In Utero Transplantation of Human Cord Blood Cells into Rabbits

We have previously shown that in utero transplantation (IUT) of haploidentical, bone marrow-derived CD34+ cells can be used as prenatal therapeutic approach for X-linked severe combined immunodeficiency (X-SCID) (1). To extend the possible applications of IUT, animal models are needed that can be used to address technical and biological questions, including optimal source of hematopoietic stem cells, timing, site and method of injection (e.g., open surgery, fetoscopy, transcatheter ultrasound-guided injection). Different animal models were described for either surgical or transcatheter transplantation of hematopoietic progenitor cells in utero (2). As an alternative to human bone marrow, cord blood progenitor cells were transplanted in utero in a sheep animal model applying a surgical procedure and injection under ultrasound guidance (3, 4). Ultrasound-guided IUT of human cord blood cells was also recently described in a swine model (5).

We have performed IUT in four rabbit fetuses at 26 days of gestation using 80 × 10⁶ human male cord blood mononuclear cells separated on Percoll gradient (47.5% fraction) and resuspended in PBS before transplantation. The intraperitoneal (i.p.) injection was performed under ultrasound guidance using a linear probe (10 MHz) in a pregnant female rabbit under general anesthesia. Seven rabbit kits were born at term, two of which died shortly after birth. The surviving animals showed normal growth rate and no clinical symptoms of graft-versus-host disease (GVHD) during an observation period of three months.

DNA was isolated from peripheral blood of the animals, 27 days after IUT (23 days after birth) and engraftment was determined by polymerase chain reaction amplification of the human amelogenin gene (AMGY) that is located on the Y chromosome. Human chimerism was confirmed by Southern blot analysis of the amplified sequences in four out of five available animals (Fig. 1A, lanes 4 – 8). Rabbit DNA quality was assessed by amplification of a rabbit specific sequence at the SAT 12 locus (Fig. 1B, lane 2–8).

In summary, ultrasound-guided IUT of human hematopoietic progenitors can result in human chimerism in rabbits. In this small-size animal model, all injected animals were clearly visible on ultrasound and survived the procedure without clinical complications. Compared to the sheep (145 days of gestation), swine (114 days), and Rhesus monkey (165 days), the rabbit has the advantage of a shorter pregnancy (31 days). In addition, in contrast to mice, the size of rabbit fetuses makes transcatheter ultrasound-guided in utero transplantation feasible. Further experiments aimed at quantifying levels of donor chimerism (i.e., percent of human CD45 cells and specific leukocyte subpopulations) and duration of engraftment will provide more detailed information on the human hematopoietic cell engraftment potential in the fetal rabbit and will determine if the rabbit IUT model can be helpful to test transplantation protocols aimed at improving engraftment, study possible applications of different sources of progenitor cells, as well as pharmacological protocols to augment donor cell engraftment (6).

**FIGURE 1.** (A) PCR analysis on DNA isolated from peripheral blood of rabbit kits 27 days after in utero transplantation of human male mononuclear cells isolated from cord blood. Chimerism was detected using a probe specific for the amelogenin gene (AMGY) on the Y chromosome by Southern blot analysis. The specificity of human Y chromosome signal is shown in human male DNA (lane 1) vs. male and female rabbit DNA (lane 2 and 3). Positive signals for human DNA were demonstrated in four out of five animals (lanes 4–8). H₂O served as PCR negative control (lane 9). The primers used for the AMGY gene (Genbank accession number: MS8418) specific amplification were: sense primer 5′-CTGATGGT-TGGCCTCAAGCCTGTG-3′; antisense primer 5′-GCCCAAAGTTAGTAATT-TAC-3′. PCR amplification was performed as follows: DNA was denatured for 4 min at 94°C and amplified for 30 cycles (94°C, 20 sec; 58°C, 30 sec; 72°C, 45 sec) with a final elongation step of 72°C for 7 min, and then stored at 4°C. (B) Rabbit specific sequence amplification was performed at the Sat 12 locus (GenBank accession number X89889) to demonstrate integrity of rabbit DNA. The primers used to amplify specific rabbit sequence were: sense primer 5′-CAGACCCG-GCAGTGGCATGAGAGTATG-3′; antisense primer 5′-GGGAGAGAGGGATGGATATG-3′. PCR conditions were as described for the AMGY gene.

