Allotransplantation of Cryopreserved Parathyroid Tissue for Severe Hypocalcemia in a Renal Transplant Recipient

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We report the successful allotransplantation of cryopreserved parathyroid tissue to reverse hypocalcemia in a kidney transplant recipient. A 36-year-old male received a second deceased donor kidney transplant, and 6 weeks later developed severe bilateral leg numbness and weakness, inability to walk, acute pain in the left knee and wrist tetany. His total calcium was 2.6 mg/dL and parathormone level 5 pg/mL (normal 10–60 pg/mL). He underwent allotransplantation of parathyroid tissue cryopreserved for 8 months into his left brachioradialis muscle. Immunosuppression included tacrolimus (target C0 10–12 ng/mL), mycophenolate mofetil and steroids. Within 2 weeks, the left knee pain, leg weakness and numbness resolved, and by 1 month he could walk normally. After a peak at month 2, his parathyroid hormone (PTH) level fell to <10 pg/mL; therefore at month 3 he received a second parathyroid transplant from the same donor. Eight months later (11 months after initial graft) he has a total calcium of 9.3 mg/dL, PTH level 15 pg/mL and is clinically asymptomatic. The amount of parathyroid tissue needed to render a patient normocalcemic is not known. In our case, the need for second transplant suggests that the amount of tissue transferred for an allograft may need to be substantially greater than for an autograft.

Key words: Cryopreservation, hypocalcemia, parathyroid allotransplantation

Abbreviations: ABO, blood groups; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ESRD, end-stage renal disease; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; PRA, percent reactive antibody; PTH, parathyroid hormone.

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Introduction

Hypercalcemia due to tertiary hyperparathyroidism is frequently encountered in patients with end stage renal disease (ESRD). It is successfully treated by subtotal parathyroidectomy, and if needed autotransplant of a small amount of residual parathyroid tissue. However, in 1–2% of patients severe hypocalcemia can occur, creating a difficult clinical problem (1). Herein we report the successful allotransplantation of cryopreserved parathyroid tissue to reverse hypocalcemia in an immunosuppressed kidney transplant recipient. This was done after all standard attempts to treat symptomatic hypocalcemia failed.

Methods

Patient

A 36-year-old male with ESRD from chronic glomerulonephritis received a first renal transplant from his sister in November 2003. The graft ceased to function after two acute rejection episodes, the second related to financial difficulties with the medical regimen, and was removed in March of 2006. He waited for a deceased donor kidney but had a high PRA of 75% to class I HLA. On May 17, 2009 he received a zero HLA mismatched deceased donor kidney transplant. Both the lymphocytotoxic and flow cytometry crossmatches were negative to the kidney donor. Immunosuppression consisted of thymoglobulin induction and tacrolimus, mycophenolate mofetil and low dose steroids for maintenance. On admission for the second kidney transplant his total calcium was 8.5 mg/dL (normal 8.5–10.5 mg/dL). The kidney functioned immediately and he had a nadir creatinine of 1.6 mg/dL 2 weeks later. Postoperatively, the patient complained of progressive left leg weakness and numbness contralateral to the kidney transplant, which he stated had begun a week prior. He was prescribed physical therapy.

Prior to admission for his second kidney transplant, the patient gave a history of treatment for tertiary hyperparathyroidism (hypercalcemia and elevated parathyroid hormone [PTH] levels); for which he underwent subtotal parathyroidectomy at another institution. In April 2009, for development of recurrent hyperparathyroidism (PTH level 668 pg/mL, normal range 10–60 pg/mL), he underwent resection of the remaining cervical parathyroid remnant with immediate autotransplantation into the right deltoit muscle. This operation occurred 1 month prior to his second kidney transplant.

On June 4, 2009 he was readmitted to the hospital with severe bilateral leg numbness and weakness, inability to walk, acute pain in the left knee and clinical tetany of the wrists. His total calcium was 2.6 mg/dL, magnesium 1.8 mg/dL, phosphorus 2.5 mg/dL, and albumin 4.1 gm/dL. The PTH was 5 pg/mL (normal range 10–60 pg/mL). An EKG demonstrated normal sinus rhythm.

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rhythm with a prolonged Q-T interval. He was given intravenous and oral calcium for several weeks.

On June 27, 2009 the patient was receiving daily 7 gm of oral calcium × 5, 3 gm of intravenous calcium and oral calcitriol up to 2 mg. The symptoms of tetany and lower extremity numbness were much improved, but he still could not walk without a walker. The total calcium was 5.6 mg/dL; ionized calcium 0.82 mmole/L (normal range 1.08–1.30) with a pH of 7.36 and the PTH was 6 pg/mL.

