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Clinical pancreas transplantation

P-1.1

Effect of sirolimus versus mycophenolate mofetil on glucose metabolism in pancreas and kidney transplantation: a prospective randomized study

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Objectives: Metabolic effects of immunosuppressive agents are important in pancreas or islet transplantation. The aim of our study was to compare glucose metabolism in Type 1 diabetic pancreas and kidney recipients on tacrolimus-based immunosuppression in conjunction with sirolimus (RAPA) or mycophenolate mofetil (MMF) in a prospective randomised study.
Methods: The investigation was performed in 40 insulin-independent rejection-free patients after simultaneous pancreas and kidney transplantation (with systemic venous drainage of pancreatic graft) on discharge from the hospital (0.6 ± 0.3 [mean ± SD] month post-transplant; with steroid dose 11 ± 4.3 mg/day) and subsequently at 21.2 ± 9.9 months (steroid-free). All recipients had a good function of the kidney graft. Fasting glycemia, insulin and C-peptide levels, HbA1c, IVGTT with KG-calculation were assessed in both groups. Insulin sensitivity was evaluated by HOMA-IR. Areas under the insulin/C-peptide curves during the IVGTT (AUC-IRI, AUC-CP) were used as the parameters of insulin/C-peptide secretion.
Results: The RAPA and MMF groups did not differ in age, BMI, post-transplant period and steroid daily dose. Trough levels of tacrolimus had no significant impact on any of examined parameters. KG and HOMA-IR of the whole study group significantly improved between the exams (1.0 ± 0.4 versus 1.43 ± 0.44%/minute, $p < 0.001$ and 4.1 ± 4.1 versus 2.73 ± 1.98, $p < 0.05$, respectively). We found only a significant difference between the groups in stimulated AUC-CP after steroid withdrawal.
Results of examinations in steroid-free period

	RAPA group (n=20)	MMF group (n=20)
HbA1c (%)	5.6±0.5	5.4±0.6
Fasting glycemia (mmol/L)	4.8±0.5	4.7±0.7
KG (%/min.)	1.35±0.36	1.51±0.5
AUC–IRI (mIU/L/60min.)	2062±1229	2601±1368
AUC–CP (pmol/mL/60min.)	96.6±29.8	114.7±33.7
Stimulated AUC–CP (pmol/mL/60min.)	47.6±18.8	63.8±22.6 (p<0.05)
HOMA–IR	2.6±1.7	2.8±2.2

Conclusion: Glucose tolerance measured by IVGTT significantly improved in whole study group probably due to better insulin sensitivity after steroid withdrawal. The higher stimulated C-peptide secretion in the MMF was not associated with a significant difference in IVGTT results between the groups. In steroid-free tacrolimus based immunosuppression the choice of RAPA or MMF did not lead to clinically relevant differences in parameters of glucose metabolism.

P-1.2

Hand-assisted laparoscopic living donor nephrectomy and distal pancreatectomy for simultaneous pancreas and kidney transplantion

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Background: Simultaneous pancreas and kidney transplantation (SPK) from live donor (LD) have not spread worldwide because of much invasiveness of donor operation. We introduce the hand-assisted laparoscopic (HALS) living donor nephrectomy and distal pancreatectomy for minimally invasive donor operation.
Method: Since January 2004 we performed 11 cases of LDSPK and we performed HALS donor operation in the last three cases. Donors were placed in a right lateral position. A 7 cm upper midline incision was made and handport was placed. Total two or three 12 mm ports were placed. Ureter was ligated with clip and divided. The left gonadal vein, renal artery and vein were ligated with vascular stapler. The left side kidney was extracted through midline incision. Then the spleen and distal part of pancreas was completely mobilized using ultrasonic shears and electrocautery. The splenic vein and artery were identified and mobilized. Donors were rotated to a supine position. Under direct vision through midline incision, pancreas parenchyma was dissected using ultrasound shears and the splenic artery and vein were ligated. The distal part of the pancreas and the spleen were extracted.
Results: Total operative time was 445 ± 45 minutes (mean ± SD) and intraoperative blood loss was 272 ± 213 ml. Warm ischemic time of the kidney and the pancreas were 2.7 minutes and 5 minutes respectively. The postoperative course of donors was uneventful. The graft of both kidney and pancreas functioned well.
Conclusion: Since 2000 we performed over 150 retroperitoneoscopic donor nephrectomies in living donor kidney transplantation. The advantage of this scopic technique is obvious. Thus we introduced the hand-assisted laparoscopic live donor nephrectomy and distal pancreatectomy for SPK. This technique is minimally invasive and safety and may become the standard method for SPK.

P-1.3

Unusual presentation of post-transplant lymphoproliferative disorder in two pancreas transplant recipients

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Background: Post-transplant lymphoproliferative disorder (PTLD) typically presents in the allograft, gastrointestinal tract, central nervous system, heart, lung or lymph nodes. PTLD in the head and neck region of adult transplant recipients is unusual and isolated tonsillar involvement has only been reported in two adult liver transplant recipients. Presented here are two cases of adult pancreas transplant alone (PTA) recipients who developed PTLD involving only the tonsils.
Case 1: Twenty-eight year old male underwent PTA (Donor EBV unknown, Recipient EBV-). Immunosuppression: rabbit antithymocyte globulin (rATG) induction, steroid withdrawal and tacrolimus (fk)/sirolimus (rapa)/mycophenolate mofetil (MMF) maintenance. He presented 6 months later with odynophagia and tonsillitis. EBV PCR was positive (680 DNA copies/ml). Imaging of the head, neck, chest, abdomen and pelvis revealed no additional pathology. Bilateral tonsillectomy was performed. Pathology demonstated atypical lymphoid proliferation with CD20+ staining. Immunosuppression was reduced and the patient recieved rituximab and valgancyclovir. Three years later the patient remains free of recurrent PTLD with excellent graft function. Maintenance immunosuppression currently consists of fk and MMF.
Case 2: Fifty-six year old male underwent PTA (Donor EBV +, Recipient EBV-). Immunosuppression: rATG, steroid withdrawal and fk/rapa/MMF maintenance. He presented 7 months later with acute tonsillitis. EBV PCR was positive (2100 DNA copies/ml). Imaging of the head, neck, chest, abdomen and pelvis revealed no additional sites. Unilateral tonsillectomy was performed with the rationale that one tonsil would serve as a tissue biopsy while the other would be followed for response to therapy. Pathology demonstrated polymorphous PTLD and was EBV+ and CD20+. Immunosuppression was reduced and the patient recieved rituximab and

valgancyclovir. The remaining tonsil returned to normal, the EBV PCR became undetectable and the symptoms resolved. Five months later the patient remains free of recurrent PTLD with excellent graft function. Maintenance immunosuppression currently consists of fk and rapa.
Conclusion: Tonsillar PTLD is very uncommon in adult transplant recipients but it is responsive to standard therapies. Unilateral tonsilectomy has the advantage of leaving one tonsil as a marker for response to therapy.

P-1.4

Reduced B7-H4 expression in human beta cell is associated with recurrent type 1 diabetes in a failed pancreas allotransplant

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Background: Since the late 1990's, the rate of early pancreatic graft loss from acute rejection has dropped. However, chronic pancreatic graft failure still affects more than 8% of all pancreas transplants. Previous studies have shown that type 1 diabetes (T1D) may recur in immunosuppressed pancreatic transplant recipients. Recently, we have found that a reduced expression of B7-H4 (a novel negative T-cell co-signaling molecule) in beta cells is associated with the development of human T1D. We have identified a pancreatic recipient who suffered a loss of insulin function secondary to recurrence of T1D with a decreased B7-H4 in beta cells.
Methods: Immunohistochemistry (IHC) was used to examine cell markers in tissue sections of the explanted pancreas from the transplant recipient 11 years after transplant and 9 years following his return to insulin. Immunofluorescence staining was used to investigate the co-localization of markers in cells.
Results: IHC of the pancreas sections of the recipient showed varying degrees of mononuclear-cell infiltration, from mild in peri-islet and -ductal areas to severe in fibrotic areas scattered throughout the pancreas tissue. Mononuclear cells infiltrated included CD3+, CD4+ and CD8+ T cells. Only 10% of the islets of the explanted pancreas were insulin-positive and the number of these cells was markedly reduced in the islets, while glucagon-positive cells were present in all of the islets in normal numbers. In the islets containing insulin-positive beta cells, the architectural presence of the beta cells changed from being the major cell type of a normal islet to being scant in number or only weakly staining. Insulin and B7-H4 expression in the residual islet cells were decreased while glucagon expression was slightly higher than normal controls. Amyloid bodies were absent in islets found in the head, body, and tail portions of the pancreas. Marker co-localization analysis showed that the explanted pancreas displayed expression patterns of insulin, glucagon, and B7-H4 similar to those found in patients with T1D.
Conclusions: The parallel decrease of B7-H4 and insulin but not glucagon in islets suggests a recurrence of T1D in the pancreas. B7-H4 reduction associated T1D recurrence, but not allogeneic responses, or amyloid formation was the major cause of the pancreatic endocrine failure in this patient.

P-1.5

Kidney pancreas transplantation with simultaneous allograft nephrectomy for recipients with prior renal transplant lost to BK nephropathy

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Background: BK nephropathy (BKN) is recognized as a significant, potentially reversible cause of graft failure in renal transplant (RTx) recipients. Candidacy for retransplantation with or without allograft nephrectomy is controversial. This report describes two RTx recipients who lost their grafts to BKN and subsequently underwent kidney and pancreas transplantation with simultaneous allograft nephrectomy (SPKAN).
Case 1: Thirty-three year old male with end stage diabetic nephropathy (ESDN) underwent living donor renal transplantation (LDRTx) at another center with the intention of listing for pancreas after kidney (PAK) transplantation. Immunosuppression consisted of simulect induction, steroid withdrawal and tacrolimus (fk) and mycophenolate mofetil (mmf) maintenance. He developed progressive deterioration in renal function 4 months after transplantation. BKN was diagnosed on percutaneous renal allograft biopsy. BK viral load was 6.7 × 10e8 DNA copies/ml in the urine and 130 000 DNA copies/ml in the serum. BK viremia responded well to immunosuppression reduction and cidofovir, but his renal allograft failed. Two years after the initial transplant, with an undetectable viral load, he underwent SPKAN. Immunosuppression consisted of rabbit antithymocyte globulin (rATG) induction, steroid withdrawal and fk/mmf maintenance. At 2.75 years follow-up he has excellent graft function with no recurrence of BK.
Case 2: Twenty-seven year old male with ESDN underwent LDRT× with the intention of listing for PAK. Immunosuppresion consisted of simulect induction, steroids, fk and MMF. Approximately 1 year later BKN was diagnosed on percutaneous renal allograft biopsy. BK vial load was 1.3 × 10e9 DNA copies/ml in the urine and 1.5 × 10e6 DNA copiel/ml in the serum. BK viremia responded well to immunosuppression reduction and leflunomide, but his renal allograft ultimately failed. Six years after the initial transplant, with an undetectable viral load, he underwent SPKAN. Immunosuppression consisted of rATG induction, steroid withdrawal and fk/mmf maintenance. At 1.5 year follow-up he has excellent pancreas and kidney graft function with no recurrence of BK.
Conclusion: SPKAN can be performed without recurrence in recipients who have lost a prior renal allograft to BKN.

P-1.6

Efficacy of pancreas transplantation: impact on severe hypoglycemia, diabetic ketoacidosis and glycemia related end points

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Objective: We evaluated the effectiveness of pancreas transplantation (PT) in preventing severe hypoglycemia (SH) and diabetic ketoacidosis (DKA) and impacting glycemia related end points.
Methods: We retrieved data for all PT performed at our institution from 01/01/1998 to 12/31/2006 (n = 216). Patients were considered insulin independent if they were not on insulin at pre-specified time points with normal FPG, HbA1c and C-peptide (CP) or mildly abnormal FPG and/or HbA1c with normal CP. Patients were diagnosed with post transplantation diabetes or partial allograft function if FPG was >126 mg/dl on two consecutive occasions, HbA1c was elevated and anti-hyperglycemic treatment prescribed along with CP > 200 pmol/l. Patients with a clinical picture consistent with severe insulin deficiency and CP < 200 pmol/l were diagnosed with complete allograft failure. Logistic regression and Kaplan–Meier analyses were performed.
Results: Mean age at transplant was 43.4 years (SD: 9) with 51% male. 200 patients had type 1 and 16 had type 2 diabetes. Simultaneous pancreas–kidney, pancreas alone, and pancreas after kidney transplants (PAK) were performed in 42, 67 and 107 of patients respectively. Mean age at the time of transplant was not different between completely functional, partially functional and completely failed grafts.. Mean follow up was 4.98 (4.6–5.3) years (SD: 2.7) and it was similar between these three functional states. All-cause mortality was 8%, 21% and 23% in the three graft states respectively. SH and DKA were experienced by 4.2% and 2.3% of patients, respectively. 197, 177, and 110 patients were followed for at least 1, 2 and 5 years after transplant. Insulin independence was present in 73.6%, 77.4% and 74.5% of patients at 1, 2 and 5 years, respectively. Partial function was present in 24.9%, 20.4% and 16.5% of patients at 1, 2 and 5 years, respectively. The number of patients with partial allograft function was significantly higher in PAK patients ($p = 0.01$). Complete graft failure was present in 1.5%, 2.2% and 9% respectively at the same time points.
Conclusion: SH and DKA were experienced by a minority of patients after PT followed for a mean period of 5 years and insulin independence was present in a significant proportion of the cohort.

P-1.7

Poor glycemic control following renal transplantation in type I diabetics: a call for increased utilization of pancreas after kidney (PAK) transplantation

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Background: PAK transplantation is controversial and remains limited in its application. We recently received approval to commence performing pancreas transplants. We wished to assess the quality of glycemic control in

our type I diabetics that had previously received a renal transplant and to identify potential candidates for PAK transplantation. We hypothesized that those individuals who developed ESRD were likely to have poor glycemic control following renal transplantation, despite close follow-up.
Purpose: To assess the quality of glycemic control and diabetic care in type I diabetics following isolated renal transplantation.
Methods: A retrospective review was performed of all renal transplants performed at our center from 6/03–10/07. Type I diabetics were identified. Demographic information, creatinine at last follow-up and most recent HgBA1C values were collected. 46 patients were identified. Those whose renal allograft failed (n = 4), had received a PAK (n = 1), or were incarcerated (n = 1) were excluded from further analysis.
Results: Demographic information is presented in table 1 below. Creatinine at last folow-up was 1.4 + 0.6 mg/dl. Of the 40 patients, only 5 (12.5%) had

n	40
White	26 (65%)
Black	3 (7.5%)
Hispanic	10 (25%)
Other	1 (2.5%)
Male/Female	17/23
Age	43.5±9.7 years
BMI	25.3±4.5
Follow–up	695±421 days

HgBA1C ≤ 7	5 (12.5%)
HgBA1C > 7–8	11 (27.5%)
HgBA1C > 8–9	8 (20%)
HgBA1C > 9–10	6 (15%)
HgBA1C > 10	6 (15%)

HgA1C values < 7. In 4 (10%) patients we could not find any HgBA1C values following renal transplantation, and 6 (15%) individuals had their most recent HgBA1C > 10. Sixteen patients (40%) had not had a HgBA1C value checked in > 100 days of their last follow-up.
Conclusions: The majority of type I diabetics have poor glycemic control following isolated kidney transplantation. They frequently have sub-optimal diabetic care as evidenced by the high number of patients that are not having their HgBA1C values checked routinely, despite good allograft function and transplant outcomes indicating good post-transplant care. Whatever factors contributed to these patients having poor glycemic control resulting in ESRD prior to transplantation seem to persist after renal transplantation. PAK transplantation is likely the best opportunity for optimal glycemic control in this selected group of individuals.

P-1.8

Pancreas autotransplantation combined with total pancreatectomy for recurrence of pancreatic cancer: a case report

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Total pancreatectomy (TP) is sometimes required for the treatment of pancreatic cancer (PC). Unfortunately, TP always results in unstable diabetes. Recently, living donor pancreas transplantation has been started in our institute. We performed pancreas autotransplantation (PAT) to prevent metabolic disorder using living donor pancreas transplant technique. We herein report a case of a 61-year-old female patient who underwent TP combined with PAT for recurrence of PC. This patient had been type 2 diabetes mellitus and treated with metformin hydrochloride (500 mg/day). Her hemoglobin A1c was 6.5% and C-peptide was 0.9 ng/ml, and urinary C-peptide was 9.5 μg/day at preoperative examination. Primary surgery (pylorus-preserving pancreaticoduodenectomy) for PC was performed in 2006 and histological examination proved R0-resection for pancreatic cancer (pT3pN1pM0, Stage IIB). Three years after surgery, recurrence of PC was found by follow-up computed tomography (CT) scan. Total remnant pancreatectomy was performed for recurrence of PC. Recurrent tumor was solitary, 1.0 × 1.0 cm in size and located in body of pancreas. There was no evidence of invasion or metastasis to the tail of pancreas in the preoperative imaging study and histological examination of frozen section during surgery.

P-1.9

Celsior versus Wisconsin solution in pancreas transplantation

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Celsior solution (CS), which has recently become available, that might theoretically offer a new means for improving graft preservation quality. The present prospective, randomized study was designed to evaluate the efficacy of CS compared with University of Wisconsin (UW) for pancreas allografts. Between January 2001 and January 2007, 88 patients underwent pancreatic transplantation, including the last 30 consecutive simultaneous pancreas kidney patients who were randomly assigned to either CS or UW. There was no case of graft thrombosis in either group. There were two cases of pancreatitis in the UW group compared with one in the CS group. No case of primary nonfunction occurred in either group. There were two cases of early duodenal stump fistulae in the CS group that required transplantectomy, whereas this complication was not observed in the UW group. Relaparotomy in the UW group was required in three cases due to infection and treated by close drainage that which, progressed to fatal sepsis in one patient. In the UW group with 6 months of follow-up, there were 12 patients insulin free. In the CS group, six patients underwent relaparotomy, three for transplantectomy and the others for intra-abdominal infection, which was fatal in two cases. In the CS group with 6 months of follow-up, there were 10 patients insulin free. Two patients died with functioning grafts. These results provided indirect evidence that CS solution is at least as safe as UW to mitigate postreperfusion graft edema and pancreatitis, as well as graft thrombosis.

P-1.10

Intraabdominal bleeding following simultaneous pancreas–kidney transplantation treated with angiographic embolization

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Significant early bleeding is one of the surgical complications following simultaneous pancreas–kidney transplantation that has historically shaped the procedure. The consequence, exploratory laparotomy, carries high morbidity levels and elevated costs for the health system. Angiographic intervention is already a common procedure for the treatment of late, but not early, vascular complications. We describe a case of an early vascular complication that was successfully treated with angiographic embolization in a to simultaneous pancreas–kidney transplant patient.