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Successful Liver Transplantation in Two Cases of Metastatic Gastrointestinal Stromal Tumors

Orthotopic liver transplantation (OLT) for malignant liver tumors can be performed if long-term results are expected to be similar to those of OLT for benign liver diseases. OLT is thus a recognized treatment for hepatoblastoma and epithelioid hemangioendothelioma (EHE) (1). Until now, liver metastases of neuroendocrine tumors are the only metastatic tumors which have been accepted for OLT (2).

We re-evaluated two cases of OLT for unresectable liver metastases of primary mesenchymal tumors. Both cases showed c-kit positivity for the initial gastric tumors and the liver metastases, allowing their classification as gastrointestinal stromal tumors (GISTs) with liver metastases. The GIST of the second patient was part of a Carney triad, a rare tumor syndrome that includes pulmonary hamartochondromatosis, extra-adrenal paraganglioma, and adrenocortical tumors (3).

Case 1

In 1996, a 39-year-old male patient underwent partial resection of the stomach for a leiomyosarcoma (LMS) of 4.5 cm. Four years later, multilobar liver metastasis was diagnosed (Fig. 1A). Neither chemotherapy nor chemoembolization were effective. In the same year, OLT of the right liver lobe from a living donor was performed. Follow-up examinations have not shown any tumor recurrence over a period of 48 months.

Case 2

In 1989, a then 29-year-old female patient underwent a Billroth II operation for a mesenchymal tumor of the stomach. In 1991, a paraganglioma of 3.5 cm was resected at the left carotid bifurcation. In 1997, a computed tomography scan of the abdomen revealed several liver nodules of up to 1.6 cm in diameter and a tumor in the left adrenal gland of 1.5 cm. The following year, atypical liver resection of the segments III, V, and VIII and cholecystectomy were performed (Fig. 1B). The tumors were histologically classified as EHE. A spread into other liver segments could not be excluded. In preparation of OLT, two nodules of high CT density in the right lung were resected. Examination revealed central ossifying hamartochondromas of 4.0 and 2.5 cm. In 1999, OLT from a cadaveric donor was per-
formed. Follow-up examinations during the past 69 months have not shown any tumor recurrence.

Resection of liver metastases is a treatment option for metastatic mesenchymal tumors, including GISTs (4). Common chemo- and radiotherapies are ineffective. OLT has not yet been reported for metastasis of sporadic GIST or within the Carney triad. When the liver metastases become apparent, neither the diagnosis of GIST was made, nor was the therapy with the selective tyrosine kinase inhibitor Imatinib (Gleevec) for GIST available. OLT was the successful treatment in both cases with a long-term, recurrence-free survival.

In advanced GIST, a sustained response with Imatinib can be achieved in more than half of the patients but without complete disease-free state (5). On the other hand, tumor progress under Imatinib therapy occurs in 11% of the cases (6), and resistance against Imatinib can be observed after long-term use of the drug. Therefore, because GISTs almost exclusively metastasize to the liver, tk4OLT should be considered for advanced metastatic GIST if local resection is not feasible and Imatinib therapy is ineffective.

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Tissue Remodeling in a Bioartificial Fibromuscular Patch Following Transplantation in a Human

Tissue engineering applications might help to overcome currently encountered limitations in reconstructive surgery in many surgical subspecialties (1). Recently we reported the successful reconstruction of a tracheobronchial defect in a patient using a bioartificial airway patch (2). Here we describe the remodeling of the transplanted bioartificial tissue.