The previous week a request was made to the Cleveland Clinic Innovative Practice Committee, a subcommittee of the Cleveland Clinic Institutional Review Board (irb.ccf.org), for permission to perform a third-party parathyroid allotransplant. Permission was granted since this patient was a failure of maximal medical therapy for hypocalcemia using oral and i.v. calcium and calcitriol. A repository of stored cryopreserved parathyroid tissue at our center was screened for a suitable donor. There were several constraints on the donor source of stored parathyroid tissue, which included frozen storage for less than a year, the absence of exposure to Human Immunodeficiency Virus (HIV), Hepatitis B and C, as well as HLA phenotypes, to which the recipient was not already sensitized, and ABO compatibility. Since such antibody screening and tissue typing were not done routinely on parathyroidectomy patients, the donor pool was limited to four individuals who were either on the kidney transplant wait list or had already received a kidney transplant at our center.

The first parathyroid allotransplant was performed on July 1, 2009. The thawed parathyroid tissue was placed into the brachioradialis muscle in the left forearm under local anesthesia and sedation. About 20 single 1- to 3-mm fragments of parathyroid tissue were placed into individual muscle pockets. The implanted parathyroid tissue was confirmed to be both benign and hypercellular during pathological evaluation when it was recovered prior to cryopreservation.

Immunosuppression
After the kidney transplant in May 2009 the recipient was maintained on tacrolimus 3 mg b.i.d (target C0 8–10 ng/mL), mycophenolate mofetil 500 mg b.i.d (target C0 2–4 mg/L) and prednisone 5 mg daily. After the parathyroid allotransplant the tacrolimus target was increased to C0 10–12 ng/mL the mycophenolate mofetil was increased to 1 gm b.i.d and the prednisone kept at 10 mg daily for 3 months.

Monitoring of parathyroid cell allograft
Levels of circulating PTH (pg/mL) were measured weekly in both the left and right arm. Both total calcium (mg/dL) and ionized calcium (mmole/L) were measured twice weekly.

Development of de novo posttransplant donor specific antibodies to the parathyroid donor class I and class II HLA mismatched antigens was monitored using the Luminex bead assay (One Lambda, Canoga Park, CA, USA). Tests were run at each follow-up visit (Table 2).

Results

Written informed consent was obtained from both the recipient and the parathyroid tissue donor. The donor selected was a 40-year-old male on hemodialysis with ESRD from glomerulonephritis who was waiting for a deceased donor kidney. He had undergone 3½ gland parathyroidec-tomy for tertiary hyperparathyroidism with cryopreservation of the excised parathyroid tissue on November 8, 2008. He had negative serology for the HIV, Hepatitis B and C and Cytomegalovirus (CMV), but was Epstein Barr Virus (EBV) positive. The recipient was both CMV and EBV seropositive.

Within 2 weeks of the first parathyroid cell allograft the symptoms of left knee pain, and leg weakness and numbness resolved, and the patient began walking with a cane for support. By 1 month postparathyroid transplant, the patient could walk normally, absent any support.

Total and ionized calcium levels remained in the 5–8 mg/dL and 0.7 to 1.0 mmole/L range, respectively, for several months (Figure 1). During this time the patient was
recieving calcium carbonate 500 mg hourly while awake. Serum PTH levels quickly increased from baseline, but stayed in the 10–15 pg/mL range for several months (Figure 2). The patient was given daily oral calcitriol 0.5–2 mg since his kidney transplant, and had hydroxy-Vitamin D levels of 35–80 ng/mL throughout the follow-up period.

The patient had magnesium measured on alternate months during follow-up. He received oral magnesium oxide 800 mg b.i.d since the first parathyroid transplant, and had levels maintained between 1.6–2.4 mg/dL. Serum phosphorus was measured monthly during the 10-month follow-up period and varied between 3.7–6.6 mg/dL. All but two determinations were in the normal range (2.5–4.5 mg/dL).