P-1.11

Pancreatic islet cells antibodies in diabetic patients submitted to pancreas transplantation

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Several pieces of evidence suggest an autoimmune etiology of diabetes mellitus type 1. To trace patients who are susceptible to the disease, we utilized islet cells antibodies (ICAs). The aim of this study was to evaluate the presence of ICAs among diabetic patients undergoing simultaneous transplantation of the pancreas and kidney (SPK). Twenty-six diabetic patients received an SPK, 12 of whom were included in this analysis. The indirect immunofluorescence method was utilized for quantitation of ICAs. The types of ICAs were no different following transplantation of the pancreas. The serum levels of pre-existent ICAS in diabetic patients undergoing SPK with immunosuppression were not reduced, and they did not interfere with the function of the implanted pancreas over a period of 60 days.

P-1.12

One hundred pancreas transplants at a brazilian institution

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Background: After decades of controversy surrounding the therapeutic validity of pancreas transplantation, the procedure has become accepted as the preferred treatment for select patients with Type 1 Diabetes Mellitus. In Brazil simultaneous pancreas–kidney transplantation has gained wide acceptance since the 90's after specific federal legislation classified those patients as priority for organ allocation. At our center a pancreas transplantation program was started on January 2001.
Objective: Report our experience with 100 pancreas transplants performed in a period of 7 years.
Method: Between January 2001 and January 2008, 100 patients underwent pancreatic transplantation at our center, 88 simultaneous pancreas kidney transplantation (SPK) and 12 pancreas transplantation alone (PTA). All of these were primary transplants. Pancreas graft management of the exocrine drainage technique involved enteric drainage in eight (all SPK) and bladder in 92 cases. The recipient systemic venous system was used for the pancreas graft venous effluent in all cases.
Results: Overall results show that the number of functioning pancreatic grafts is 64 after 100 performed. Graft losses were: rejection (eight cases), venous thrombosis (nine cases) arterial thrombosis(one case), or surgical complications such as anastomotic leak (three cases), perigraft infection (10 cases), pancreatitis of the graft(five cases). Most cases of pancreatitis (80%) when preservation time was of more than 18 hours. Rejection was observed less frequently in SPK recipients five cases (5/92 recipients) than PTA recipients (3/12). Death was observed in 24 cases. Death was related in most cases to infection (20 cases). These patients were managed mainly by wide drainage and antibiotic therapy. Three of those were managed unsuccessfully by transplantectomy. We observed a decrease on infection rate related to death after we stopped to routinely perform induction therapy on SPK recipients without and increase on rejection. The four other recipients who died were due to major hemorrhagic stroke, cardiac arrest (two cases) and car accident.
Conclusion: Despite surgical and immunosuppressive complications, is that pancreas transplantation is highly effective therapy for diabetes mellitus, and after 7 years of program, and 100 transplantations performed, there is still a role in the diabetes treatment for allograft transplantation in a near future.

P-1.13

Ten years experience with partial extraperitoneal placement of enteric drained pancreas transplant

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Background: Pancreas transplantation was first performed at CHUM-HND in 1984. Since then, the surgical technique has evolved in multiple aspects. The location of engraftment changed over time. Presently, a partial extraperitoneal approach in the iliac fossa is used with peritoneal separation between vascular and enteric anastomosis and follow-up is done with doppler US and biopsy when required. The aim of this retrospective study is to report on clinical results of this technique.
Patients and methods: One hundred patients who received pancreas transplant between 1999 and 2009 are reviewed. Data include: Graft and patients survival; quality of life (QoL) using a structured patient interview by questionnaire and comparing between patients who received PKT and a cohort of patients who received kidney transplant alone (KTA) during the same period. Biopsy proven acute rejection (BPAR) and their impact on graft survival analyzed by receiver operating characteristics (ROC).
Result: Ten years patient and graft survival are respectively 87% and 80%. QoL measurement showed some evidence that PKT patients were better adjusted to their vocational, domestic, and social environment than KTA ($p < 0.02$). Furthermore, there was high proportion of PKT patients (50%) compared to only 24% of KTA who were working at the time of the study. ROC analysis showed that occurrence of more than one rejection has a significant effect on graft loss (95% CI (0.74–0.94) $p = 0.001$. sensitivity = 0.90; specificity = 1–0.28).
Conclusion: Partial extra peritoneal Pancreas transplantation is a reliable technique to treat type 1 diabetic patients.

P-1.14

The outcome of pancreas transplantation from marginal donors in Japan

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Objectives: A total of 69 cases of pancreas transplantation (PTx), comprising of 52 from deceased donors (DDs), two from non heart beating donors (NHBDs), and 15 from living donors, were performed since "The Organ Transplant Law"(Oct.1997) until Dec.2008. Organ donation has been still limited because of the strict donor criteria in Japan. Further, most of donors are considered marginal because of the poor donor conditions. The effectiveness of PTx from marginal donors (MDs) was examined in this study.
Methods: Fifty-four cases of PTx from DDs and NHBDs were examined . The majority of PTx was SPK category with enteric drainage. Tacrolimus-based induction therapy (mostly, basiliximab) was used.
Results: The average donor age was considerably older (43 years; 18–72) with donors over age 40 comprising 65% of the total. Fifty-nine percentage in the cause of death was due to cerebrovascular accidents. According to the Kapur's critria, 76% in our series could be considered marginal. The ratio of liver to pancreas grafts was 1.0 (56/54) in Japan while 3.26 in all American OPOs. Thus, to increase blood supply into pancreatic head, a gastroduodenal artery was reconstructed as much as possible when liver procurement was performed. Mean total cold ischemic time of pancreatic was 11 hour 42 minutes. Recipients, consisting of 28 men and 26 women, were in their 30's (61%) and in their 40's (31%). Mean duration of dialysis was 5.7 (0–17) years. The recipients had a quite longer mean waiting time of 1035 (111–3167) days. Twenty-three patients on the waiting list died of diabetic complications. Only one patient died of cardiac event postTx. Ten pancreatic grafts were lost by thrombus (5) in the acute phase and some reasons (5) in the chronic phase. Nine out of 10 graft failures came from MDs. Pancreas graft survivals (GS) at 1, 3 and 5 years postTx were 86%, 81%, and 74%, respectively. In contrast, kidney GS in SPK were 92%, 92%, and 71%, respectively.
Conclusion: Despite poor donor conditions, we have obtained the satisfactory results so far. Most of pancreatic graft failures occurred in PTx from MDs. Further evaluation should be examined to identify the risk factors in MDs.

P-1.15

Efficacy of immediate post-transplantation CT angiography in pancreas transplantation

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Graft thrombosis at immediate post-transplantation period is still a heel of Achilles in pancreas transplantation. In this study we evaluate the efficacy of computed-tomography angiography on monitoring of graft patency at immediate post operative period. One hundred and twelve pancreas transplant recipients who underwent pancreas transplantation including simultaneous pancreas–kidney (SPK), pancreas after kidney (PAK) and pancreas transplantation alone (PTA) between October 1992 and May 2009 in a single center, were enrolled in this study. We routinely used heparin (subcutaneous or systemic) during and post pancreas transplantation and switched to oral anticoagulation (warfarin). Oral anti-coagulation was continued for 3 months post-transplantation in PTA or PAK, and for 1∼3 months post-transplantation in SPK. We had monitored graft thrombosis with color doppler ultrasonography (US) untill July 2005, and after then we have performed CT angiography for the monitoring of graft thrombosis at post operative day 2. We divided all the recipients into two groups, color doppler US monitoring group (Group A) and CT angiography monitoring group (Group B). In this study we retrospectively analyzed the efficacy of diagnosis for graft thrombosis between two groups according to imaging modality. Finally graft survival was analyzed with Kaplan–Meier Methods. We have performed 51 pancreas transplants (28 SPKs, 4 PAKs and 19 PTAs) in Group A, and 61 pancreas transplants (34 SPKs, 5 PAKs and 22 PTAs) in Group B. We diagnosed total vascular thrombosis in two recipients (3.9%) and partial venous thrombosis in one recipient (2.0%) which was confirmed negative by exploration in Group A ($n = 51$), and in 1 (1.6%) and 17 (27.9%) in Group B ($n = 61$) respectively. Total veinous thrombosis of the graft in three recipients could not be saved, however partial thrombois in 17 patients in group B were resolved by conservative management. There was no contrast media-related

complication in group B. There was no difference in graft survival between recipients with partial thrombosis and patients without thrombosis in group B. Partial venous thrombosis at immediate post-transplantation period had no effect on graft survival. Evaluation of graft patency with CT angiography was safe and efficient for screening of vascular thrombosis and management of pancreas transplantation.

P-1.16

Epidermal nerve fibres in pancreas transplant recipients: follow-up after 2.5 years of normoglycemia

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Background: Skin biopsy with epidermal nerve fibre (ENF) counts may be used to analyze possible nerve regeneration in patients with type 1 diabetes mellitus (DM) and neuropathy following pancreas transplantation (PTx). We report on the results of lower limb ENF counts in normoglycemic PTx recipients at 2.5 years of follow-up.
Methods: Skin biopsies were performed using a 3-mm punch from lower thigh and upper calf areas of 20 PTx recipients (simultaneous kidney and pancreas in 18, pancreas after kidney in one, and PTx alone in one patient; of mean [±SD] age 47 ± 9 and DM duration of 27 ± 9 years) at the time of PTx and at 30 ± 5 months post-transplant. Fourteen sex- and age-matched healthy controls (C) were also examined. After fixation and freezing, 40-μm sections were stained using rabbit polyclonal antibody to the panaxonal marker PGP 9.5 followed by mouse anti-rabbit IgG antibody conjugated with rhodamine. Samples were imaged with a digital camera mounted on a microscope equipped for fluorescence. The average number of ENF per mm length of epidermis was derived.
Results: Insulin-independence with excellent metabolic control (HbA1c: PTx 5.4 ± 0.5 versus C 5.6 ± 0.3%; $p > 0.05$) and adequate kidney graft function (S-creatinine 1.3 ± 0.3 mg/dl) were achieved in PTx recipients. Severe depletion of ENFs was present in baseline skin biopsies from PTx recipients (PTx versus C, thigh: 0.7 ± 1.2 versus 11.4 ± 4.2; calf: 0.3 ± 1.0 versus 8.0 ± 3.0 ENF/mm; $p < 0.001$) with total ENF absence in thigh and calf samples of 12 and 17 PTx recipients, respectively. No significant change was seen at follow-up (thigh: 1.5 ± 2.3; calf: 0.3 ± 0.7 ENF/mm; $p > 0.05$ versus baseline) with absence of ENF fibres in 8 thigh and 15 calf biopsies.
Conclusions: Lower limb epidermal nerve fibre depletion was not improved following establishment of normoglycemia in pancreas transplant recipients. Although still longer follow-up periods may be needed, these results confirm the presence of structural, poorly reversible neuropathy in pancreas transplant recipients. Besides vascular disease and immunosuppressive treatment, this may represent an additional risk factor for the occurrence of diabetic foot complications in the post-transplant period.

P-1.17

Surgical complications in simultaneous pancreas and kidney transplantation (SPK) with enteric exocrine drainage and systemic venous drainage

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Background: Surgical technique for SPK has undergone many changes over time. Venous thrombosis and anastomotic leak represent the most frequent complications with an incidence of 2–10% each, and they remain the main causes of early pancreas loss after transplantation. In this study we reviewed surgical complications occured in 83 consecutive SPK which have all been performed with the same surgical technique at our centre.
Methods: From September 1996 to May 2009 we performed 83 SPK with enteric exocrine drainage by a mechanical duodenum-ileum anastomosis and systemic venous drainage on the inferior caval vein. Donors and recipients mean age were 30 ± 8 years (15–48) and 40 ± 8 years (23–59) respectively. Mean pancreas cold ischemia time was 8 ± 1 hours. Immunosuppressive therapy was based on TAC + MMF + steroids.
Results: After a mean follow up of 61 ± 34 months, 5-year actuarial survival rates were 99%, 97% and 91% for patient, kidney and pancreas respectively. We had one early pancreas loss due to venous thrombosis. No anastomotic leak occurred; 18 pts had enteric bleeding only one requiring reoperation and three patients had hemoperitoneum. Five patients lost their pancreas due to acute pancreatitis.
Conclusions: In our experience, the use of a surgical technique in SPK with mechanical enteric exocrine drainage and systemic caval venous drainage has lead to a very low incidence of surgical complications such as venous thrombosis and enteric leakage. Acute pancreatitis, which is mainly related to donor risk factors, is currently the principal cause of early pancreas loss after transplantation.

P-1.18

Percutaneous embolization of peri-duodenal varix due to portal hypertension in a patients with kidney–pancreas transplantatation

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Vascular complications associated with pancreas transplantation are not uncommon and are cause of serious complications. We report a case of a 32 years old woman with a hystory of 28 years of insulin depedent diabetes mellitus and celiac disease. She underwent liver transplantation when she was 22 years old for acute hepatitis. After 7 years the patiens developed end stage kidney disease and began haemodialisis and listed for a kidney–pancreas trasplantation. This one was performed successfully when she was 29 years old with enteric diversion (Roux intestinal loop recostruction).

After 5 years was admitted in our hospital with serious intestinal bledding and poor liver function. The ultrasound showed a pattern like a arteriovenous fistula near the head of the pancreas. CTscan was not diagnostic and arteriogram showed the presence of mesenteric varix and a shunt mesenteric-cava through the duodenum of the pancreatic graft. The liver biopsy and portal pressure gradient showed a portal hypertension and a liver cirrhosis. In order to take time to wait for a new liver the patients underwent percutaneous embolization of mesenteric varix through jugular access. The procedure was uneventfull and the patient was successfully transplanted 2 months later. Pancreas function was always satisfactory.

P-1.19

Analysis of inhaled anesthetic agents in pancreas transplantation

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Objective: Recent anesthesia research suggests that certain inhaled anesthetic agents have an improved capacity to ameliorate ischemia-reperfusion injury in transplanted organs. This study compares three primary inhaled agents, isoflurane, desflurane and sevoflurane in pancreas transplantation. Primary outcomes included early graft loss and perioperative serum lipase levels, as well as graft function and survival.
Methods: Data were extracted using a retrospective review of all pancreas transplants between 2003 and 2009 ($n = 308$), with an extensive review of all recipient and donor demographics, as well as post-transplant outcomes. The choice of primary anesthetic agent was random and at the discretion of the anesthesiologist without input from the surgeon. Early graft loss included loss of graft function for any reason within 7 days of transplant. Serum amylase and lipase levels were measured daily in the immediate post-transplant period. Serum HbA1c and C-peptide levels were recorded for any available value up to 1-year post-transplant and the most recent values available.
Results: The 308 transplants included simultaneous pancreas–kidney (SPK, 56%), pancreas after kidney (PAK, 24%), and pancreas transplant alone (PTA, 20%). The sevoflurane group was more likely to include isolated pancreas transplantation (either PAK or PTA, $p < 0.001$). The isoflurane group had the highest rate of early graft loss and the worst graft survival at 1-year; this group also had the highest peak serum amylase and lipase in the first 30-days post-transplant. The sevoflurane group had the lowest serum lipase levels for each of the first 7 days post-transplant.
Conclusion: These results suggest that sevoflurane is the best inhaled anesthetic agent as this group had the lowest perioperative lipase levels and better than expected outcomes given the preponderance of isolated pancreas transplants in this group. Isoflurane had the worst outcomes, with the highest early graft loss and lowest 1-year graft survival.

P-1.20

Is there a benefit of induction therapy and calcineurin inhibitor postponing?

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Background: Kidney Delayed Graft Function (KDGF) is a prevalent complication after Simultaneous Pancreas–Kidney Transplant (SPK). The role of induction therapy and postponing calcineurin inhibitors (CI) for management of KDGF is still controversial.
Methods: From 1996 to 2009, 122 SPK were retrospectively analyzed regarding the occurence of KDGF . KDGF was defined as the need of dialysis during the first week post-transplant. Only patients with KDGF were selected for this study and divided in Group 1 – use of induction (OKT3 or Thymoglobulin) and delayed CI (beginning from postoperative day 7 to 10) and Group 2 – without induction and immediate use of CI. Time for recovery of KDGF was analyzed in the two groups. This period of recovery included all post-transplant days up to the last session of dialysis needed.
Results: KDGF occurred in 25 patients (20.5%) after SPK, of which 15 with (Group 1) and 10 without (Group 2) induction therapy. There was no difference between the two groups regarding cerebrovascular event as cause of donor death, recipient gender and age, preemptive transplant and cold ischemia time. Group 1 presented higher donor age (31.6 versus 24 years, $p = 0.042$). Time of recovery of KDGF was similar between the two Groups (14.6 versus 14.1 days, $p = 0.298$).
Conclusions: There was no benefit of using induction therapy and postponing CI for abbreviation of period of recovery of KDGF after SPK.

P-1.21

Surgical technique video description of retroperitoneal placement of pancreas graft with portal-enteric drainage

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Background: Difficulties were reported in arterial reconstruction in pancreas transplantation (PTx) with portal-enteric drainage (PED) especially in male recipients with omental obesity. Furthermore, since the graft is usually located intraperitoneally in a central position, graft accessibility for percutaneous biopsy may also be problematic. We herein show the video of a recently described method for PTx with PED, in which the pancreas graft is located in the retroperitoneal space behind the right colon mesentery.
Methods: Between April 2001 and March 2009 a total of 159 recipients were scheduled for PTx with PED. Pancreas were procured en-bloc with liver and kidneys. No venous extension graft was used, while a donor iliac artery bifurcation Y graft was employed routinely. In the recipient, the abdomen was entered through a midline incision. The right colon was mobilized allowing exposure and dissection of both the superior mesenteric vein (SMV) and the right common iliac artery (CIA). The venous anastomosis was created end-to-side between donor's portal vein and recipent's SMV. The arterial anastomosis was then created end-to-side between donor's Y iliac graft and recipient's CIA. Enteric drainage was obtained through a Roux-en-Y jejunal limb. The pancreas graft was eventually placed in the right retroperitoneal space, being covered by the ascending colon and its mesentery.
Results: Retroperitoneal PTx with PED was carried out in 158 out of 159 recipients (99.4%): 88 SPK (66 using a cadaveric kidney and 22 a live donor kidney), 58 PTA and 12 PAK, irrespectively of recipients' body max index or size of SMV. Arterial anastomosis was always performed with ease, despite keeping the Y iliac artery graft extremely short. In a recipient of a pancreas after kidney graft, systemic drainage was preferred to portal effluent due to intraoperative diagnosis of liver cirrhosis. Relaparotomy rate was 14.5%. Three (1.9%) pancreas grafts were lost due to thrombosis; no additional grafts were lost because of surgical complications. Percutaneous biopsy was always possible: 20 biopsies were performed (75% diagnostic) in 15 recipients. One-year patient and graft survival were 95.1% and 88.3%, respectively.
Conclusions: The technique shown in this video has distinctive technical advantages as compared to the "classical" method in which the graft is located intraperitoneally.