In a 58-year-old male, a bioartificial fibromuscular patch was implanted into a 1.5 × 1.5 cm large tracheobronchial anastomosis defect (onset 4 weeks postoperatively) as previously described in detail (2). Briefly, the patient had undergone right carinal pneumectomy for relapsing non–small-cell lung cancer following operation and radiation (60 Gy) 4 years earlier. The defect had caused pleural empyema and pyocelepticaemia which were treated by thoracostomy and medically. At time of patch implantation, an omentum major transposition and right subscapular myo-

plasty combined with a thoracoplasty were performed.

The patch had been generated within 5 weeks from a 3 × 4 cm skin biopsy that was obtained at the thoracostomy site. Autologous muscle cells (MC) and fibroblasts (Fb) were isolated enzymatically from the biopsy and cultured for 2 weeks (37°C, HAM-F12/MEM culture media; volume ratio 50:50). An acellular 24 × 36 mm collagen network (derived from a porcine jejunal segment) served as carrier matrix. Autologous Fb and MC in a cell ratio of 95:5 were seeded for 3 weeks on the matrix (37°C, HAM-F12/MEM culture media; volume ratio 50:50).

Patch integrity was controlled by fibroscopies 1, 3, 6, and 12 weeks following implantation. We detected patch matrix reorganization resulting in increased mechanical stability and a defect area decrease from 12 × 9 mm to 9 × 5 mm. A transtracheal patch biopsy performed 12 weeks after implantation documented a harmonic cellular distribution pattern with a surprisingly altered patch composition representing 80% MC and 20% Fb (Fig. 1).

Numerous clinical and experimental studies surveyed bioartificial implants for a wide range of clinical applications, including tracheal reconstruction (1). As yet, the venture to generate a bioartificial

FIGURE 1. Cellular patch composition. Overall cell number doubled within 3 months. Fb represented the cellular majority during tissue culture before implantation (in vitro). Three months after implantation tissue remodeling resulted in a dominant MC fraction.
tracheal substitute did not provide a convincing graft (3) and currently autologous repair tissues (i.e., pericardial patches) represent dependable alternatives for limited tracheal reconstruction that result in scar tissue formation and contraction of the airway defect (4).

In our presented case, we repaired a limited defect in the tracheobronchial system with a tissue engineered autologous tracheal patch. Analogous to clinical experiences with autologous pericardial patches, we detected a decrease of defect size over time. In contrast to previous findings, patch biopsy revealed a profound change of tissue composition within the first 3 months following implantation: the MC:Fb cell ratio had reversed in favor of MC. This unexpected increase of smooth muscle cells in the patch matrix cannot be explained by cicatrization. The underlying mechanism, however, remains unknown and cannot be explained by our data. Either the privileged ingrowth of patient MC or a survival benefit of implanted MC could explain these findings. In the light of previous unexpected (sometimes fatal) clinical experiences with bioartificial grafts (5), further studies are needed to address questions regarding the healing behavior and mechanisms of bioartificial implants.

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was acutely ill with persistent retching and blood pressure of 190/89 mmHg. The transplant allograft in the left lower quadrant was firm, enlarged, and minimally tender, with an audible bruit. Anuria developed, and the serum creatinine was 6.7 mg/dL.

After emergent hemodialysis, an ultrasound demonstrated a circumferential fluid collection of the allograft. The presence of circumferential fluid collection, recent biopsy, and anuric renal failure led us to consider the diagnosis of Page Kidney. Figure 1 is an arteriogram of the allograft. At the lower pole is a moderate-sized pseudoaneurysm which was treated with embolization. Also seen is the attenuation of the demarcation at the cortex with a blush-like appearance, a visual effect of external compression.

The patient then underwent open surgery for evacuation of the peritransplant hematoma. Anuria had been present at this time for nearly 24 hr. Evacuation of the hematoma was followed by the immediate formation of urine. Postoperatively, the blood pressure fell to 150/85 mmHg and urine output increased to 3 L/day by the second postoperative day. By the fourth postoperative day, the serum creatinine had returned to 1.4 mg/dL and the patient was discharged.