Although he felt well, the patient could not be weaned off oral calcium, and by 3 months the PTH had fallen <10 pg/mL. Therefore, on October 7, 2009 (day 97) a repeat parathyroid allograft was placed just below the first in the left brachioradialis muscle under local anesthesia and sedation. The parathyroid cells came from a larger frozen aliquot from the same donor as the first graft. Immunosuppression remained the same except for a bolus of 500 mg i.v. methylprednisolone. In a pattern similar to the first parathyroid allograft, PTH levels in the ipsilateral arm rose to the 10–15 pg/mL range for 2 months, but then increased further to 25 pg/mL during the third month (Figure 2). This was accompanied by a rise in total calcium over 8 mg/dL and ionized fraction over 1 mmole/L (Figure 1). After 26 weeks the patient was weaned to one TUMS (regular strength, calcium carbonate 500 mg) p.o. t.i.d, and has remained on this dose due to his osteoporosis. At 44 weeks he maintains a total calcium of 9.3 mg/dL and has a PTH of 15 pg/mL. During the entire 11-month follow-up the patient did not experience any rejection episodes of the renal allograft, and has maintained a creatinine of 1.6–1.8 mg/dL.

Throughout the postoperative period the recipient was monitored for any de novo donor specific HLA antibodies. The parathyroid donor was HLA mismatched for HLA-A30, −B58, Cw3, Cw7, DR4 and DQ8 (Table 1). There was no antibody detected to any of these mismatched antigens at any time postparathyroid transplant. DQ8 antibodies were positive presedone kidney transplant, but became negative postkidney transplant and remained negative on subsequent posttransplant monitoring (up to day 329), Table 2. The second kidney donor was a zero HLA-A, −B and −DR mismatch deceased donor.

Discussion

While severe hypocalcemia after parathyroidectomy for hyperparathyroidism is uncommon, it has been observed in about 1–2% of patients (1). Those at risk are usually patients who have had total thyroidectomy or more extensive resection of parathyroid glands, whether for primary hyperparathyroidism or for parathyroid disease typically associated with renal disorders (secondary or tertiary hyperparathyroidism). As early as 1976, it was recognized that parathyroid autotransplantation is a successful intervention to prevent permanent hypoparathyroidism (2). However, this unintended complication can nevertheless occur, causing dependence on high-dose calcium and vitamin D supplementation with long-term risks of paresthesias, multiorgan calcinosis and renal failure.

Parathyroid cell allotransplantation has been described sporadically over the last 30 years as a potential therapy for permanent hypoparathyroidism (3–10). A number of
In vitro techniques to diminish the immunogenicity of as the cell implants are subsequently rejected through immunosuppression. The results have not been durable, methodologies have been tried, including microencapsulation of parathyroid cells and allotransplantation without immunosuppression. The results have not been durable, parathyroid cells in engineered microcapsules of degassed 2% sodium-alginate (8). These techniques may prolong survivals compared to unmodified tissue.

Simultaneous kidney and parathyroid transplantation from the same deceased donor has been reported with the recovery of circulating PTH (10,12). If available, this can be a viable approach to the problem of both renal and parathyroid organ failure, and presents no additional histocompatibility challenge to the recipient. In such cases, healthy and fresh donor parathyroid tissue may be advantageous for allograft implantation, since the expression of class I and II HLA phenotypes on normal parathyroid tissue appears to be downregulated (13). Whereas parathyroid tissue from patients with either hyperplastic or adenomatous glands demonstrated increased expression of class I and II HLA class I and class II antigens (11). Additional novel approaches have included dispersal of cells in culture with HLA antibody coated microspheres to remove HLA-rich stromal cells (5); a temporary transfer of irradiated human parathyroid cells to a murine ‘interim host,’ by placing them under the mouse kidney capsule for several weeks prior to clinical transfer (4); and encapsulating parathyroid cells in engineered microcapsules of degassed 2% sodium-alginate (8). These techniques may prolong survivals compared to unmodified tissue.

Methodologies have been tried, including microencapsulation of parathyroid cells and allotransplantation without immunosuppression. The results have not been durable, as the cell implants are subsequently rejected through the usual mechanisms of the host alloimmune response. In vitro techniques to diminish the immunogenicity of parathyroid tissue have been attempted by digestion with collagenase and filtering the tissue though a nylon mesh. Such dispersal can reduce the numbers of accompanying macrophages and dendritic cells that are rich in surface HLA class I and class II antigens (11). Additional novel approaches have included dispersal of cells in culture with HLA antibody coated microspheres to remove HLA-rich stromal cells (5); a temporary transfer of irradiated human parathyroid cells to a murine ‘interim host,’ by placing them under the mouse kidney capsule for several weeks prior to clinical transfer (4); and encapsulating parathyroid cells in engineered microcapsules of degassed 2% sodium-alginate (8). These techniques may prolong survivals compared to unmodified tissue.