P-1.22

Pancreas transplant alone in child: a case report

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Background: Pancreas transplantation alone (PTA) remains a rare procedure and may be indicated for selected brittle diabetic patients. There have been only a few pancreas transplants (PT) performed in pediatric patients worldwide.
Case Report: This report presents a thirteen-year-old male patient with type 1 diabetes mellitus since four years old. After multidisciplinary evaluation and close follow-up, PTA was indicated based on unstable diabetes and frequent episodes of hypoglycemic unawareness demanding frequent hospital admissions. A whole organ bladder and systemic drained PT was performed. Immunosuppression included thymoglobulin, tacrolimus, mycophenolate mofetil and steroids. Early outcome was uneventful and the patient was discharged 12 days after surgery normoglycemic and insulin-independent. There was a first episode of acute rejection (grade II) 20 days post-transplant successfully treated with corticosteroids. A second episode (grade IV) occurred 13 months post-transplant and required treatment with thymoglobulin and conversion from steroid to sirolimus. The patient is currently on the 42nd month of follow-up in use of tacrolimus, sodic mycophenolate and sirolimus, experiencing good clinical outcome, an insulin-free state and significant improvement in quality of life. According to medical literature this is the youngest patient submitted to PTA in the world.

P-1.23

Simultaneous pancreas–kidney transplant in a HIV recipient – case report

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Background: After the development of highly active antiretroviral therapy (HAART) for HIV patients, an increasing interest in organ transplantation for this selected population has arisen. There is a lack of reports about pancreas transplant in HIV recipients.
Case Report: We report the case of a 43-year-old man HIV + who presented type 1 diabetes for 25 years and end-stage-renal disease, on dialysis therapy during the last three years. His CD4 count was 830 cells per milliliter and a negative viral load was achieved after 3 months on antiretroviral therapy. His nutritional status was favorable and no opportunistic infections had occurred. A simultaneous pancreas–kidney transplant was performed from a 19-year-old deceased donor victim of trauma. Pancreas technique was enteric-portal drainage and a no induction immunosuppression protocol was employed including the use of tacrolimus, sodic mycophenolate and steroids. In the postoperative period, there was a kidney delayed graft function requiring hemodialysis for 14 days. In the postoperative day 11, a kidney biopsy showed mild rejection, successfully treated by steroids. The patient was discharged after 22 days normoglycemic, insulin-independent, and with serum creatinine of 1.9 mg/dl. Currently, his outcome has been uneventful, without readmissions or any opportunistic infections. After 5 months post-operatively, viral load is negative and CD4 count is 460 cells per milliliter. The current serum creatinine is 1.1 mg/dl and no insulin has been required.

P-1.24

Pancreas allograft preservation with UW alone or mixed solutions for donor aorta flush – is there a difference?

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Background: Since the advent of new preservation solutions for organ transplantation, different protocols of donor aorta flush have been used for procurements. Whether UW alone or mixed solutions through donor aorta plays a role on post-transplant outcome remains controversial.
Methods: From 2005 to 2009, 111 pancreas transplants (PT) were retrospectively analyzed regarding the type of donor aorta flush. There were 64 simultaneous pancreas–kidney, 39 pancreas after kidney and eight pancreas transplant alone. These patients were divided in Group 1 – flush with UW alone ($n = 81$) and Group 2 – flush with mixed solutions ($n = 30$). Donor aorta was always flushed with 2 liters of solutions. In Group 2, the first liter

used was diversified among Eurocollins, Soltran, Celsior or HTK(according to the liver team) solutions and the second liter was always UW. Several parameters were studied and compared between the two groups: donor age, cerebrovascular event as cause of death of donors, recipient age and gender, cold ischemia time, peak and 10th post-operative day serum amylase and lipase, 1-year patient and pancreas survival and the occurrence of pancreas technical complications (pancreatitis, thrombosis, leak and ischemia/reperfusion injury).
Results: The single variable with significant difference was the peak amylase, higher in Group 1 (469.0 versus 238.6, p = 0.012). Cold ischemia time was similar in the two Groups (14.6 vs 13.9 hours, p = 0.450). One year patient (86.4 versus 86.6%) and pancreas (76.5 versus 76.6%) survival were also similar as was the rate of technical complications (22.2 versus 20%).
Conclusions: The use of a preservation solution different from UW for the first liter of donor aorta flush did not influence the outcome of PT. Flushing donor aorta with mixed solutions (UW and other) has achieved results at least as good as those obtained with UW alone.

Clinical islet transplantation

P-2.1

Results of islet autotransplantation after extended pancreatectomy for benign disease of the pancreas and their significance for live donation

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Backgroud: Islet autotransplantation is successful in the prevention of surgical diabetes after pancreas resection for chronic pancreatitis (CP), with insulin independence rates of 50% at 1 year. We report our experience with islet autotransplantation after extensive pancreatectomy for the resection of benign tumors of the pancreas and compare the results with those of autologous donors with CP and donors with brain death (DBD).
Methods: Between January 1992 and December 2008, 12 patients underwent extensive left pancreatectomy for benign lesions located at the neck of the pancreas. One patient had complete traumatic section of the pancreas. Eleven tumours were separated from the specimen and sent for extemporaneous pathological examination. After unequivocal diagnosis of benignity, the rest of the specimen was processed and unpurified pancreatic digest was infused into the portal vein. Isolation results were compared with those obtained from 10 CP patients or 303 DBD.
Results: Tumours were seven cystadenomas and three insulinomas. Mean islet yields were 248'121 IEQ vs 110'290 in CP (p = 0.03) and 345'201 in DBD (p = 0.89). Normalized to weight of pancreatic tissue processed, we isolated 5'895 IEQ/gram vs 1'457 in CP (p = 0.007) and 3'932 in DBD(p = 0.005), and transplanted 3'839 IEQ/kg body weight versus 2'196 in CP (p = NS). Median follow-up for benign disease was 90 months, one patient died from unrelated causes after 12-years. After a 7.5-year follow-up, all patients have positive basal and stimulated C-peptide levels and normal HbA1c, and 11/12 patients are insulin-free.
Conclusion: Islet autotransplantation after extensive pancreatic resection for benign disease is a successful procedure. Pancreatic surgical specimens (a situation near-identical to live donation) yield higher numbers of islets per gram of tissue and similar total islet numbers as whole organs from DBD.

P-2.2

Islet function is similar in alloislet and autoislet transplant recipients, despite the lower islet mass of islet autotransplants

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Background: Alloislet transplant is a promising treatment for type 1 diabetes. Despite high rates of insulin independence early after islet allotransplant, islet longevity remains suboptimal. Possible contributing factors include autoimmune recurrence, alloimmune rejection, or toxicity of immunosuppressant medications. In contrast, islet autografts, infused at the time of pancreatectomy for chronic pancreatitis, are not subject to these variables. We compared islet function in autograft and allograft recipients at a similar duration post-transplant.
Methods: We enrolled 8 autograft and 8 allograft recipients, matched for similar duration posttransplant (mean 2.1 ± 1.2 years) and BMI (22.4 ± 3.2 kg/m^2). Patients were insulin-independent or required only minimal insulin. Patients underwent a 4-h oral glucose tolerance tests (OGTT), intravenous glucose tolerance test (IVGTT), and arginine stimulation test (AST) at a glucose of ~150 mg/dl.
Results: Age, gender, BMI, duration posttransplant, and hemoglobin A1c levels were similar between groups. Alloislet recipients received significantly more islet equivalents per kg body weight (IE/kg) than the autograft patients (9,958 ± 6,229 IE/kg vs 4,589 ± 1,232 IE/kg, p = 0.03). The mean glucose excursion (area under the curve, AUC) during OGTT did not differ between groups. There was no difference in the acute insulin response to glucose (AIRg) and arginine (AIRa) (Table 1).

	Alloislet	Autoislet
AUC glucose (mg/dL*min)	42,303 ±1,303	45,970 ±10,445
AIRg, ratio peak:baseline	4.2 ± 2.7	7.4 ± 8.0
AIRa, ratio peak:baseline	4.4 ± 2.3	3.9 ± 1.6
Presented as mean ± standard deviation		

Conclusions: In conclusion, autograft recipients demonstrated similar acute insulin secretion and glucose excursion as allograft recipients, despite receiving less than half as many islets. Either transplanted islets function better in the autotransplant setting, or, more likely, they have increased engraftment and survival. Lack of autoimmune or alloimmune destruction or lack of immunosuppressive drug toxicity may account for the similar functional capacity of islet autografts despite a lower transplanted islet mass.

P-2.3

ET-Kyoto ductal injection and density adjusted purification combined with potent anti-inflammatory strategy facilitated single donor islet transplantation

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Background: One of the current issues of clinical islet transplantation is necessity of multi donors for achieving insulin free status. We developed modified islet isolation method which includes modified ET-Kyoto ductal injection and density adjusted purification for non-heart-beating donor (Kyoto method). Recently we adapted the Kyoto islet isolation method for brain-dead donor and achieved high success rate of clinical islet isolation. In this study, we used this modified islet isolation and potent anti-inflammatory strategy to examine the possibility for single donor islet transplantation.
Method: Two islet isolations were conducted in September and October 2008. Ductal injections were performed at the procurement site using modified ET-Kyoto solution (1 ml/g pancreas) and both pancreata were preserved by two-layer (oxygen charged perfluorochemical/ modified ET-Kyoto solution) method. Pancreas was digested using Ricordi method and islets were purified by density adjusted continuous density gradient with ET-Kyoto solution and iodixanol. Donor and islet characteristics were shown in Table 1. Anti-thymocyte globulins were administered at day 0, 2, 4 and 6 as immunosuppressive induction. For anti-inflammatory strategy we used Eternacept and Anakinra. Eternacept was given at day 0, 3, 6 and 10 and Anakinra was given from day 0 to 7. Tacrolimus and Mycophenolate mofetile were used for maintenance.
Results: Both two patients have achieved insulin independence with excellent glycemic control after single islet infusion. Average SUITO indexes between day 3 and 30 were 29.2 and 45.3 respectively, while SUITO index more than 26 represents that the patients had enough functional islet mass for insulin independence (Matsumoto S. et al. Transplant Proc 2005;37:3435). The most recent SUITO indexes after 6 months were 46.7 and 56.0 respectively.
Conclusion: Our modified islet isolation protocol combined with potent anti-inflammatory strategy made it possible to achieve insulin independence after single donor islet transplantation.

Table 1 Donor and Islet Characteristics (P-2.3)

	Case 1	Case 2
Donor age (y)	57	55
Donor BMI (kg/m2)	25	32
Pancreas weight (g)	87	128
Cold ischemic time (min)	148	162
Total islet yield (IE)	543,225	927,227
Islet yield / patient weight (IE/kg)	9,895	12,216
Purity (%)	60	60
Viability (%)	96.8	98.8
Tissue volume (mL)	5	5

P-2.4

Super high dose islet transplantation is associated with high SUITO index and prolonged insulin independence

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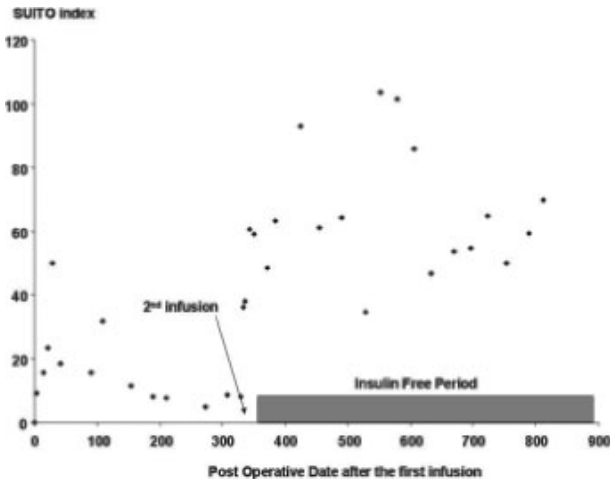
Background: One of the current issues of clinical islet transplantation is difficulty of achieving prolonged insulin free status. It has been shown that functional islet mass gradually decreased after islet transplantation. We developed SUITO index which reflected engrafted islet mass. The formula of SUITO index is that the fasting C-peptide (ng/ml)/(fasting glucose-63 mg)×1,500. SUITO index more than 26 represents that the patients had enough functional islet mass for insulin independence (Matsumoto S. et al. Transplant Proc 2005;37:3435). In this study, we have shown that super high dose islet transplantation was associated high SUITO index and prolonged insulin free status.

Method: Two islet isolations were conducted in February 2007 and January 2008. Ductal injections were performed at the procurement site using ET-Kyoto solution and both pancreata were preserved by two-layer (oxygen charged perfluorochemical/ET-Kyoto solution) method. Islets were isolated using Ricordi method. Both isolated islets were transplanted into a type 1 diabetic patient. The patient was 58 years old and body weight was 57 kg. Islet yield were 514,467 IE and 872, 174, purities were 49% and 85% and viabilities were 91% and 99% for the first and second islet transplantations respectively. Therefore she received total 24,327 IE/kg body weight. The immunosuppression was based on the Edmonton protocol.

Results: After the first islet transplantation, the average SUTO index for the first month was 24.6. The glycemic control became stable and daily insulin dose became less than half but did not achieve insulin free status. After the second islet transplantation, the average SUITO index for the following 1 month was 48.5 and she became insulin free with excellent glycemic control. Her SUITO index remained high and average SUITO index after the first month was 65.9. The most recent SUITO index was 70 at POD 812 with insulin free status.

Conclusion: Super high dose islet transplantation (>24,000 IE/kg) was associated with high SUITO index and prolonged insulin independence.



P-2.5

Pancreatic ductal preservation imroves islet isolation outcome in autologous transplantation for chronic pancreatitis

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Background: For patients with Chronic Pancreatitis (CP), total or partial pancreatectomy followed by autologous islet transplantation offer promise to eliminate pain and also avoid post-surgical diabetes. Maximization of islet yields from often fibrotic and inflamed organs is crucial for achieving post-transplant insulin independence. We adapted pancreatic ductal preservation method developed for deceased donor pancreata toward auto-islet isolations and report on the improved outcome.

Methods: Eleven patients (2 men, 9 women; ages 22–47 years) underwent total (n = 10) or partial (n = 1) pancreatectomy for the treatment of CP refractory to maximal medical management. Pancreata were preserved in UW solution without ductal cannulation (control) in initial three cases and eight pancreata were preserved with pancreatic ductal injection using ET-Kyoto solution followed by the two-layer preservation method (ductal preservation group). Islets were isolated by modified Ricordi method and were purified only in one case. All islet infusions were performed under general anesthesia into the portal venous system with portal pressure monitoring.

Results: When compared to the control group, total islet yields were higher in the ductal preservation group (129,313 ± 89,421 IE vs 466,885 ± 262,319 IE) and islet yield/gram pancreas weight showing significant increase (1,336 ± 791 vs 5,794 ± 2,267 IE/g; $p < 0.01$)). Pellet size was also higher in ductal preservation group (5.33 ± 0.6 vs 11.9 ± 7.0) suggesting that this method of preservation effectively protected pancreatic tissue against autolysis. Post-transplant islet function as evidenced by first month basal c-peptide was also higher (1.4 ± 0.7 vs 2.1 ± 0.9). There were no major technical complications related to the infusion. In two cases, due to atrophy of exocrine tissue the purity of islets obtained was greater than 65%.

Conclusions: Our results suggest that higher islet yields can be achieved even from chronically inflamed and fibrotic organs using ductal preservation. The techniques applied for islet isolations from cadaver pancreata are showing promise for pancreata from CP patients. These results may justify further expansion of total or partial pancreatectomy followed by islet auto transplantation for the treatment of CP.

P-2.6

Influence of autoimmunity in clinical islet transplantation evaluated by type 1 diabetes-specific auto-antibodies

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Background: Recurrence or persistence of autoimmunity in clinical islet transplantation, evaluated by type 1 Diabetes-specific auto-antibodies (AAbs) may contribute to islet graft dysfunction and loss. Auto-reactive CD45RO+ memory and CD8+cytotoxic T-cells against type 1 diabetes-related epitopes seem to be involved in post-transplant re-activation or persistence of autoimmunity against generic beta-cell antigens coming from the infused islets.

Objectives: To determine the possible influence of recurrent or persistent autoimmunity in clinical islet transplantation on islet graft function and survival by evaluation of type 1 Diabetes-specific AAbs during post-transplant follow-up.

Methods: Forty-four patients with long-term type 1 Diabetes, who received an islet transplant at our institution from 2000 to 2007, were monitored for anti-GAD65, anti-IA2 and anti-insulin AAbs, and classified as negative or positive (persistent positive or converter-to positive) according to their levels and changes during follow-up (positivity defined by two or more values above normal reference levels, in at least one type of AAbs).
Results: Forty islet transplant recipients were AAbs positive for at least one type of AAbs. At monitoring, for anti-GAD65 AAbs, 22 patients were negative, 14 persistent positive, 8 converter-to positive; for anti-IA2, 27 were negative, 10 persistent positive, 7 converter-to positive; for anti-insulin, 12 were negative, 19 persistent positive, 13 converter-to positive. Kaplan–Meier curves for islet graft dysfunction (41 recipients with dysfunction, 3 insulin-free) and failure (23 recipients C-peptide negative, 21 with function) showed that, of the types of AAbs, none was singularly a determinant factor for outcomes, with only the positivity for anti-insulin AAbs shortening islet graft function (negative vs persistent positive and converter- to positive, $p < 0.05$). When considering AAbs cumulatively, positivity for any type of AAbs resulted in poorer outcomes, with shorter graft function and survival (negative vs positive, $p < 0.05$).
Conclusions: Type 1 Diabetes-specific AAbs can persist or recur after clinical islet transplantation despite immunosuppression. Positive anti-insulin AAbs showed to negatively influence islet graft function, while any positivity for AAbs can contribute to reduce islet graft function and survival.

P-2.7

Immune monitoring in clinical islet transplantation by assessment of induced-activation of CD4+ T-lymphocytes

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Background: Immune monitoring in clinical islet transplantation is mainly based on different immune responses combined with drugs trough levels, metabolic parameters and clinical status, none alone being completely reliable in preventing graft dysfunction or failure, infections or immunosuppressive side-effects.

Immuknow™ by Cylex® is a simple FDA-approved test used for immune monitoring in solid organ transplant recipients. It detects the capability of activation of CD4+ T-lymphocytes stimulated by phytohemagglutinin, measured as ATP production, the levels of which significantly correlate with clinical outcomes.
Objectives: To define CD4+ T-cell activation levels in clinical islet transplantation correlated with clinical outcomes, to help in managing graft stability and to reduce immunological and immunosuppressive risk.
Methods: During a 2-year follow-up, 176 samples from 26 islet transplant recipients with functioning grafts were analyzed using the Immuknow™ assay (at least two tests for each patient). Recipients were categorized in groups according to islet graft function (stable, dysfunction or failure), episodes of infections or serious side-effects. To better characterize the study group, 68 patients with long-term type 1 Diabetes and 53 healthy subjects were used as age- and sex-matched control groups.
Results: Recipients with stable islet grafts had ATP levels (mean ± SD) of 216 ± 47 ng/ml, those with islet graft dysfunction or failure of 370 ± 69ng/ml, while those with infections or toxic side-effects of 91 ± 57 ng/ml.