This case report represents a serious complication of kidney biopsy, an acutely developing Page Kidney that resulted in severe hypertension and acute renal failure. It also documents for the first time a multidisciplinary approach to Page Kidney from nephrologists, radiologists, and transplant surgeons. In the transplant population, external compression of a solitary kidney causes acute renal failure, which may have devastating and permanent consequences (4, 5). In the present report, early recognition of Page Kidney and immediate radiologic and surgical intervention were paramount in saving the transplanted kidney.

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Living Donor Liver Transplantation from Donor with Previous Upper Abdominal Surgery

Experience with and technical improvements in left-lobe donation have led to the use of right-lobe grafts in adult-to-adult living-donor liver transplantation (LDLT) to overcome the problems encountered with “small-for-size” grafts. We started a program using right-lobe LDLT for adult patients in 1998 to mitigate accumulating problems (1).

Donor candidates were limited to relatives up to the third civil degree, or spouses or their equivalents of the recipient, who showed a strong voluntary will to donate. During the period from June 1990 to September 2004, 1038 LDLTs were performed in 990 patients at Kyoto University Hospital. Right-lobe LDLT was first carried out in February 1998, and we have since performed 360 right-lobe LDLTs. Of these, five donors had previous history of upper abdominal surgery (Table 1). The median follow-up period of the five donors was 79 months (range 53–114 months). There were three laparoscopic cholecystectomy for cholecystolithiasis, open cholecystectomy for polyp in one, and distal gastrectomy for perforated gastric ulcer in one. Dense adhesion was seen in hepatic hilus during the donor operation but was successfully managed with previously reported our surgical technique (2).

Operative cholangiography was obtained by 24G needle puncture through common bile duct in four cases. One donor showed persistent fluid collection diagnosed ultrasonography without any clinical symptom, which was closely observed until discharge without treatment.

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Relation</th>
<th>Previous operation</th>
<th>Operation time (min)</th>
<th>Blood loss (g)</th>
<th>Hospital stay (days)</th>
<th>Complications</th>
</tr>
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<tbody>
<tr>
<td>39</td>
<td>Sister</td>
<td>Cholecystectomy</td>
<td>391</td>
<td>160</td>
<td>11</td>
<td>None</td>
</tr>
<tr>
<td>55</td>
<td>Father</td>
<td>Distal gastrectomy</td>
<td>333</td>
<td>200</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>54</td>
<td>Brother</td>
<td>Laparoscopic cholecystectomy</td>
<td>469</td>
<td>30</td>
<td>22</td>
<td>Fluid collection</td>
</tr>
<tr>
<td>56</td>
<td>Husband</td>
<td>Laparoscopic cholecystectomy</td>
<td>380</td>
<td>200</td>
<td>16</td>
<td>None</td>
</tr>
<tr>
<td>57</td>
<td>Father</td>
<td>Laparoscopic cholecystectomy</td>
<td>476</td>
<td>420</td>
<td>10</td>
<td>Sepsis</td>
</tr>
</tbody>
</table>

TABLE 1. Right lobe living donors with upper abdominal surgery

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None of the donors and recipients showed biliary and vascular complications during the follow-up period. One recipient with blood type incompatible graft died from sepsis 7 month after transplantation.

Among 355 donors without upper abdominal surgery, the donor age was 41.6 ± 11.5 years (range 19–64), body weight was 61.3 ± 10.9 kg (38.4–107.2), and body mass index was 22.8 ± 3.2 kg/m² (16.6–34.3). Duration of the donor operation was 405.9 ± 81.6 min (195–669) and blood loss was 276.5 ± 246.0 g (10–2300). The hospital stay was 195–669 and blood loss was 276.5 ± 246.0 g (10–2300). The hospital stay was (195–669) and blood loss was 276.5 ± 246.0 g (10–2300). The hospital stay was 16.3 ± 9.6 days (6–114). There was no difference about the donor perioperative parameter between the donors with or without upper abdominal surgery.