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<table>
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<th>Time after parathyroid Tx</th>
<th>DQ8 bead</th>
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<td>Week 1</td>
<td>1090</td>
<td>839</td>
<td>342</td>
</tr>
<tr>
<td>Week 2</td>
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<tr>
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<td>Month 11</td>
<td>1169</td>
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Table 1: HLA antibody status of both donors and the recipient

Table 2: Posttransplant screening for donor-specific antibody

The use of tissue banking to cryopreserve parathyroid tissue after parathyroidectomy for future autografting is not universally available. The cryopreservation technique has been reported by several investigators, and the method utilizes standard processes of tissue handling and step-wise preparation for freezing and storage in liquid nitrogen that have not significantly changed with time (15–17). Nevertheless, a recent publication reported that this resource is available to fewer than 30% of surgeons who perform parathyroid surgery (18). Where available, these tissue banks provide a source of parathyroid tissue for those who have insufficient parathyroid hormone production and are traditionally reserved for potential autotransplantation (17,19). For example, patients with secondary hyperparathyroidism related to kidney failure and long-term dialysis inevitably develop secondary hyperparathyroidism with multigland parathyroid hyperplasia. At time of parathyroid surgery, the subtotal or near-total parathyroidectomy excises parathyroid tissue in quantities that are many times larger than any quantity that might be needed for possible autotransplantation (20). Cryopreserved tissue can theoretically be used for allotransplantation but since the stored tissue is intended for use in the same patient, screening for transmissible viral pathogens and tissue typing is rarely performed. This would require specific permission and deferral of any additional expense from the parathyroid donor. Some of these issues could be obviated if the parathyroid donor was a transplant wait list candidate that had previously undergone such screening, as in our case.

An additional important consideration for allotransplantation using stored cryopreserved parathyroid tissue is the quantity of tissue necessary for successful reversal of hypoparathyroidism. While generous amounts of excess parathyroid tissue are usually frozen for future autografting, sufficient sample size would need to be available for

<table>
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<th>Patient</th>
<th>ABO</th>
<th>HLA A</th>
<th>HLA B</th>
<th>HLA Bw</th>
<th>HLA Cw</th>
<th>Dr</th>
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<tr>
<td>Parathyroid/kidney recipient HLA phenotypes</td>
<td>O</td>
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<td>44, 62</td>
<td>2, 16</td>
<td>7, 11</td>
<td>52, 53</td>
<td>2, 7</td>
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<td>Parathyroid/kidney recipient anti-HLA antibodies (pretransplant)</td>
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<td>7, 27, 42, 61, 73, 81, 8201</td>
<td>1, 9, 51, 103</td>
<td>4, 5, 6, 8, 9</td>
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<td>Kidney donor HLA phenotypes</td>
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<td>2, 29</td>
<td>44, 62</td>
<td>3, 16</td>
<td>7, 11 52, 53</td>
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<tr>
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<td>88, 62</td>
<td>4, 6</td>
<td>3, 7</td>
<td>4, 11 52, 53</td>
<td>2, 7</td>
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Luminex HLA detection assay: controls <500 MFI (mean fluorescent intensity).
both the intended recipient and for a potential allograft recipient. There is an expected loss of viable parathyroid cells in the freeze-thaw process. It has been reported that about 71% of cryopreserved parathyroid tissue will be viable for the first 24 months of frozen storage, with viability inversely proportional to the length of time stored (19). The negative effect of cryopreservation no doubt contributes to the relatively modest success rates reported with delayed parathyroid autotransplantation of 40–60% (15,17,21). Alternatively, the freezing of parathyroid tissue may contribute to a loss of surface HLA expression and contribute to reduced immunogenicity. The precise amount of cryopreserved parathyroid tissue needed to render a patient normocalcemic is not known. In our case, the need for second transplant points out that the amount of tissue transferred for an allograft may need to be substantially greater than for an autograft.

At the present time, durable results have only been reported in parathyroid allotransplantation when immunosuppression to prevent rejection is administered. A commonly used regimen in kidney transplantation of tacrolimus, mycophenolate mofetil and steroids appears to permit the recovery of PTH secretion. The optimal agents needed for parathyroid allotransplantation are not known. The use of these agents, which are associated with significant morbidity and toxicities, would therefore be limited to those patients with hypoparathyroidism accompanied by an additional need for transplantation and immunosuppression. The primary transplant organ would dictate the regimen employed. Finally, if a local cryobank is not available, frozen parathyroid tissue could be shipped to alternate sites for allotransplant with appropriate arrangements and screening.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

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