Long-term type 1 diabetic patients had ATP values of 400 ± 99ng/ml, while healthy subjects of 469 ± 67 ng/ml. Significant differences were found amongst islet transplant recipients, long-term type 1 diabetic and normal subjects, except for ATP levels at islet graft dysfunction or failure that approximated ($p = ns$) those at baseline (Figure). In islet transplant recipients, significant positive correlations were seen amongst ATP levels and either CD4+ T-lympocyhytes ($p < 0.01$), fasting plasma glucose ($p < 0.01$) and HbA1c ($p < 0.05$).
Conclusions: This retrospective observational study has defined the levels of induced-activation of CD4+ T-cell in clinical islet transplantation. Immuknow™ assay can be of utility for immune monitoring in clinical islet transplantation, to reduce the risk of graft dysfunction or failure, infections or side-effects.

P-2.8

Initiation of a islet isolation program in The Netherlands

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Background: Our aim was to set up a human islet isolation facility in the Netherlands for clinical islet transplantation. Islet isolation procedure and islet assessment The islet isolation laboratory was allocated human (heart-beating-)donor pancreases for clinical and research purposes since its initiation in 2006. Isolation and purification of islets ($n = 53$ procedures) was performed at the GMP-clean room facility of the Leiden University Medical Center.
Results: Isolation and subsequent purification typically resulted in islet cultures of 60–90% purity. Islets were cultured for 1–7 days with preservation of islet morphology and insulin content for the duration of the culture period. The average yield of isolated islets per donor pancreas (weight: 102 ± 22.9 g) was 504.800 ± 294.000 islet equivalents ($n = 26$ during the past year), with low rate of cell death ($< < 5\%$ PI+) and high viability ($> > 80\%$ FDA+). Islet yield was positively correlated with BMI ($r = 0.51$, $p < 0.01$); [$p < 0.01$; 371.500 ± 168.800 (BMI < 25, $n = 16$) and 718.200 ± 332.400 (BMI > 25, $n = 10$)] and age [$p < 0.05$; 560.700 ± 321.400 (age < 50 years, $n = 19$) and 353.000 ± 166.100 (age > 50 years, $n = 7$)]. GIIS results in 4–10× insulin release over baseline. Isolation of islets from non-heartbeating donor pancreas resulted in a good islet yield (519.300 ± 207.500 IEQ, $n = 3$) and GIIS (> 5 fold) Six clinical islet transplantations were performed in three patients. In one patient a mixed meal test was performed after the second Tx and showed a maximum C-peptide response of 4.24 nmol/l.
Conclusion: This initial outcome illustrates the current feasibility, procedural integrity and efficacy of the islet isolation program in the Netherlands. Non-heartbeating pancreas may be a potential source for clinical transplantation in the near future.

P-2.9

Kyoto islet isolation method allows efficient islet retrieval from young donor pancreas for clinical use

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Background: It has been well known that donor factors influence the outcome of islet isolation and transplantation. Several groups reported that lower isolation results were achieved with pancreata from donors younger than 20 years of age. Since it is difficult to separate the islets efficiently from the surrounding exocrine tissue, mantled islets are generated during islet isolation from young donor pancreas. This leads to the result of poor recovery rate after purification. On the other hand, it has been reported that young door islets offer high qualitative and clinically appealing features. We have developed a new islet isolation protocol, called the Kyoto Islet Isolation Method, based on the Ricordi method. It includes the modified two-layer (M-Kyoto/perfluorochemical [PFC]) method of pancreas preservation, pancreatic ductal protection, and new islet purification solution (Iodixanol-based solution). The technology enables us to make diabetic patients insulin independent using islets from 2 or 3 pancreata from Donation of Cardiac Death (DCD) donors. The purpose of this study is to assess the efficacy of Kyoto Islet Isolation Method for young donor pancreata.
Methods: We performed four cases of islet isolation using pancreata from donors younger than 20 years of age with the Kyoto Islet Isolation Method. We evaluate the outcomes of these islet isolations by comparing to ones of islet isolations using older donors in islet yield before and after purification, purity and viability.

Results: Young donor (n = 4) age was 17.3 ± 2.5 years (14–20). Islet yields before purification was 568,846 ± 42,154IE and after purification was 426,264 ± 58,981IE. Viability was 98 ± 2% and purity was 55 ± 5.8%. All preparations were eventually transplanted to the patients. Older donor (n = 21) age was 48.6 ± 12.2 years (22–62). Islet yields before purification was 544,576 ± 293,786IE and after purification was 374,318 ± 200,614IE. Viability was 97 ± 4% and purity was 48.6 ± 13.4%. Sixteen of 21 preparations met all the criteira for our product release. The outcomes of islet isolation using young donors with the Kyoto Islet Isolation Method were comparable to that of using older donor.
Conclusions: Kyoto Islet Isolation Method allowed efficient islet retrieval from young donor pancreata. It is thought that this method encourages the utilization of islets derived from young donors for islet transplantation.

P-2.10

Effects of exercise on glucose homeostasis in clinical islet transplantation
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Islet transplantation (IT) can restore insulin independence and eliminate severe hypoglycemia in subjects with T1DM but long-term outcomes are not optimal. Exercise training improves insulin sensitivity (SI), is the first line of therapy for T2DM (together with diet), prevents progression to T2DM, and may enhance β-cell function. Regular physical activity may improve long-term islet allograft function by improving glucose homeostasis. We evaluated 25 IT recipients (18 Islet Alone, 5 Islet After Kidney and 2 Islet + Bone Marrow) who received 1 to 3 islet infusions, immunosuppression with sirolimus-tacrolimus. Mychophenolic acid was used to replace either drug in case of drug intolerance. At 3 and 6 monthly intervals, subjects completed a physical activity/duration questionnaire, underwent a 5 h mixed meal tolerance test (MMTT) and had HbA1c measured. Fasting glucose and C-peptide, 90-min glucose and C-peptide, MMTT stimulation index, and glucose and C-peptide area-under-the curve (AUC) were analyzed. We assessed differences in MMTT variables and HbA1c between time-points where subjects reported physical exercise and time-points with inactivity. Exercise activity time-points (n = 99) were significantly associated with an 8.9 mg/dl reduction in fasting glucose and a 3,813 mg/dl×min reduction in glucose AUC as compared to time-points with inactivity (n = 42). β-cell secretory responses were not significantly different. When adjusting the data for insulin and duration of transplant (post operative day), statistical significance was lost although fasting glucose and AUC glucose remained lower during exercise time points and 90 min C-peptide and C-peptide AUC were higher. In this retrospective analysis, exercise activity resulted in improved fasting glucose and AUC glucose. However, results were not statistically significant after adjusting for insulin and duration of transplant. It is possible that a larger sample size may be required to demonstrate significant differences. The better glucose disposal observed during exercise time points may be the result of improved SI, a well known effect of exercise in healthy and T2DM subjects. Also, the higher C-peptide AUC observed during exercise time points after adjusting for insulin and post operative day may be reflective of better β-cell secretory capacity and glucose disposal. Randomized prospective studies are required in order to confirm this hypothesis.

Immunosuppression

P-3.1

Exendin-4 in addition to tacrolimus improves viability in cultured beta cells
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Background: Islet transplantation (IT) to replace beta cell mass in DM1 patients is an attractive therapy, but clinical results have been disappointing in part because of progressive loss in graft function. One explanation for this is damage to beta cells by immune-suppressors. The incretin effect is defined as the augmented insulin secretory response to glucose delivered to the gut relative to that achieved by intravenous glucose when plasma levels of glucose are comparable. The principle incretin, GLP-1, and its long acting analog, exendine, have a myriad of beneficial effects on the beta cell as well as protecting beta cells from a variety of toxic agents. Tacrolimus is the main immune-modulator drug used in islet transplantation, but there is evidence that it could be toxic for the beta cell. In this work we conducted a series of experiments evaluating the viability of a beta cell line, MIN-6, in culture, exposed to tacrolimus (tac) and exendine (ex).
Methods: We used MIN-6 cells in culture and evaluated the viability of the cells exposed to different concentrations of tac and ex with the MTT assay. The cells were cultured at 37°C, 5% CO2 for 24 h in media supplemented with tac 10 nM, ex 10 nM, or ex 1000 nM or a combination. After this period the cells were exposed to MTT, which can be metabolized to purple crystals of formazan only in living cells, and the optical absorbance evaluated. The results are expressed as % of viability in comparison with control condition.
Results: With the concentration of tac used, no cellular damage was detected compared with control. With ex 10 nM and ex 1000 nM cells showed enhanced viability (136% and 130% $p < 0,05$). However, in the presence of tac 10 nM, ex 10 nM loss its beneficial effect, whereas with tac 10 nM and ex 1000 nM the cell viability was 221%, better than control group, tac group and ex 1000 nM alone group ($p = 0.002$/ 0.007/0.0008).
Conclusions: In a cell line model of the beta cell the use of ex 1000 nM in combination with tac 10 nM enhanced the cellular viability, representing a potential therapeutic choice in order to improve the clinical results of IT.

P-3.2

Induction with single dose alemtuzumab subcutaneously in simultaneous kidney pancreas (SPK) transplantation
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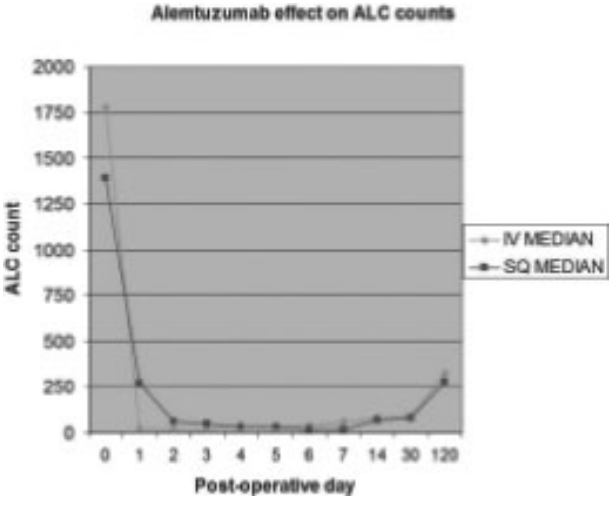
Purpose: Although not approved for induction, alemtuzumab has been shown to be an effective induction agent. Until recently, giving alemtuzumab intravenously (IV) was sole method of administration. The subcuantaneous (SC) route has been demonstrated to be safe and effective route in the kidney transplant and leukemic population with less side effects. We sought to determine if subcutaneous of administration of alemtuzumab is effective in SPK transplant recipients.
Methods: All patients who received SPK transplants with negative cross-match from January 2007 until July 2008 and received alemtuzumab as induction were included. Patients were stratified into two groups based on method of administration of one dose of 30 mg of alemtuzumab, intravenous or subcutaneous prior to reperfusion. All patients received tacrolimus



and mycophenolate mofetil and a rapid steroid taper (off by POD #4). There were 10 patients in the IV group and 23 in the SC group. Protocol kidney biopsies were done on post-operative day 30 and 120. We looked at the following endpoints: absolute lymphocyte count daily for the first 7 days and days 14, 30, and 120, clinical acute rejection, subclinical acute rejection on protocol biopsies, kidney and panceas graft survival.
Results: Absolute lymphocyte counts in the two groups are shown in Graph-1. Acute rejection and graft survival rates are shown in Table 1.
Table 1. Rejection and graft survival rates.

	IV = 10 patients	SQ = 23 patients	P Value
Kidney graft loss	zero	zero	NS
Pancreas graft loss	1 (10%)	3 (13%)	NS
Clinical rejection	zero	1 (4%)	NS
Subclinical Rejection	1 (10%)	2 (9%)	NS

Conclusions: The drop in absolute lymphocyte count at all time points, acute rejection and graft survival rates in the SC alemtuzumab group are similar to those in the IV alemtuzumab group. SC administration of alemtuzumab is an effective induction agent in SPK recipients.

P-3.3

AMT, an inducible nos inhibitor, attenuates neogrowth and prolongs graft survival of alginate-poly-L-lysine-alginate microencapsulated islets
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To study the effect of an inducible nitric oxide synthase (iNOS) inhibitor on the non-specific inflammatory reaction induced by alginate-poly-L-lysine-alginate (APA) embedded islets, we co-encapsulated islets with silica gel-entrapping 4H-1,3-Thiazin-2-amine,5,6- dihydro-6-methyl monohydrochloride (AMT) and transplanted microcapsules intraperitoneally to syngeneic healthy mice to access capsular neogrowth and graft viability. To sustain AMT releasing, solution mixture of tetramethoxysilane and AMT were incubated for 7 days allowing silica network formation. The diameter of crushed gel particles was 2.04 ± 0.78, 2.35 ± 1.00, 2.42 ± 1.17 and 2.97 ± 1.43 mm (each group has n = 3) for preparations containing 0, 30, 60 and 120 mg AMT, respectively. All gel preparations have AMT sustained release for at least 200 h and the time prolongs to at least 600 h when the AMT-containing silica gels were embedded in APA capsules. Histological examination of retrieved subcutaneous silica grafts revealed no cellular infiltration. Twelve healthy B6 mice were randomly separated into three groups. Mice in group A received intraperitoneally (IP) empty APA microcapsules. Group B mice received IP 300 APA containing islets and group C mice received IP 300 APA containing islets and 2.5 mg/ml of silica gel prepared by adding 60 mg AMT. The degree of capsular neogrowth was scored as 0, 1, 2, 3 and 4 which stands for no cell adhesion, < 25%, 25–50%, 50–75% and > 75% cell adhesion on capsular surface, respectively. Two weeks following transplantation, the neogrowth score of retrieved APA microcapsules was 1.65 ± 0.41 (n = 3), 3.23 ± 0.41 (n = 5) and 2.08 ± 0.43 (n = 4) for mice in groups A, B and C, respectively (A vs B and B vs C, $p < 0.05$; A vs C, $p > 0.05$). The number of dithizone-stained islets in the retrieved APA capsules was 2 ± 2 and 20 ± 4 for mice in groups B and C, respectively ($p < 0.05$). In conclusion, AMT, an iNOS inhibitor, attenuates surface neogrowth and protects APA-encapsulated islet from non-specific inflammatory destruction.

Sustained release and high local concentration of AMT can be achieved by entrapping AMT in silica-gel and co-encapsulating islets with silica gel-AMT in alginate-poly-L-lysine-alginate microcapsules.

P-3.4

Successful pancreas-renal transplantation without induction therapy
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Most pancreas transplant centers initially use immunosuppression with antilymphocyte induction because the pancreas appears to be a highly immunogeneic organ. Although the addition of an antilymphocyte agent provides enhanced immunosuppression in the early posttransplant period, it is associated with added costs and adverse reactions. In this study we evaluated the safety and efficacy of tacrolimus (Tac), mycophenolate mofetil (MMF), and steroid immunosuppression without induction after simultaneous kidney–pancreas transplantation (SKPT). Six patients (30%) displayed rejection episodes with a mean follow-up of 12 months (range = 4–18 months). No graft was lost due to rejection. The results of this series suggest that SKPT can be safely performed without induction therapy.

P-3.5

Mediated expression of the immunomodulatory molecule PD-L2 in human islets maintains stable *in vitro* islet function
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Background: Islet allotransplantation is a developing therapy to restore independence to exogenous insulin in Type 1 diabetic patients. Early loss of transplanted islets due to alloimmunity coupled with the islet specific toxicity of conventional immunosuppressant drugs has prompted research into alternative means to protect the islet allograft. The binding of Programmed Death Ligand-2 (PD-L2) to its receptor PD-1 expressed on activated T-cells elicits a negative signal that leads to inhibition of T cell proliferation. Therefore, the aim of this study was to express PD-L2 in human pancreatic islets using an adenoviral construct in order to confer protection from alloreactive T cells.
Methods: Human PD-L2 cDNA was cloned 'in frame' with the EGFP sequence to generate a PD-L2-EGFP gene construct, which was then used to prepare the recombinant adenoviral vector, Adv-PD-L2-EGFP. Adenoviral particles for transfection were produced using HEK-293A cells. PD-L2 binding to the PD-1 receptor was assessed by flowcytometry using HEK-293A transfectants. Islets were transduced with varying viral titres ranging from 2×10^3 pfu/IEQ to 3×10^6 pfu/IEQ for 48 h. Adenoviral vector blank was used as a control. Both the expression of PD-L2 on the surface of islet cells and islet quality were verified by flowcytometry. Insulin secreted by transduced islets was determined by ELISA.
Results: FACS analysis showed that Adv-PD-L2-EGFP induced PD-L2 on HEK-293A cells, which were able to functionally bind to its PD-1 receptor. Moreover, Adv-PD-L2-EGFP induced the expression of PD-L2 in up to 10–30% (n = 3) of islet cells at 1.5x10^6pfu/IEQ, without increasing the level of necrotic cells compared to untreated islets. Finally insulin ELISA showed that islets transduced with AdV-PD-L2-EGFP were able to secrete insulin after stimulation with a high glucose environment.
Conclusion: Adenoviral mediated expression of PD-L2 in human islets did not affect the *in vitro* function of human islets. PD-L2 molecules induced by the Adv-PD-L2-EGFP bound to PD-1 receptor. Accordingly the immunomodulatory effects of PD-L2 to protect islets against immune injury warrants further investigation as a promising strategy for islet transplantation.