The current successful results of LDLT have established this technique in the treatment of end-stage liver disease. These techniques have expanded the potential donor pool and decreased the waiting list mortality. Our present experience demonstrated that living donation from the donor with upper abdominal surgery could be done with cautious operation procedure. However, special attention to assure the donor safety should be necessary, which must be the first priority in this treatment modality.

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Schistosoma Mansoni Infection and Liver Graft

A 23-year-old male diagnosed with posthepatitis B cirrhosis underwent an orthotopic liver transplant in June 2002. This patient had suffered from oesophageal variceal bleeding in March 2001, was treated by endoscopic ligature, and had undergone lamivudine treatment since December 2001. He was classified Child Pugh B8. The donor was a 59-year-old man who had died from intracerebral hemorrhage. The patient’s biological hepatic tests were normal, as was his liver ultrasound.

At the end of the transplant procedure, a hepatic biopsy of the graft was performed. Histological examination showed two granulomas surrounding eggs of Schistosoma. Two 2-day treatments of praziquantel (Biltricide) were administered as specific antischistosomal treatment on the second and third postoperative days and 2 weeks later for another 2 days. The postoperative course was uneventful with a moderate rejection on day 21 which was easily treated with an increase of tacrolimus. Six months after the liver transplant, the patient developed HHV-8 primary infection and underwent several liver biopsies none of which showed Schistosoma eggs or liver fibrosis. Schistosomiasis is a parasitic disease which causes liver fibrosis. Eggs trapped in the liver parenchyma induce granuloma and inflammation that can lead to periportal fibrosis and portal hypertension. Liver fibrosis can be prevented by early administration of antischistosomal drugs (1).

To our knowledge this is the first reported case of a liver transplant with a graft infected by Schistosoma Mansoni. Impact of Schistosomiasis on graft outcomes after kidney transplants have been reported and it has been shown that there was no significant incidence of acute or chronic rejection, provided that the patients are treated using antischistosomal drugs 1 month before the transplant procedure (2). In our case, treatment of the recipient with praziquantel efficiently prevented any consequence of this incidental Schistosomiasis of the liver graft. Such treatment was not considered as a contraindication to the use of praziquantel considering the absence of associated liver fibrosis.

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Venous Thromboembolism in Renal Transplant Recipients

It was a tremendous honor to have one of our articles (1) mentioned in a recent letter and response in Transplantation (2, 3). We would like to clarify that our primary hypothesis in the cited article was to assess the relationship between post-transplant renal insufficiency and risk of late (more than 1 year posttransplant) risk of venous thromboembolism (VTE), or to use the authors’ preferred terminology, thromboembolic complications (TEC). Thus, we used hospitalizations attributed to cytomegalovirus (CMV) as an adjustment variable, because the association of renal insufficiency with venous thromboembolism might be mediated through a number of mechanisms, and the authors themselves have explored CMV as a potential contributor to TEC (4). We did not find a significant association between hospitalized CMV infection and later risk of subsequent TEC, but late TEC might differ substantially from early TEC, which would more likely be directly related to surgical procedures.

We agree with Kazory and Ducloux on the limitations of registry data, namely the inability to independently confirm outcomes or predictor variables, and thus the possibility of misclassification. In an earlier article (5), we did explore associations between CMV serology and subsequent risk of pulmonary embolism. We found that positive recipient (but not donor) CMV serology was significantly associated with pulmonary embolism in unadjusted, but not adjusted analysis. Donor-positive to recipient-negative CMV serology was not associated with pulmonary embolism, although naturally this is a different outcome than the combination of deep venous thrombosis and pulmonary embolism.

Although clearly these studies do not exclude the possibility that CMV is a true risk factor for TEC, in terms of relative risk it would likely be behind other risk factors such as advanced age and obesity, as well as risk factors that in our opinion have been given inadequate attention, such as polycystic kidney disease and systemic lupus erythematosus.

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