH study, which included only low risk patients, only 13.0% were able to be weaned off immunosuppression for more than a year.[15]

Over several years, having tried mechanistic target of rapamycin with calcineurin inhibitors (both tacrolimus and cyclosporine), rituximab, and bortezomib (Valcade, Takeda) in a number of patients who developed B-cell/plasma cell activity (AMR) without encouraging response (like in this patient), we decided to try belatacept. The posttransplant lack of de novo DSA development 7 y after transplant in kidney recipients[9] (belatacept 1.9% versus control 17.8%; $P < 0.001$) was a major reason behind the decision.

Belatacept is a fusion protein involving the Fc fragment of human IgG1 and the extracellular domain of cytotoxic T lymphocyte–associated antigen 4. It selectively inhibits T-cell activation through costimulatory blockade via engagement with CD80 or CD86. Importantly, de novo DSA develops via indirect pathways[9,13] and germinal center responses.[16] Although belatacept reportedly can inhibit DSA development,[9] whether it does so via interference with T follicular helper–B-cell crosstalk is uncertain.[16,17] Notably, however, the patient's DSA MFI against DQ4 and DQ8 has not changed during the 4+ y following belatacept therapy.

Notably, belatacept has received a black box warning from the US Food and Drug Administration against its use in liver transplantation. Why the liver trial[14] failed is not the focus of this report. However, as mentioned above, the drug has shown remarkable results in kidney transplantation, and we are treating the immune system, not the transplanted organ, liver, or kidney.

A particularly intriguing potential indication is treatment of persistent low-grade immunological injury in long-term surviving liver allograft recipients. In this circumstance, DSA is frequently associated with chronic liver inflammation and progressive fibrosis, but aggressive conventional treatment is feared because of toxicities and complications like infections and tumors; moreover, conventional treatment usually does not improve the ultimate outcome.[4,7] Hopefully, this reported case will be confirmed in other similar patients. If so, formal trials might be considered not only in late-onset TCMR/AMR but also in nontransplant autoimmune disease.

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