P-3.6

Lower rate of acute rejection with rapamycin vs mycophenolate mofetil in simultaneous pancreas–kidney transplant recipients: 8-year follow-up
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Background: The optimal immunosuppression for kidney–pancreas transplantation long-term remains to be identified. This is a prospective randomized, single center study, evaluating the effect of Rapamune vs Mycophenolate Mofetil in kidney–pancreas transplant recipients.
Methods: A total of 159 SPK recipients who were randomized, 80 into the Mycophenolate Mofetil (MMF) arm, and 79 in the Rapamycin (Rapa) arm, starting in September 2000. Patients received zenapax (2 doses; 1 mg×kg) and Thymoglobulin (5 doses; 1 mg×kg), induction immunosuppression as well as tapering steroids and Tacrolimus. End points included patient, pancreas and kidney graft survival as well as acute rejection.
Results: Actuarial patient survival at 3, 5, and 7 years post-transplantation was: 91% + 3%, 83% + 5%, and 83% + 5% in the MMF arm; 88% + 4%,

81% + 5%, and 76% + 7% in the Rapa arm (p = 0.50, logrank test). Kidney-specific death-censored actuarial graft survival at 3, 5, and 7 years post-transplantation, were as follows: 89% + 4%, 85% + 4%, and 78% + 6% in the MMF arm; 99% + 1%, 92% + 4%, and 89% + 5% in the Rapa arm (p = 0.09, logrank test). Pancreas-specific death-censored actuarial graft survival at 3, 5, and 7 years is as follows: 94% + 3%, 94% + 3%, and 91% + 4% in the MMF arm; 97% + 2%, 97% + 2%, and 97% + 2% in the Rapa arm (p = 0.29, logrank test). Kidney specific (biopsy-proven) acute rejection occurred in 21 of the 80 patients in the MMF vs 8/79 patients in the Rapa arm (p = 0.01). Pancreas specific acute rejection (biopsy-proven or clinically suspected) occurred in 22/80 in the MMF arm vs 6/79 in the Rapa arm (p = 0.002). GI symptoms led to dose reductions or withholding MMF in the first year, 21% in the MMF group vs 1% Rapa group, p = 0.0003).
Conclusion: Excellent kidney and pancreas graft survival can be achieved using MMF or Rapa based immunosuppression in this long-term study. Rate of AR for pancreas and biopsy-proven kidney transplantation appears to be statistically significantly better with Rapa vs MMF. The difference appears to be better Rapa tolerability in this patient population with type 1 diabetes where gastroparesis occurred in 60% of the patient population.

P-3.7

Bortezomib spares plasma cells protective for tetanus toxoid during removal of HLA antibodies

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Background: Bortezomib is a promising new agent in solid organ transplantation due to its ability to affect antigen presentation and antibody production. However questions about its ability to delete protective immunity have emerged. Herein, we report data supporting bortezomib's ability to spare protective tetanus toxoid producing plasma cells.
Methods: Five-living donor renal transplant (txp) patients (pts) were treated with bortezomib (1–2 cycles of 1.3 mg/m^2×4 doses) to delete clones pre-txp or remove HLA antibodies post-txp removal. Serial measurements of HLA antibody were conducted weekly before, during, and after treatment via single antigen bead on Luminex (One Lambda Inc). Tetanus toxoid IgG was measured quantitatively via ELISA (ARP Inc, Belmont, MA).
Results: Two cycles of Bortezomib were used for clonal deletion in 4/5 patients. One patient received a single cycle of bortezomib for post-txp HLA antibody removal. Pre-Bortezomib HLA level [mean fluorescent intensity (MFI)] ranged from 10,000 to 1,200. In all four cases HLA antibody was completely removed to below 1,000 MFI. Tetanus Toxoid IgG levels are shown in Figure 1. All patients maintained stable tetanus toxoid IgG levels despite 1–2 cycles of bortezomib therapy. No patients experienced infection or peripheral neuropathy.
Conclusions: This data indicates that bortezomib therapy spares plasma cells protective for tetanus toxoid despite removal of HLA antibodies. This possibly suggests that the highly active plasma cells may be more sensitive to bortezomib therapy compared to resting protective plasma cells.

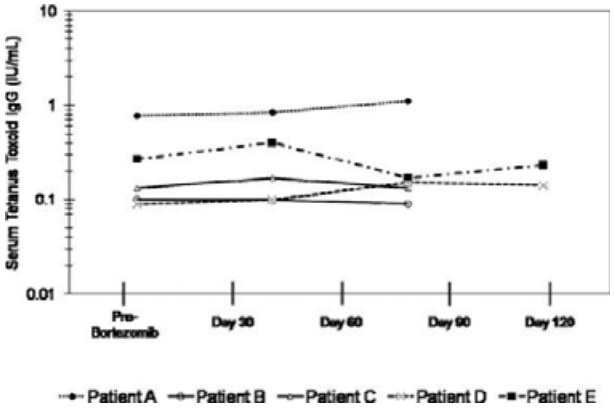

Figure 1. Tetanus toxoid levels before and after treatment with 1–2 cycles of bortezomib.

Ethics

P-4.1

The solidarity of science and self-sufficiency: why efforts to enhance organ donation should remember the needs of research

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Part one: Contribution to research as a neglected motivation for deceased donation

The persistent shortage of human organs, cells and tissue for transplantation continues to receive global attention, however the lack of such materials for use in scientific research is largely unrecognized by the public. This paper argues that efforts to increase deceased donation should include a renewed emphasis on the possibility of contributing to society through posthumous participation in research concerning the treatment of organ failure and disease.

Part two: The principle of solidarity, as manifested in scientific research, is also a key element of general approaches to increasing donation such as the pursuit of self-sufficiency

This paper examines the principle of solidarity in the context of scientific research and in the provision of transplantation services. The collective obligations of society to meet needs for transplantation, embodied in the concept of self-sufficiency, will be outlined. The role of researchers in establishing international collaborations in the pursuit of common goals will be discussed as a possible model for cross-border exchanges and sharing of human body components that contrasts with purely commercial outsourcing. The solidarity of science will be suggested as an inspiration for national and global societies seeking to pursue self-sufficiency in transplantation as a global public good for health.

P-4.2

Ethical issues involved in xenotransplantation: current trends

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Background: Recent scientific developments indicate that transplantation of organs, tissues or cells between species may at some stage become a realistic option, ultimately resulting in an increased supply of organs to meet medical demand. However, in order to allow the clinical application of xenotransplantation (XT), several immunological, biosafety, physiological, regulatory, social and ethical issues still need to be adequately addressed. This communication aims to consider a framework of ethical principles within which specific procedures can be assessed. Three key issues are analyzed in this communication: safety-for both the transplant recipient and the broader community-; human ethics and animal ethics.
Methods: In order to write this communication we have taken into account current articles about this subject, different legal texts and several international and national documents related with this field.
Results: We have considered the practical ethical issues with the conduct of XT research. These issues can broadly be classified as those that relate to human beings (the ethical conduct of animal-to-human trials) and those that relate to animals (the ethical conduct of animal-to-animal studies and the welfare of source animals for clinical trials).
Conclusions: Based on these considerations, several principles have been identified to be met before XT research involving humans is allowed to proceed. Given the current state of our knowledge, serious ethical consideration is needed before an animal-to-human trial in XT can be approved. Two groups of people can be distinguished whose rights may be affected in XT research involving humans: individual XT recipients and the wider community. Secondly, several principles have been identified before XT research involving animals is allowed to proceed. In addition, the genetic modification of animals for use as the source of XT products raises ethical issues. With regard to the latter, we have concluded that as long as the type of modification does not interfere significantly with the overall characteristics of the animal species, it is not considered ethically unacceptable. The wider social significance of XT has included questions about justice in the allocation of sources.

Immunobiology-Tolerance

P-5.1

Transforming growth factor beta 1 (TGF-β1) and rapamycin synergize to inhibit TCR-induced human CD4+ and CD8+ T cell proliferation

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Background: The major hurdle with clinical islet transplantation for patients with type 1 diabetes is a gradual decline in insulin independence. This decline may reflect drug toxicity to islet β cells in part. Therefore, refined immunosuppressive protocols or a better alternative to systemic drug administration would be useful to maintain long-term insulin independence. Recent studies have also demonstrated that transforming growth factor beta 1 (TGF-β1) is a critical regulator of thymic T cell development as well as key player in peripheral T cell homeostasis, tolerance, and T cell differentiation during the immune response. TGF-β1 promotes the differentiation of induced Treg cells that would provoke transplantation tolerance. In the present study, *in vitro* experiments were performed to determine whether TGF-β1 could suppress anti-CD3-stimulated human CD4+ and CD8+ T cell responses. Furthermore, we addressed the question if TGF-β1 and rapamycin, key immunosuppressant in clinical islet transplantation, can synergize to inhibit these immune responses.
Methods: Human peripheral blood mononuclear cells were labeled with CFSE to monitor subsequent cell divisions. Cells were stimulated with either anti-CD3/anti-CD28 or anti-CD3 alone in the presence or absence of TGF-β1. In some experiments, rapamycin was added to the culture medium at different concentrations. Flowcytometric analysis was performed after staining surface CD4 and CD8. The percentage of cell proliferation was determined by low CFSE population, using CD4 or CD8 gating.
Results: TGF-β1 suppressive activity to CD4+ or CD8+ T cells was minimal against anti-CD3/anti-CD28 stimulation. On the other hand, TGF-β1 inhibited both CD4+ and CD8+ T cell proliferation induced by anti-CD3 alone stimulation. Combination of TGF-β1 and rapamycin produced a synergistic inhibition of both anti-CD3/anti-CD28- and anti-CD3 alone-stimulated T cell responses.
Conclusions: These studies suggest that local delivery of TGF-β1 at the islet transplantation site with low dose rapamycin would potently inhibit T cell response *in vivo* and would be more effective to achieve long-term insulin independence with lower doses of immunosuppressants.

P-5.2

Identification of novel parasite-derived compounds that promote long-term islet allograft survival

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Background: Parasites can evade immune clearance in mammals by deviating protective Th1-type responses to permissive Th2-type responses. From this we aimed to identify if there were novel parasite-derived molecules that might promote allograft tolerance.
Methods: Gel permeatin chromatography analysis of Fasciola hepatica excretory/secretory products (ES) revealed two distinct protein fractions; Mass spectrometry identified these as Thioredoxin peroxidase (Prx) and Cathepsin L (CL). The effect of ES, Prx and CL on Th1-responses and allograft rejection was determined.
Results: Macrophages isolated from mice administered with ES or Prx showed markers of alternative activation. Prx-treated macrophages promoted increased IL-4 but decreased IFNγ production by T cells. In contrast, mice administered with CL reduced macrophage inflammatory products TNF-α, IL-6 and IL-12. Thus, ES components can collectively suppress Th1-type responses and drive a Th2 bias. For allograft rejection studies recipient mice received injections starting day-1 then every second day for a total of nine injections. Mice were given a full MHC-mismatched islet allograft (BALB/c islet- > C57BL/6) on day 0. Control mice had an islet allograft mean survival time (MST) = ~18 days (n = 10); 100% of ES treated mice failed to reject the islet allograft for > 100 days (n = 3); 40% of Prx treated mice failed to reject the islet allograft for > 100 days (n = 5); and 30% of CL treated mice failed to reject the islet allograft for > 100 days (n = 5). No adverse effects or reactions from treatments were observed.
Conclusions: Parasite derived compounds can promote long-term allograft survival. Further challenges to identify required combinations of ES components to achieve long term islet tolerance and to decipher the mechanisms involved remain.

P-5.3

Effect of costimulation blockade treatment on allogeneic islet graft survival in the alloimmunized mice

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Background: Initial treatment with anti-CD40L and CTLA-4Ig is sufficient to prolong allograft survival in naïve mice model but not in immunized recipients or in bigger animals such as human. In our previous study, we showed that addition of anti-LFA-1 antibodies improved pig islet graft function in Immuno-competent mice when long-term acceptance was induced by CTLA4Ig/anti-CD40L. Anti-LFA-1 antibodies may be beneficial to block the memory T cell responses. In this study, we, first, examined the effect of costimulation blockade treatment on allogeneic islet transplantation using allo-immunized mice as recipients (study1). Immune antibodies can cause hyper acute rejection or may accelerate the rejection by enhancing antigen processing and presentation. Therefore, in study2, we investigated the effect of immune antibodies on allograft survival by blocking T cell response with costimulation blockade treatment.
Methods: C57BL/6 mice were immunized by transplantation of Balb/c islets under left kidney capusles. Alloreactive antibodies were confirmed by staining of Balb/c spleen cells and subsequent flowcytometry analysis. In study 1, immunized and STZ-induced diabetic C57BL/6mice received 200 Balb/c islets under right kidney capsules and were divided into three groups, Group 1: control antibody injections. Group 2: anti CD40L and CTLA-4 Ig injections. Group 3: anti CD40L, CTLA-4 and anti LFA-1 antibody injections. In study 2, naive sera and immune sera were pooled from 10 C57BL/6 mice in each group. Naive STZ-induced diabetic C57BL/6 mice were transplanted with 300–500 Balb/c islets under kidney capsules and 50 μl of pooled sera was i.p. injected immediately after transplantation. Graft function was estimated by daily monitoring of blood glucose and body weight.
Results: There were no significant differences of graft survival among the three groups. In a pilot study with immune sera, a mouse that received the costimulation blockade treatment had a functional graft longer than 90 days even in the presence of preformed antibodies.

Study 1		
	n	Graft survival (days)
Group1 Control	7	0, 4, 5, 8, 9, 13, 14
Group2 2Ab treatment	5	4, 4, 8, 8, 16
Group3 3Ab treatment	7	4, 6, 8, 13, 14, 17, 26
Study 2		
+ immune sera	3	6, 12, 12
+ immune sera + 3Ab treatment	1	>90*

P-5.4

Resistance of diabetes in aged nod mice is mediated by CD4+CD25+FOXP3+ Regulatory T cells

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Although most of female NOD mice spontaneously develop diabetes, some aged female NOD mice do not develop diabetes in pathogen-free housing. We investigated the role of CD4+CD25+FoxP3+ T cells in aged NOD mice. Diabetes was monitored in NOD mice in six groups (N = 100 each) for over 60 weeks. Lymphocytes from diabetic NOD mice were adoptive transferred to aged NOD mice without diabetes. Lymphocytes or CD4+CD25+ T cells or CD4+CD25- T cells from aged NOD mice without diabetes were co-transferred with lymphocytes from diabetic NOD mice to NOD.scid mice. Nondiabetic aged NOD mice were treated with cyclophosphamide. CD4+CD25+FoxP3+ T cells in pancreatic lymph nodes, spleen and peripheral blood of aged NOD mice were measured. At the age of 60 weeks, 74% to 79% total mice in each group developed diabetes. Only 3 of 25 aged NOD mice developed diabetes at 12 weeks after adoptive transfer of lymphocytes from diabetic mice. When 1×10^7 lymphocytes from aged NOD mice were co-transferred with 5×10^6 lymphocytes from diabetes NOD mice into NOD.scid mice, all 10 NOD.scid mice developed diabetes mice at 6 weeks after adoptive transfer. However, when 2×10^7 lymphocytes from aged NOD mice were co-transferred, none of 12 NOD.scid mice developed diabetes at 6 weeks and 6 of 12 NOD.scid mice developed diabetes at 12 weeks after adoptive transfer ($p < 0.01$). When 1×10^6 CD4+CD25- T cells was co-transferred with 5×10^6 splenocytes from diabetic NOD mice into NOD.scid mice, diabetes was developed in all 6 NOD.scid mice. In contrast, none developed diabetes at 8 weeks and only 1 of 6 mice developed diabetes at 12 weeks, when 1×10^6 CD4+CD25+ T cells was used. The percentages of CD4+CD25+Foxp3+ T cells in CD4+ T cells in the pancreatic lymph nodes, spleen and peripheral blood were 20.1 ± 5.3%, 14.6 ± 4.1% and 9.8 ± 3.5% in aged NOD mice; and 5.8 ± 1.5% ($p < 0.01$), 8.6 ± 0.7% ($p < 0.01$) and 6.4 ± 1.7% ($p < 0.05$) in control diabetic NOD mice. When a low dose of cyclosphamiade was given, diabetes was developed in 15 of 20 young NOD mice and in 3 of 17 aged NOD mice at 8 weeks old. However, when the high dose was given, 11 of 19 aged NOD mice had diabetes ($p < 0.01$). One week after treatment with the high dose, the level of CD4+CD25+Foxp3+ T cells in pancreatic lymph nodes was 6.96 ± 1.71%. Our data demonstrated that immunoregulation through CD4+CD25+Foxp3+ T cells mediated the resistance of diabetes in aged NOD mice.

P-5.5

Hormone resuscitation of BD porcine donors reduces pancreatic inflammation

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Background: Islets for clinical transplantation are derived almost exclusively from brain-dead donors. Brain death results in a series of haemodynamic, neurohormonal and pro-inflammatory perturbations, all of which are thought to contribute to donor organ dysfunction. Pancreatic islets are particularly susceptible to damage during brain death and its sequelae. Observations of pituitary hormone depletion after brain death (BD) suggested that hormone replacement (HR) may ameliorate some of its metabolic consequences, and improve the quality of donor organs.
Aims and Methods: In this study, we used a pig model of BD to compare the effects of HR and standard pressor support with noradrenaline (NAd) on haemodynamic parameters and markers of pancreatic function and inflammation. BD was induced by inflation of an extradural Foley catheter. All pigs received iv fluids to maintain CVP between 0–5 mmHg, and HR or NAd were commenced 1 h post-induction of BD. The starting dose of NAd was 3.3 μg/min, titrated to maintain a MAP of 60–70 mmHg. Pigs in the HR group received a 4-drug cocktail comprising vasopressin, methylprednisolone, T3 and insulin. Pigs were monitored for 6 h after induction of BD, with blood, urine and pancreas sampling at 0, 1, 3 and 6 h post-BD.
Results: Haemodynamic status of the donor pigs as indicated by MAP, heart rate, cardiac output stroke work and renal perfusion was significantly improved in the HR group, compared with the NAd group ($p < 0.05$). Blood levels of amylase and lipase were equivalent between the two groups. Histological examination of the pancreas revealed increased necroinflammatory foci in the NAd group (cellular necrosis score 2.0 ± 0.6 vs 1.0 ± 0.7 for HR, $p < 0.05$). Gene expression for various inflammatory markers and for insulin was measured by RT-PCR. Expression of HSP-72, IL-6 and MCP-1 was upregulated in the NAD group but downregulated in the pigs receiving HR. Insulin gene expression at 6 h post-BD increased by 1.5 fold in the NAd group, and decreased 2-fold in the HR group compared to baseline values. Reduced Insulin staining at 6 h post-BD was evident in a similar proportion of pigs from each group.
Conclusions: HR of porcine BD donors resulted in improved haemodynamic parameters and reduced pancreatic cellular necrosis and inflammation, but did not confer any detectable benefit in preserving islet insulin content.

P-5.6

Anti-CD45RB therapy effectively prevents the rejection of islet allograft by islet-reactive CD4 and CD8 cells in a TCR transgenic model

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Therapy with monoclonal antibody targeting CD45RB reliably induces tolerance to allografts in rodent transplant models. Despite extensive study on the role of CD45 molecule, the mechanisms underlying anti-CD45RB antibody induced tolerance *in vivo* are not fully understood. Herein, we have aimed to dissect the immunological mechanisms by anti-CD45RB therapy using a TCR transgenic model of islet allograft rejection. TCR transgenic mice specific for hemagglutinin (HA) provided antigen-specific T cells for adaptive transfer into INS-HA transgenic mice expressing HA molecules on pancreatic islet β cells. Adaptive transfer of TS1 or CL4 cells, expressing the HA specific MHC class II-restricted TCR CD4+ or I-restricted TCR CD8+ T cells, respectively, via IV, failed to induce hyperglycemia. However, when transferred under the kidney capsule along with HA islets, CL4 cells induced hyperglycemia, while TS1 cells did not, suggesting activated CL4 cells have diabetogenic properties. Furthermore, co-transfer of CL4 and TS1 cells on the kidney capsule induced hyperglycemia both with and without HA islets. In another application of the TCR transgenic model, islets were prepared from INS-HA mice and then transplanted into streptozotocin-induced diabetic TS1 or CL4 mice. Compared to syngenic Balb/c control mice, which show permanent INS-HA islet survival, TS1 and CL4 mice reject the INS-HA islet; however, anti-CD45RB therapy effectively prevented the rejection of the INS-HA islet grafts in TS1 and CL4 mice. These data indicates that interference of CD45 molecule with monoclonal antibody is able to prevent the autoimmune attack by both islet-reactive CD4 and CD8 T cells. Further understanding of the tolerogenic properties of anti-CD45RB therapy in TCR transgenic mice will not only provide insight into the actions of this potentially clinically relevant agent, but may also define pathways of tolerance induction *in vivo*.

P-5.7

Hyperbaric oxygen therapy prevents autoimmune diabetes onset in NOD mice

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Background: Hyperbaric Oxygen Therapy (HOT) has been attributed numerous properties, including immunomodulation and tissue repair. Aim of our study was to assess the effects of HOT on autoimmune diabetes development in Non-Obese Diabetic (NOD) mice.
Methods: Female NOD mice were monitored for glycosuriae (positive glycosuria confirmed with nonfasting glycemia >350 mg/dl) to detect diabetes onset. Experimental groups included: (1) Four-weeks old mice treated daily with 60 min HOT (2.0 atm) and untreated controls; (2) Ten-weeks old mice treated daily with either HOT or hyperbaric ambient air (Air) starting 1 week before a single intraperitoneal injection of cyclophosphamide (CyP; 200 mg/kg) to induce accelerated autoimmune diabetes.

Results: Spontaneous diabetes onset occurred in 85% of control mice (n = 20; median: 20.5 weeks, range 16–26 weeks). HOT significantly reduced diabetes occurrence to 45% (n = 20; range 19–29 weeks; p = 0.002 log-rank test). In another set of experiments, 80% of the mice in the control group receiving CyP without hyperbaric treatment developed diabetes (n = 20; median: 15 days, range 11–21 days). A similar pattern was observed in the Air-group, with 80% diabetes incidence (n = 10; median: 14 days, range 11–14 days). HOT significantly reduced diabetes occurrence to 40% (n = 20; range 11–14 days; p = 0.02 vs control and p = 0.04 vs Air).
Conclusions: Our data indicates that HOT can significantly reduce autoimmune diabetes incidence in NOD mice, both spontaneous and CyP-induced onset. Both the safety profile and its non-invasiveness (with virtually absent side effects) make of HOT a suitable candidate to further exploration of its possible clinical applications, including in diabetes prevention trials as single strategy or in combination with other treatments.

Islet culture

P-6.1

Comparison of fresh and cultured islets from human pancreata

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Background: For clinical islet transplantation, many centers have recently introduced the culturing of human islets prior to transplantation because it provides many benefits to clinical islet transplantation, such as flexibility for evaluation of isolated islets and pretreatment of patients. However, isolated islets deteriorate rapidly in culture. In the present study, we compared human fresh islets to cultured islets with *in vitro* and *in vivo* assay.
Method: Thirty-four pancreata from brain-dead donors, which were procured from either Southwest Transplant Alliance (Dallas, TX) or LifeGift (Fort Worth, TX) between May, 2007 and April, 2009, were used in this study. Human islet isolation was conducted in the standard Ricordi technique with modifications introduced in the Edmonton protocol. Isolated islets from human pancreata were cultured for 6, 24, 48, and 72 h at 37°C and 5% CO_2 in culture medium and evaluated by islet count, stimulation index, ATP contents, and transplantation into diabetic nude mice.
Results: After culture for 24, 48, and 72 h, islet yield significantly decreased from 2,000 to 1,738 ± 26 IE (13% loss), 1,525 ± 30 IE (24% loss) or to 1,298 ± 18 IE (35% loss), respectively. The ATP contents were significantly higher in the 6 h-cultured group (near fresh group) than in 48 h-cultured group. The stimulation index was relatively higher in the 6 h-cultured group than in 48 h-cultured group. Human islets with or without culture were transplanted into diabetic nude mice. The attainability of post-transplantation normoglycemia was significantly higher in fresh group than in the culture group. Intra-peritoneal glucose tolerance testing (IPGTT) showed that the blood glucose levels of mice transplanted with fresh islets were significantly lower than with cultured islets at 30, 60, 90, and 120 min after injection.
Conclusion: These data suggest that human islet transplantation without culture could avoid the deterioration of islets during culture and improve the outcome of islet transplantation. Based on these data, we have transplanted fresh islets without culture for our current clinical islet transplantation protocol.

P-6.2

Liraglutide, a long-acting human glucagon-like peptide 1 analogue, improves human islet survival in culture

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The culture of human islets is associated with approximately 10–20% islet loss, occasionally preventing transplantation. Pre-conditioning of the islets to improve post-culture yields would be of immediate benefit, with the potential to increase both the number of transplanted patients and their metabolic reserve. In the present study, the effect of liraglutide, a long-acting human glucagon-like peptide 1 analogue, on cultured human islets was examined. Culture with liraglutide (1 μmol/l) was associated with a preservation of islet mass (33 ± 23 and 39 ± 13% more islets at 24 and 48 h, compared to control; $p \leq 0.01$ at 48 h) and with the presence of larger islets ($p \leq 0.01$ at 48 h). These observations were supported by reduced apoptosis rates after 24 h of treatment. We also demonstrated that previously-treated islets do not retain a functional advantage in the absence of liraglutide, both utilizing *in vitro* glucose-induced insulin release tests and *in vivo* marginal mass islet transplantation in C57BL/6-RAG-/- mice. However, when liraglutide is adminitered continuously, its protecting effects are presereved, suggesting that treatment with liraglutide should be continued after transplantation. Overall, these data demonstrate the beneficial effect of liraglutide on cultured human islets, preserving islet mass. They support the design of clinical studies looking at the effect of liraglutide in clinical islet transplantation.

P-6.3

Culture of impure human islet fractions in the presence of alpha-1-antitrypsin prevents insulin cleavage and improves islet recovery

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Background: Exocrine acinar cells are commonly co-transplanted with islets [impure preparations] in auto and allotransplantation. Proteases are generally released by acinar cells along with insulin by islets in during pretransplant culture and post transplant. We hypothesized that released proteases cleave the insulin molecule and that addition of alpha 1 antitrypsin (A1AT) to impure islet culture blocks insulin cleavage and improves islet recovery and function.
Method: Trypsin, chymotrypsin and elastase (TCE) activity and insulin levels were measured in culture supernatants of pure (n = 5) and impure (n = 5) islet fractions from deceased donors. SDS-PAGE was used to detect insulin band after incubation of insulin with proteases. The effects of A1AT supplementation (0.5 mg/ml) [n = 4] on TCE activity, insulin levels, and islet quality were assessed using standard assays. The ultrastructure of islets exposed to TCE and control medium was examined by transmission electron microscopy (TEM).
Results: Proteases (TCE) levels in culture supernatant were directly proportional to percentage purity of islets [pure, impure and highly impure]. Low levels of insulin were detected in culture supernatants when high protease levels were present. Insulin levels measured in supernatants of 2,000 IE aliquots of impure and highly impure islet preps were 61% and 34% compared with pure preps, respectively. Incubation of insulin with commercially available proteases (TCE) or exocrine acinar cell supernatant cleaves the insulin molecule as assessed by SDS-PAGE gel. Addition of A1AT to impure islet preparations reduces protease levels and restores normal insulin levels [by ELISA and SDS-PAGE] in culture supernatants. A1AT improved insulin levels to 98% in impure and 78% in highly impure fractions compared to pure islet fractions. A1AT supplementation improved postculture recovery of islets in impure preps compared to non-treated control (80% vs 32%). Islet viabilitymeasured by membrane integrity tests was similar in both control (97.5%) and A1AT treated group (97.9%). TEM results revealed damaged beta cell microorganelles after exposure to proteases (TCE).
Conclusion: Culture of impure human islet fractions in the presence of A1AT prevents insulin cleavage and improves islet recovery.

P-6.4

***In vitro* effects of IGF-1 on Human Islet function and quality**

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Islet culture prior to transplantation is critical for repairing and recovering cells damaged during the isolation procedure. Islets have insulin and IGF-1

receptors and both insulin and IGF-1 are involved in signal transduction of the insulin gene. Current culture media containing insulin alone may be improved with the addition of IGF-1. The purpose of this study is to investigate the effects of IGF-1 on human islet function, viability and integrity. Human islets (n = 8) with purity >70% and viability >90% were cultured in serum free medium with and without 10 nM IGF-1 for 24 (n = 3), 48 (n = 2), and 72 (n = 3) h. Percentage of islet recovery was determined by islet count and viability was determined using FDA/PI. Laser Scanning Cytometry was used to quantify β-cell insulin expression and apoptosis by Tunnel assay. Insulin secretion and oxygen consumption rate (OCR) were measured by dynamic perifusion assay. The following results show the percentage difference for the 24, 48, and 72 h IGF culture conditions as compared to the control. Islet recovery was 10.64%, 1.22%, and 5.93% greater. β-cell apoptosis was 4.20%, 2.83%, and 5.03% lower. OCR was: 4.98%, 3.1%, and 4.04% greater. In conclusion, islets cultured in media supplemented with IGF-1 demonstrated an improvement of islet recovery and integrity. Tunnel staining revealed anti-apoptotic effects of IGF-1 to islet β-cells.

P-6.5

Use of human serum or human albumin for human islet culture

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Background: After human islet isolation islets are usually incubated for 48 or 72 h in CMRL 1066 supplemented with 0.5% human serum albumin instead of whole human serum. Whole serum is usually used for standard tissue culture due to the presence of growth factors necessary for cell viability. However, it has been suggested that medium supplemented with human albumin preserves islet mass and function better than whole human serum.
Objective: To evaluate and compare the effect of human serum and human albumin added to culture medium on viability and insulin secretion in human islets.
Methods: Islets were isolated from 7 multiorgan donors according to the Ricordi's method and cultured for 72 h in standard culture flasks with CMRL 1066 supplemented with 0.5% human serum albumin (HSA-islets) or 10% human AB serum (HS-islets). After 24 h and 72 h incubation viability was evaluated by acridine orange/propidium iodide staining, and islet cell function by measuring glucose-stimuled insulin secretion (GSIS) and expressed as insulin stimulation index (SI) and percentage of insulin secreted in relation to total insulin content.
Results: Viability, after 72 h incubation, was higher in HS-islets vs HSA-islets (91 ± 2% vs 75 ± 6%) (p = 0.024). DNA content was not different betwen both groups neither 24 h nor 72 h incubation. Insulin content was significatively lower after 24 h incubation in HS-islets vs HSA-islets (877 ± 194 vs 1321 ± 395 ng/mgDNA) (p = 0.046) but no differences were found after 72 h. Basal insulin secretion was not different between both groups but GSIS was increased in HS-islets vs HSA-islets after 72 h incubation (37.3 ± 13.5 vs 26.4 ± 9.3 ng/mgDNA) (p = 0.027). Percentage of stimulated secretion in relation to total insulin was higher in HS-islets vs HSA-islets after 24 h (6.08 ± 0.93 vs 3.97 ± 0.64%) (p = 0.005) and 72 h (4.82 ± 1.59 vs 2.94 ± 0.71%) (p = 0.062) and, finally, SI was also significatively increased at 72 h incubation in HS-islets vs HAS-islets (12.0 ± 2.6 vs 5.26 ± 0.50) (p = 0.026).
Conclusions: Our results suggest that CMRL1066 supplemented with human serum increases islet viability and function and could help to preserve islet mass and insulin secretion before transplantation in diabetic patients.
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P-6.6

Together they can: escaping anoikis by establishing cell-cell contacts in the human pancreatic cell line "hPan"

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Background: Besides insulin therapy, transplantation of islets rather than the whole pancreas is a promising option to treat type 1 diabetes. Innovation and improvement on this topic however is hindered by the lack of knowledge regarding the mechanisms determining cell survival and settlement in a new localization. Identifying cellular and molecular key players in such a setting will increase the effectiveness of human cell replacement therapies such as islet transplantation. The purpose of this study was the establishment of an *in vitro* model system suitable to study human pancreatic cell survival under adherent as well as non-adherent conditions.
Methods: We recently created a human pancreatic cell line termed "hPan". This cell line was established by retroviral infection of a primary culture of human islet cells with vectors coding for h-Ras, E1A and hTERT. The hPan cells were cultured under normal standard adherent conditions as well as under rotary conditions (Synthecon, Houston, TX, USA).
Results: Interestingly, hPan cells enter anoikis upon loss of cell-matrix contacts. Only those cells which manage to form cellcell contacts in rotating suspension cultures avoid anoikis and build up organoid-like structures. The integrity of cellcell contacts therefore compensates for the loss of cell-matrix contact-mediated survival signals in hPan cells and prevents apoptosis.
Conclusions: As anoikis is involved in a wide diversity of tissue-homeostatic as well as oncogenic processes, our model system is ideally suitable to study cell survival strategies in the context of islet transplantation as well as in cancer. Gained knowledge promises not only to advance our understanding of normal and cancer cell physiology but also permit us to exploit these differences therapeutically.

P-6.7

Visualisation of mitochondrial heterogeneity in stressed human pancreatic cells

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Background: Insulin secretion is known to involve complex events in which the mitochondria play a central role in the generation of signals that couple glucose detection to insulin secretion. Besides being key players for normal beta cell function, mitochondria are also of pivotal importance as cell stress transducers. One important feature of the mitochondrial response to stress stimuli is a certain degree of heterogeneity. The aim of the present study was to develop a combination of mitochondria-specific fluorescent stains enabling the easy visualization of mitochondrial heterogeneity in living human pancreatic cells by means of real time live confocal microscopy.
Methods: We used the human pancreatic cell line hPan as a model system and stained simultaneously with the fluorescent probes, tetramethylrhodamine methyl ester (TMRM), which detects mitochondrial membrane potentials and the reactive oxygen species (ROS)-sensitive dye dichlorodihydrofluorescein diacetate (DCF).
Results: By combining these two probes with the use of a spinning disc confocal imaging system, which allows confocal microscopy of living cells, we were able to visualize the mitochondrial heterogeneity within the pancreatic cell line

hPan under laser-induced stress conditions. Interestingly, we were able to identifiy a subpopulation of mitochondria which are resistant to the laser induced cell stress conditions.
Conclusions: Such an approach will facilitate future research addressing the contribution of pancreatic mitochondrial subpopulations to physiological as well as pathological cellular responses such as experienced during the course of islet transplantation. Future studies will further characterize the laser induced cell stress resistant subpopulation of human pancreatic mitochondria.

Islet encapsulation

P-7.1

Islet transplantation in pre-vascularized collagen chambers placed subcutaneously or in the omentum: a comparative study

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Background: Many sites of transplantation have been investigated in an effort to optimize the function and viability of the transplanted islets. Transplantation sites such as the omentum and subcutaneously have been previously examined in independent studies however these sites of transplantation have not been directly compared together. Here we report on a direct comparison of the outcomes of transplanting syngeneic islets into pre-implanted polypropylene chamber devices located in the omentum or subcutaneously of diabetic Lewis rats.
Methods: Transplantation of syngeneic Lewis rats at 10,000 IEQ/kg into pre-implanted chamber devices located in the omentum or subcutaneously. Parameters such as blood glucose, IVGTTs, weights, insulin levels in the blood, and histology were measured and analyzed.
Results: Islet survival and function was significantly better when transplanted in the omentum (7/10 reversed) vs subcutaneously (4/9 reversed). Islets in both chamber transplant sites responded similarly to IVGTT challenge and were functional as determined by immunohistological examination and insulin levels. Interestingly, we observed a significantly faster and sustained normalization of blood glucose in the Lewis rats containing islets transplanted into the omental chambers compared to the other groups ($p < 0.0001$). A significantly higher density of vasculature and collagen was present in the omentum chambers compared to the subcutaneous group which would suggest that the development of vascularization is paramount to the functioning of the transplant.
Conclusions: Based on these results, the omentum is the preferred transplantation site when using a polypropylene device to harbour islets.

P-7.2

Surface modification of islets with PEG-lipid for improvement of graft survival in intraportal transplantation

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Background: Transplantation of islets of Langerhans (islets) is a promising technique for treating insulin-dependent diabetes mellitus (type I). One unsolved issue is the early graft loss due to inflammatory reactions triggered by blood coagulation and complement activation that occurs immediately after transplantation into the liver through the portal vein. Several proposed approaches for improvement of the graft survival include heparin coating and covalent poly(ethylene glycol) (PEG) conjugation. We previously have studied the improvement of graft survival by modification of islet surfaces using amphiphilic PEG-conjugated phospholipid and bioactive molecules. Here we analyzed the effect of PEG-modification on the improvement of graft survival immediately after intraportal transplantation into streptozotocin-induced diabetic mice.
Methods: The surface of hamster islets was modified with PEG-lipid. PEG-lipid modified islets (PEG-islets) were transplanted into the liver through the portal vein of streptozotocin-induced diabetic mice. We measured the graft survival periods and blood insulin levels immediately after intraportal transplantation to determine the cell damage to islets. Histocytochemical analyses of liver were also performed post intraportal transplantation.
Results: The graft survival of PEG-islets was significantly prolonged compared with bare islets in livers of diabetic mice. Reduction of blood insulin level within 60 min after transplantation of PEG-islets suggests that the cell damage observed immediately after transplantation could be suppressed by surface modification with PEG in comparison with bare islets.
Conclusion: Our approach for the improvement of graft survival will be useful in the clinical setting.

Reference
Teramura Y and Iwata H. Transplantation (2009) in press.

P-7.3

Effect of diabetes on oxygenation and neovascularization for subcutaneous encapsulated islets

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Background: We previously desmonstrated, in non-diabetic rats, that high-mannuronic (high-M) alginate provides a sufficient oxygenation (30–40 mmHg) for encapsulated islets survival after subcutaneous transplantation. However, diabetes can decrease the implant vascularization by micro-/macrovascular lesions. Therefore, we investigated the neovascularization and oxygenation for high-M alginate subcutaneously transplanted in diabetic rats.
Methods: Non-diabetic ($n = 8$) and diabetic (by STZ 85 mg/kg iv, 4 weeks prior transplantation; $n = 8$) rats were transplanted with subcutaneous high-M 3% v/v implants. Diabetes was confirmed by a pathological NFBG (489 ± 16 mg/dl vs 118 ± 3 mg/dl for diabetic and control rats, respectively, $p < 0.05$). Diabetic vasculopathy was confirmed by Von Kossa staining for calcification deposition on vessels. Oxygen pressures (pO_2) were weekly measured *in vivo* by Electonic Paramagnetic Resonance up to 4 weeks post-transplantation. After graft explantation, neoangiogenesis (surrounding the implant) was assessed by histomorphometry (counting of newly-formed vessels). Additional non- ($n = 6$) and diabetic ($n = 8$) rats were subcutaneously transplanted with encapsulated pig islets (8,000 IEQ) and followed 8 weeks post-transplantation. Porcine C-peptide was weekly followed (in rat sera) and islets survival was assessed by insulin content after graft explantation.
Results: A significant higher pO_2 was found for non-diabetic vs diabetic rats (36 ± 8 vs 26 ± 5 mmHg, respectively; $p < 0.05$) at 1 week post-implantation. However, similar pO_2 were measured in both groups at 2/3/4 weeks (25 ± 1 mmHg). No difference of angiogenesis was observed between both groups after graft explantation. After pig islets transplantation in diabetic rats, normoglyceamia was totally achieved up to 60 days post-transplantation. No significant difference of porcine c-peptide sera levels (0.137 ± 0.058 vs 0.147 ± 0.033 ng/ml) and insulin content per encapsulated islets after explantation (419.2 ± 219.7 vs 381.1 ± 160.5 μU/ml/islet) was found between diabetic and non-diabetic rat recipients, respectively.
Conclusions: Although a delay of oxygenation was observed in diabetic recipients, diabetes does not significantly affect the oxygenation and the survival of encapsulated islets in the subcutaneous tissue.

P-7.4

The theracyte device protects islet allografts in the immunized host

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Background: Previous studies, both in humans and rodent models, have shown that the TheraCyte immuno-isolation device is allo-protective in non-sensitized recipients. We have also shown that pre-implantation of the device, allowing neovascularization of the device (surface before islet transplantation, significantly improves the graft survival and function. In this study we evaluated the immunoprotective capacity of the TheraCyte device in immunized recipients using an allogeneic rat model.
Methods: Survival of encapsulated and free Lewis rat islets, transplanted into naive or immunized streptozotocin-induced diabetic Wistar–Furth rats, was compared. The devices were implanted 1 month before immunization. The recipients were immunized by transplantation of approximately 1,000 Lewis rat islets under the left kidney capsules. Six weeks after immunization, alloreactive antibodies in sera were examined by flowcytometry. Only rats positive for alloreactive antibodies were used as immunized recipients for islet transplantation. One thousand Lewis rat islets were transplanted either under kidney capsules or into preimplanted devices. Graft function was evaluated by daily blood glucose measurement. Cured animals were followed up to 6 months. OGTT and IVGTT were

examined at 1, 3 and 5 months after transplantation. In cured rats, graftectomy was performed to confirm the reoccurrence of diabetes. Grafts were also examined histologically.
Results: Allospecific antibodies were detected in both naive and immunized hosts after free islet transplantation. Encapsulated islets functioned up to 6 months in both naive and immunized hosts. During the 6 months, immunized hosts remained positive for alloreactive antibodies while naive recipients remained negative. In the encapsulated islet transplantation, there were no significant differences found in OGTT or IVGTT between the groups. After 6 month viable islets were surrounded by donor fibroblasts in the devices, in both naive and immunized hosts.

	free islet transplantation		encapsulated islet transplantation	
	naive	immunized	naive	immunized
n	5	3	4	4
preformed allo-antibody	-	++	-	++
allo-antibody after TX	++	++	-	++
graft survival	9 days	6 days	>6 months	> 6 months

Conclusion: The TheraCyte™ device protect islet grafts from rejection in allo-immunized recipients.

P-7.5

Pre-implantation of cell encapsulation devices allows for greater survival of transplanted cells

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Background: The therapeutic strategies to treat type 1 diabetes have been limited by several factors such as long term complication for insulin injection and limited numbers of donor pancreata for either whole organ transplant or islet transplant. Genetically engineered human cell lines that can produce insulin could be one of the options to overcome those limitations if they can be safely transplanted, such as using encapsulation. Encapsulation materials can be made biocompatible and immune protective, however, survival of the transplanted tissue throughout the handling and transplant procedure is equally important. In cell therapies, loss of transplanted cells have been observed soon after implantation. This study examines the impact of pre-implantation and device size on the survival of encapsulated insulin producing cell transplants.
Methods: Non-islet human cell line, G80 was genetically engineered to constitutively express the Red Fluorescence Protein (dsRed). DsRed G80 cells were then loaded into pre-implanted or freshly implanted devices with nominal internal volume capacities of 4.5 µl or 20 µl. *In vivo* survival in encapsulation devices was monitored using Xenogen's IVIS spectrum imaging system.
Results: Over the first 3 weeks, very little loss (0–18%) of fluorescent dsRed G80 cells was observed in pre-implanted devices. In contrast, freshly implanted 4.5 µl devices a 70–80% loss of fluorescence or cell survival was observed at 1 week post implantation with very little recovery seen over the next 2 weeks. While similar losses were observed at week one with freshly implanted 20 µl devices, a 50–60% increase in fluorescence was observed from week 2 to week 3, suggesting cell expansion/recovery.
Conclusion: Pre-implantation allows encapsulation devices to vascularize first and therefore maintain transplanted cells, while in freshly implanted devices, cells are lost during the healing/vascularizing process.

P-7.6

Islet transplantation using macroencapsulation device

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Stochastic microencapsulation of individual islets within droplets of hydrogels is the most common procedure being used worldwide. The random placement of islets within these droplets results in some tissue exposure by inadvertent disposition on the edge of the droplet, with consequent sensitization of host immunity and loss of graft function. We investigated the foreign body response in rats to thin alginate sheets made with three types of mannuronate-rich alginate from *Macrocystis pyrifera*: NovaMatrix SLM-100 alginate from FMC biopolymers (Gp1), a similar viscosity alginate purified in our laboratory of Keltone HVCR from Monsanto (Gp2), and a higher viscosity alginate purified in our laboratory from *M. pyrifera* freshly harvested in La Jolla, California (Gp3). Sheets of these alginates measuring 300 µm thick and 1.5 cm^2 were made from 3% solutions (Gp1 and 2) or 0.6% solutions (Gp3) of alginates in normal saline. Sheets were sutured on the peritoneal wall or subcutaneously and recovered 2 weeks post implantation in rats. The sheets were assessed *in situ* for gross fibrosis, collagen deposition and inflammation on a scale of 0–3, with 0: no reaction and 3 indicating complete encapsulation within a fibrotic collagen capsule. Sheets made with Novamatrix (Gp1) implanted IP were uniformly found within fibrotic capsules, with an average rating of 3+; Gp1 sheets implanted SC were graded 2 with some brown granular material surrounding the implant. Sheets made from repurified Keltone HVCR (Gp2) implanted IP were graded 3, with adhesions to the liver. However, Gp2 sheets implanted SC displayed minimal fibrosis in two of three examples. Gp3 sheets implanted IP were better disposed, with an average grade of 2. Gp3 sheets implanted SC were graded 1, with some cellular proliferation seen with two of three sheets but little collagen deposition. Alginate source differ in the host response depending on the location of implantation. The SC site offers advantages of less host fibrosis and ease of surgical access. However, the SC site is relatively acellular and avascular, which limits the oxygen flux available for the rapidly metabolizing islet tissue. Implantation IP offers better oxygen flux and the insulin produced will be delivered via the physiologically relevant portal system.

Islet Xenotransplantation I

P-8.1

Evaluation of tolerance induction and reversal of autoimmunity by porcine thymus and islet xenotransplantation in NOD mice

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Pig islet xenotransplantation represents an attractive candidate to treat diabetes and could solve the human donor shortage. Recent reports demonstrated feasibility using neonatal and adult porcine islets in preclinical studies. Nonetheless, in order for islet xenotransplantation to become a viable treatment option for well-managed type 1 diabetics, graft survival must be achieved with minimal or no immunosuppression. In addition to rejection, recurrent autoimmunity in the grafted islets must be avoided. Xenogeneic thymus transplantation is an effective approach to achieving T cell tolerance across xenogeneic species barriers, leading to porcine skin graft acceptance in mice and promoting kidney xenograft survival in baboons. However, it remains unknown whether xenogeneic thymus transplantation reverses autoimmunity, whether human autoimmunity could affect a xenograft from a highly disparate species such as the pig and whether reversal of autoimmunity is essential for achieving cure through islet xenotransplantation. In this study, we assessed the autoimmune status of fetal porcine (FP) thymus (THY) grafted NOD mice and examined pig islet xenograft survival in diabetic and non-diabetic FP THY grafted mice. NOD mice were thymectomized at 3 weeks of age and diabetic as well as non-diabetic mice received FP THY grafts under the kidney capsule following conditioning with 5.5Gy TBI and T cell depleting mAbs. Porcine islets, SLA-matched to the thymus donor, were transplanted underneath the opposite kidney capsule 1–3 days after FP THY transplantation. FP THY engraftment will be followed by comparing peripheral CD4 reconstitution with that in control, age-matched animals receiving the same treatment without a thymus graft. Preliminary results demonstrated successful diabetes reversal with the above regimen at 5 weeks post-transplantation. Islet xenograft survival will be continued to be monitored by glucose measurements as well as histological analyses following CD4 reconstitution 8–16 weeks post-transplantation. MLR assays will clarify the degree of tolerance to the porcine donor that is achieved in these mice. These studies will determine the potential of FP THY transplantation to induce tolerance to xenogeneic islet grafts in the presence of anti-islet autoimmunity.

P-8.2

Improved method of porcine pancreas procurement with arterial flush and ductal injection enhances islet isolation outcome

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Background: Several pancreas procurement procedures have been used for porcine islet isolation. However, the impact of procurement technique on islet isolation outcome has not been extensively studied. Commonly procurement is done in slaughterhouse or animal facility without applying human surgical procurement techniques. Flushing of the pancreas with cold preservation solution is not feasible due to non-surgical procurement and logistics related to pig size and location of procurement. We evaluated an advanced procurement technique for porcine islet isolation designed to reduce warm ischemic damage as well as to remove blood content by implementing arterial flush and ductal injection of cold preservation solution.
Method: Pancreases procured from adult Landrace pigs after cardiac death were divided into one of three different surgical protocols: pancreatectomy utilizing (a) only surface cooling (Group 1, n = 24), (b) surface cooling and ductal injection with cold preservation solution prior to panctreatectomy (Group 2, n = 12), and (c) surface cooling, ductal injection and a vascular flush of cold preservation solution selectively flushing through the celiac trunk and the superior mesenteric artery (Group 3, n = 14). We have assessed the islet isolation results and islet quality with both in vitro and in vivo assays.
Results: Significantly higher purified islet yield was obtained in Group 3 (491496 ± 50.294 (mean ± SEM) IEQ vs 334850 ± 23,350 IEQ for Group 1, p = 0.001; 365679 ± 29254 IEQ for Group 2, p = 0.02). Islet yield per pancreas in Group 3 was also significantly higher compared with other groups (2209 ± 231 IEQ/g vs 1418 ± 128 IEQ/g for Group 1, p = 0.001; 1512 ± 131 IEQ/g for Group 2, p = 0.01). Measurements of islet viability after 7 days culture assessed by oxygen consumption rate per DNA showed that islets obtained from Group 3 had the highest value among 3 groups (217 ± 24 nmol/min_mgDNA vs 180 ± 29 for Group 1, p = 0.03; 210 ± 50 for Group 2, p not significant). Sustained normoglycemia was observed in diabetic nude mice transplanted with 2000 IE islets from all groups.
Conclusion: This study demonstrates that an advanced pancreas procurement technique including ductal injection and selective arterial flush with cold preservation solution can provide significant improvements on porcine islet isolation outcomes.

P-8.3

The road to successful islet xenotransplantation

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A discussion is provided summarising 15 years of Living Cell Technologies' research into the transplantation of alginate encapsulated neonatal porcine islets. Emphasis is given to the safety and efficacy of various in vivo animal models and the translation of these pre-clinical studies to human therapeutic trials. Important milestones attained during this transition include the acquisition of a swine herd free of any micro-organism capable of infecting humans, successful accreditation of the medical testing laboratory responsible of herd and recipient testing, an additional GMP licensed laboratory with rigorous procedures to extract islets from pancreata and stringent Conditions for the release criteria of the encapsulated cells. The latter required a crucial purification of alginate to eliminate endotoxins, proteins polyphenols, and a critically defined encapsulation process to produce blemish free, biocompatible, durable capsules of defined nanoporosity. Other completed objectives include the molecular characterisation of Porcine Endogenous Retrovirus (PERV) within the donor herd, the confirmation of the PERV non-transmitting phenotype and finally the development of a selective breeding program to enhance safety of recipients of the porcine tissue. The culmination of this research has placed LCT in a strong position to attain regulatory and ethical approvals for human clinical trials.

P-8.4

Rapid quantitative assessment of the porcine pancreas biopsy predicts islet yield

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Background: The cost of islet procurement from donor pigs is greatly increased by use of organs that produce low yields. In this study we developed an assessment system using morphometry of dithizone stained pig pancreas to enable the preselection of donor organs that would have acceptable islet yields.
Method: Porcine (age: 0.5–5.5 y.o.) pancreas biopsy slices with 2 mm thickness taken at the procurement were soaked in dithizone solution for 5 min and then photographed under a dissecting microscope . The islet was evaluated prior to islet isolation by converting the islet numbers (IC) to islet equivalents (IE), then decided IE/cm^2, IE/IC, % >150 μm islets, and >% 200 μm islets. These parameters were evaluated in three different areas of pancreas (duodenal lobe, splenic lobe, and connecting lobe, n = 42 each). Stepwise multivariate linear regression analysis was performed to assess for correlations with islet yield (IE/g pancreas) and decide which area has the most predictive value. To identify other predictors including donor and islet isolation procedure variables, we performed binary logistic regression analysis with significant variables from the univariate analysis (n = 67). For this analysis, the pigs were categorized into high (n = 23) and low (n = 44) groups according to islet yield (cut off: 2000 IE/g).
Results: Islet equivalents per centimeter squre of splenic lobe and connecting lobe were significantly correlated with islet yield (r = 0.433, p = 0.002 for splenic; r = 0.413, p = 0.003 for connecting). IE/IC and % 200um islets of connecting lobe were also correlated with islet yield (r = 0.266, p = 0.04 and r = 0.288, p = 0.03, respectively). Stepwise multivariate linear regression analysis revealed that IE/cm^2 of splenic lobe significantly predicted islet yield. Binary logistic regression analysis indicated that IE/mm^2 of splenic lobe was the only parameter that significantly correlated with successful pig islet isolation (p = 0.01, Odds ratio: 3.605). Variables associated with donor and islet isolation procedure, such as age, sex, ischemic time or enzyme lot, were not significantly correlated with islet yield in this study.
Conclusion: Our study suggests that islet morphometry of splenic lobe biopsies using dithizone stain can be a reliable predictor of islet yield from pig pancreas. This rapid assessment facilitates immediate selection of pig donor organs that are more likely to provide acceptable islet yields.

Tuesday, October 13, 2009

Islet Xenotransplantation II

P-9.1

The age of porcine sertoli cells is critical for providing immune protection of porcine islets xenografted into rats and mice

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Islet transplantation is a viable cell replacement therapy with great potential to effectively cure type 1 diabetes mellitus (T1DM). The supply of high quality donor pancreata and harsh immunosuppressive drug regimes are two major obstacles that need to be overcome to progress the field. Several limitations have prevented islet transplantation from emerging as a standard of care for T1DM. Sertoli cells can provide an immune privilege to the islets of Langerhans when co-transplanted, thus bypassing the need for immunosuppression. Research to-date has focused on the use of porcine neonatal Sertoli cells and has neglected the potential for the use of Sertoli cells of adult porcine origin. Thus, we set out to investigate whether it was an advantage to using Sertoli cells of adult origin as opposed to neonatal. We isolated Sertoli cells of varying ages from pigs ranging from 8-days to 5-years of age and screened for various markers. We observed based on Real Time PCR, protein expression profiles and functional in vitro assays that there

is an ''ideal'' age bracket where adult Sertoli cells can impart their immune-modulatory functions. In addition, FasL mRNA levels as determined by Real-Time PCR were 12–15 fold higher in expression when comparing the different age brackets ($p < 0.001$). To investigate functions in vivo, we then xenotransplanted adult Sertoli cells under the kidney capsule of diabetic nude BALB/c mice and immune-competent FVB/n mice. In parallel, we extended these studies by xenotransplanting labelled adult Sertoli cells into polypropylene chambers previously transplanted subcutaneously in STZ-induced diabetic Lewis rats. We concluded that adult Sertoli cells did not affect the functioning of the co-transplanted islet cells as assessed by the presence of porcine insulin production. Also, our novel labelling technique was useful to monitor transplanted adult immune-modulatory pig Sertoli cells in these and other cell therapeutic applications.

P-9.2

Xenotransplantation of microencapsulated porcine islet cells in diabetic rats

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Background: Xenotransplantation of microencapsulated porcine islet cells might be a possibility to overcome the shortage of human donor organs. Several materials for microencapsulation of cells are described in literature which all show severe disadvantages. NaCS is easy to produce, does not show any cytotoxicity and cell lines survive for a nearly unlimited time-spam after microencapsulation. However, this material has not been tested for microencapsulation and xenotransplantation of porcine islet cells.
Methods: Porcine islet cell isolation and purification was performed according to a newly modified Ricordi method. Porcine islet cells were microencapsulated with NaCS. Diabetes was induced in Sprague Dawley rats by intraperitoneal injection of STZ. Only rats that showed polydipsia, polyuria and blood sugar levels higher than 400 mg/dl over a time period of 14 days were used for the experiments. Microencapsulated porcine islet cells were transplanted under the kidney capsule of the animals. Blood sugar levels were monitored on a weekly basis, porcine C-Peptide levels and insulin levels were measured using ELISA. Intravenous glucose tolerance testing was performed once a months. After 4 months, the animals were sacrificed, the kidney containing the microencapsulated porcine islet cells was retrieved and processed for histological and immunohistochemical examination.
Results: After xenotransplantation of microencapsulated porcine islet cells diabetes was reversed in rats. Animals stayed normoglycaemic up to four months. Functionality of transplanted porcine islet cells was detected by insulin measurement and detection of C-Peptide. After scarification, histological and immunohistochemical evaluation showed no signs of fibrosis or inflammation in the surrounding tissue. Viability of microencapsulated porcine islet cells after explantation was proven by immunohistochemical viability stains.
Discussion: It is feasible to reverse diabetes in rats by transplanting porcine islet cells microencapsulated in NaCS. Rats stayed normoglycaemic until the end of the study period. No signs of fibrosis could be detected in the surrounding tissue. NaCS seems to be a promising material for microencapsulation of porcine islet cells in order to treat diabetes. Further studies have to be carried out to show long term survival of transplanted porcine islet cells microencapsulated in NaCS in diabetic rats.

P-9.3

Creating a prevascularized site for islet transplantation using a V.A.C.®-GranuFoamTM and HBO in rats

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Background: Naturally, islet cells are highly vascularized in the pancreas. This physiological vessel structure is damaged during the isolation process. Therefore, isolated islet cells dependent on diffusion of oxygen and nutrients from the surrounding tissue. After transplantation a lot of freshly isolated islet cells become apoptotic because of hypoxia. Thus, insulin independency can not be achieved because of graft dysfunction. The aim of the study was to show, that it is feasible to create a prevascularized site in rats, using a V.A.C.® (Vacuum Assisted Closure) GranuFoamTM, that is normally used in wound healing and HBO (hyperbaric oxygenation) to induce angiogenesis.
Methods: Fourty Sprague-Dawley rats are divided in five groups and the V.A.C.®-GranuFoamTM is implanted in the subcutaneous tissue. According to the V.A.C.®-Therapy, a drainage is used to suck the secretion and to accelerate wound healing and vascularization of the V.A.C.®-GranuFoamTM. HBO is administered to the different groups at different timepoints for at least one week after implantation to a maximum 1 week prior and 3 weeks after implantation. After the experiments, the blood flow is measured using Szintigraphy. Moreover the V.A.C.®-GranuFoamTM is explanted and processed for histology and immunohistochemistry to assess angiogenesis.
Results: It is feasible to create a prevascularized site in the subcutaneous fatty tissue of rats, using a V.A.C.®-GranuFoamTM and HBO. Angiogenesis is not induced without HBO within one month after implantation of V.A.C.®-GranuFoamTM but within 2 weeks after implantation of the system and HBO therapy. Vessels are not only distributed in the outer parts of the V.A.C.®-GranuFoamTM, the whole sponge-like V.A.C.®-GranuFoamTM is pervaded by new vessels. HBO therapy prior to the implantation does not have a significant influence on vessel growth.
Conclusion: As ischemically damaged islets are likely to undergo cell death or loose functionality due to hypoxia, the use of the V.A.C.®-GranuFoamTM and HBO might be a promising method to create a prevascularized site to achieve better results in islet transplantation.

P-9.4

***In vivo* increase of alpha cells in pig islets by low dose of stz improves insulin secretion**

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Background: Human and pig islets differ by their structures (60%/25% vs 90%/8% for β/α cells, respectively) and functions (stimulation index at ~12 vs 2 for a G1-15 mM, respectively). An increase of intracellular cAMP is required to improve insulin release by pig ß cells. Glucagon may play a crucial role by stimulating ß cell. Therefore, we investigated the possibility to modify in vivo the pig islet structure (in native pancreas) in order to increase the proportion of α cells per islet and then to possibly improve insulin production by isolated islets.
Methods: Selected doses (0, 30, 50, 75, 100 mg/kg) of streptozotocin (STZ), were injected in 27 young pigs to assess the effect of STZ on α cells into native pancreatic islets. Pancreatic insulin/glucagon contents were measured by hormonal extraction/radioimmunoassay and the islets remodelling was quantified by histomorphometry for α/ß cells proportion at 3 months post-transplantation. After the selection of STZ-dose increasing α cells by 20% in islets, nine additional pig pancreas (STZ-treated and Ctrl) were procured and digested (by Liberase PI). Isolated islets were tested in vitro for glucose stimulation.
Results: A significant correlation was found between the dose of STZ and (i) the pancreatic content of insulin ($p < 0.05$, $R = -0.86$) as well as (ii) the proportion of ß cells inside islets ($p < 0.05$, $R = -0.84$). A maximum of 50 mg/kg STZ was required to obtain the optimal remodelling with a significant destruction of ß cells (74% vs 51% of ß cells/islet for STZ < 50 vs STZ > 50 mg/kg; $p < 0.05$) and a concomitant increase of the proportion of α cells/islet in native pig pancreas (26% vs 48% of α cells/islet for STZ < 50 vs STZ > 50 mg/kg; $p < 0.05$). This remodeling was essentially found in small islets (50–200 μm). At 3 months post-STZ treatment [(30 mg/kg, $n = 6$) and (50 mg/kg, $n = 3$)], pig islets were isolated and compared to normal isolated islets ($n = 3$). A higher proportion of α cells was obtained in STZ-modified islets than Ctrl ($p < 0.05$). After in vitro stimulation, isolated STZ-pig islets demonstrated a significant higher glucagon content (65.4 ng/ml vs 21.02 ng/ml, $p < 0.005$) and insulin release (144 μU/ml vs 59 μU/ml, $p < 0.05$) without cAMP raising agent such as Fsk than Ctrl animals, respectively.
Conclusions: Streptozotocin low dose (< 50 mg/kg) can modified in vivo the pig islets structure and improve their functions after isolation.

P-9.5

The effects of glucagon-like peptide 1 (GLP-1) and gastrin on the proliferation and differentiation of neonatal pig pancreatic cell clusters (NPCCs)

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Islet transplantation is a promising therapy for cure of type 1 diabetes, however, donor shortage hampers wide application of this treatment option. Neonatal pig pancreatic cell clusters (NPCCs) is one of good candidates for alternative sources due to the technical easiness of isolation, maintainability in tissue culture and growth potentials in the recipient. One major drawback using NPCCs is that long time (6–12 weeks) is needed for correction of hyperglycemia of diabetic animals upon transplantation. Therefore, promotion of NPCCs differentiation toward mature phenotypes during culture period would obviate glucotoxicity to implanted NPCCs and obviously facilitate early functioning. In this study, we examined the effect of glucagon-like peptide 1 (GLP-1) and gastrin either alone or in combination on NPCCs proliferation and differentiation using RT-PCR and immunocytochemical methods. Although either GLP-1 or gastrin treatment increased the progenitor ductal cells proliferation and early differentiation of endocrine cells compared with untreated control, in particular at high concentration (100 nM), the combination of GLP-1 and gastrin (10 nM each) potently enhanced those processes as revealed by increased Ki67+ cells in ductal cells and early expression of endocrine cell markers such as insulin, glucagon, GLUT2, and PDX-1. Therefore, treatment of appropriate factors such as GLP-1 and gastrin during NPCCs culture period could promote their differentiation toward mature phenotypes and expand endocrine pools by increasing proliferation of progenitor duct cells.

P-9.6

Long-term culture of neonatal islet cell clusters demonstrates better outcomes for reversal of diabetes

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Background: Porcine neonatal islet-like cell clusters (NICC) have the potential to be a limitless source of beta-cells for replacement therapies in type 1 diabetes. However, after transplantation there is a lag time before they develop and secrete insulin in response to glucose.
Aim: To determine the optimal time point for NICC culture that produces the best *in-vivo* functional outcomes.
Methods: NICCs were isolated from 1 to 3 day old pig pancreases, and cultured for up to 4 weeks. The following parameters were determined at weekly intervals during culture: number (IEQ), FACs analysis of % beta cells and % beta cell viability, stimulation index, Insulin: DNA ratio, ATP activity, ethidium bromide and acridine orange viability staining, and gene expression levels of GLP1-R, insulin, glucagon, Caspase 3, and tissue factor (TF). At weekly timepoints during culture, NICC were transplanted under the renal capsule of streptozotocin induced diabetic SCID mice.
Results: NICCs cultured for ≥2 weeks achieved normal blood glucose levels within a mean of 37 days in all transplanted diabetic mice. NICC cultured for 1 week achieved normoglycemia in only 50% of animals and took a mean of 53 days to reach this objective. As a result of longer culture time there was however a significant loss of IEQ over time. Preliminary gene expression data indicate an increase in the level of insulin and a decrease in level of TF gene expression over time in culture, whilst Caspase 3, GLP1-R and glucagon expression remained constant.
Conclusion: Culture of NICCs for at least two weeks provided the best *in-vivo* functional outcome for transplantation.

P-9.7

Comparison of the portal vein and hepatic artery as sites for pig islet xenotransplantation in non-human primates

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Aims: Pig islets offers a potential solution to the limited human islet transplantation for type 1 diabetes. The intra-portal infusion has been commonly used for islet transplantation. However, potential risk exists in the intraportal islet infusion. In this study, we compared the portal vein and hepatic artery as sites for pig islet xenotransplantation in rhesus.
Methods: Immunosuppressed streptozotocin (STZ) -induced diabetic rhesus were transplanted intraportally (PV; n = 8) and intrahepatic arterially (HA; n = 6), respectively with 50000 neonatal porcine islets (NPIs)/kg. Graft survival and function were determined by blood glucose monitoring, and examination of porcine C-peptide and liver biopsy post transplantation.
Results: One and four HA and PV animals, respectively, died during and after transplantation procedure. All and three of the remaining PV and HA animals, respectively, became insulin-free from days 70–110 after transplantation for more than 120 days with insulin positive NPIs in their liver biopsy samples. In addition, another two remaining HA animals demonstrated partially NPI function. Porcine C-peptide and no PERV infection were detected in all the recipients.
Conclusions: The intra-portal and -hepatic artery NPI xenotransplantation achieved similar function outcomes in diabetic rhesus recipients. However, the intra-portal infusion procedure may cause unnecessary mortality.

Islet engraftment

P-10.1

Gene expression profiling of cytotoxic lymphocytes and islets in the peripheral blood for monitoring of portal islet transplants in streptozotocin-diabetic mice

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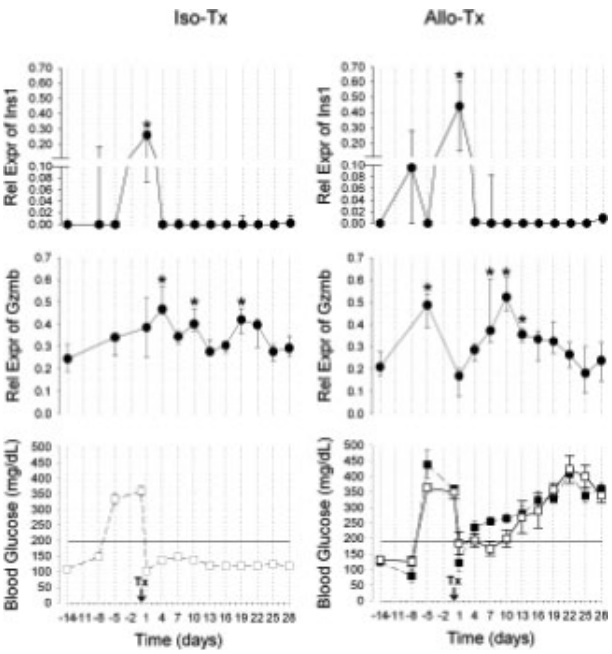
Background: The purpose of this study was to evaluate the combined measurement of gene expression levels of cytotoxic lymphocyte (CL) and



islet hormone genes for monitoring rejection and graft damage in a mouse islet transplant model.
Methods: Two groups of male mice received portal transplants from male FVB donor mice 9 days after induction of diabetes by intraperitoneal streptozotocin injection (220 mg/kg): (1) Iso Tx (FVB, n = 8) and (2) Allo Tx (C57BL/6, n = 8). Blood was sampled by tail snip. Real-time PCR was used to measure the mRNA levels of granzyme B (Gzmb), perforin 1 (Prf1), fas ligand (Fasl), insulin 1 (Ins1) and glucagon (Gcg). Data were given as median with 25 and 75 percentiles. Friedeman repeated measures analysis of variance on ranks in conjunction with Dunn's method of multiple comparisons vs control (day 14 pre Tx) was used for statistical analysis. $p < 0.05$ was considered significant.
Results: In isografts lasting normoglycemia occurred immediately after Tx. In allografts, one half of the group attained normoglycemia (< 200 mg/dl) and the other had glucose levels at ~ 50 mg/dl before loss of glucose control as result of histologically proven rejection ensued. Gzmb, Prf1, Fasl expression rose steadily and peaked before severe hyperglycemia occurred in allografts. Gzmb was the best rejection marker with a 2.5-fold and significant peak expression over baseline. This pattern differed from isografts, which had an undulating expression with low amplitude. Ins1 and Gcg were reliably detectable only on the first day post Tx. Twofold higher expression levels of Ins1 were predictive of fair blood glucose control.
Conclusions: Immunoprofiling of CL gene expression is a noninvasive method to detect ongoing rejection before overt diabetes becomes evident. As Ins1 and Gcg gene expression in blood was reliably detectable only early after Tx, their measurement may help to estimate graft damage and eventually predict metabolic control.

P-10.2

Comparison between ferucarbotran (Resovist®) and ferumoxide (Feridex®) in human islet graft monitoting

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Backgroud: Ex vivo labelling of the islets with iron oxide (SPIO) nanoparticles prior to transplantation allows MRI imaging of the graft and could be used in clinical practice to track islets rejection. The aim of the study is to compare the use of two iron nanoparticules labelling agents (ferucarbotran (Resovist®) and Ferumoxide (Endorem® or Ferridex®) in vitro and in vivo, with a radio-histological correlation.
Method: For all experiment, we used human islet preparations of high purity level (80–90%). Human islets were labelled by incubation with SPIO nanoparticles at different concentrations (ferucarbotran: 14, 56, 140 and 280 μg/ml iron, or ferumoxide: 100, 200 or 280 μg/ml of iron) for 24 h at 37°C. Static incubations were performed on 100 islets, comparing insulin response to basal (2.8 mM) or high glucose stimulation (16.7 mM), with or without cAMP stimulation at the same conditions. Insulin and Pearl's (assessment of iron content) staining were performed. Electronic microscopy analysis was performed. Labelled Islets were used for in vitro or in vivo imaging in MRI 1.5T. Liver section after organ removal was performed in the same plane as MRI imaging to get a correlation between histology and radiology.
Results: Labelled and control islets responded similarly to glucose stimulation during static incubation tests. Both had stable viabilities (80–90%). On histology, both iron particles were co-localized with insulin staining cells, but the uptake was very heterogeneous within the same preparation. Electronical microscopy showed iron inclusion in the islet stroma and beta cells at the highest concentration of Ferucarbotan, it failed to demonstrate iron in the Ferumoxide condition. MRI images of phantoms correlated with the iron cell content, using both SPIO. However, Ferumoxide demonstrated a less intense signal as Resovist. After human islet transplantation in rats, the 1.5T MRI signal was strong with Ferucarbotan, but weaker after Ferumoxide labelling, even at the highest concentration (280 μg/ml). A good radio-histological correlation was obtained after liver staining in the same plane as the MRI acquisitions.
Conclusion: Ferucarbotan appears more appropriate for human islets imaging compared to Ferumoxide, in vitro and in vivo at 1.5 T. Both SPIO are non-toxic to the islets and we confirm a good radio-histological correlation.

P-10.3

Vascularization of PPL (polypropylene) chambers for islet implantation assessed by Magnetic Resonance Imaging (MRI). A preliminary data

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Background: A novel PPL cylindrical porous chamber implanted s.c. or into the major omentum can be incorporated by connective tissue that is rich in blood vessels and therefore might serve as a support for pancreatic islet (PI) transplantation (Tx). As the PPL material itself does not emit any signals in a magnetic field, MRI could be able to assess the stage of vascularization and consequently the right time for PI implantation into the chamber.
Methods: PPL chambers (24×6 mm) were implanted s.c. (n = 7) or into the major omentum (n = 7) in Brown Norway female rats (180–200 g). The animals were scanned weekly for 1 month and then every other week for 5 months using an experimental MR Bruker Biospec 4.7T scanner equipped with a resonator coil (ø 7cm). Standard gradient echo sequence (TR/TE = 100/3.4 ms, slice thickness = 1 mm, resolution = 117×117 mm) was fast enough to eliminate movement artifacts during the in vivo scanning. . Main veins were visualized by native MR angiography (native time of flow). In five recipients (1 s.c. and 4 i.p.), diabetes was induced by intraperitoneal injection of streptozotocine (60 mg/kg) 2 months after the chamber implantation. Ten days later, when stable hyperglycemia was confirmed, labeled PI (10000 PI/kg; 48 h, ferucarbotran 5 ml/ml) were inserted into the lumen of the chamber.
Results: The implantation of chambers did not cause any complications. Tissue ingrowth was detectable by MRI one week and seemed to be completed by 5 (omental) and 8 (s.c.) weeks after implantation. Labeled PI isografts normalized blood glucose by 2 weeks after their administration into chambers in all diabetic animals. Contrary to chambers inserted subcutaneously, MR angiography detected more than one large vein in close proximity to the chambers implanted into the major omentum.
Conclusions: According to MRI, vascularization of the PPL chambers is more extensive and proceeds faster after their implantation into the omentum then in the subcutaneous space. Detection of newly formed vessels at the implantation site helps to determine the right time for administration. Labeled PI implanted into the chambers survive, normalize blood glucose levels in diabetic recipients and may be detected in T2 weighted scans for as long as 5 months.
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P-10.4

A novel technique to surgically implant a polypropylene scaffold into the omentum for the transplantation of pancreatic islets

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Background: The omentum due to its extensive vascularization and blood drainage has been utilized for a site of transplantation of islets as a cell therapy for diabetes. Here we describe the use of a novel chamber device pre-transplanted within the omentum as a vehicle for islet cell therapy
Methods: The chamber device was wrapped entirely by the omentum of Lewis rats and secured to allow subcutaneous access for cell infusions. After 4 weeks, isolated rat islets or buffer were then transplanted into the chamber at a concentration of 10000 IEQ/kg weight (n = 10).
Results: 7/10 diabetic rat recipients normalized their blood glucose levels. Histology revealed extensive vascularization and collagen formation within the device which seemed to be critical for the survival and function of the islets.
Conclusions: The use of the implanted chamber device provides an effective physiologicalenvironment for the support of pancreatic islets as a cell therapy.

P-10.5

Novel aspect of experimental islet transplantation: investigation of desirable trasplantation sites and production of diabetes model pig

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Islet transplantation has potential to become the most physiologically advantageous and minimally invasive procedure for the treatment of type 1 diabetes mellitus. The most serious problem is that long term insulin independence of five years has been substantialy still quite low. Our concern is to improve poor long term insulin independence, of which the one cause is considered to be transplant site. Currently, prepared islets are transplanted into the portal vein area in the liver of the recipients by drip-infusion method through the peripheral blanch of supramesenteric vein. However, it has been indicated that considerable recipients suffered from iatrogenic portal hypertension with elevated hepatic enzymes by intraportal dose of a certain amount of islets. Alteration of transplant site is optimal option to solve this problem. One candidate of transplant site is mesentric membrane, for which we have tried to inoculate islets on the collagen-coated non-woven fabric sheet to be wound around mesentric vessels. Another site is a bioartificial device, for which we have developed an optimal three-dimensional culture device providing immobilization scaffold affinitive for living cells. On the other hand, to evaluate efficacy of such device, a large animal diabetes model is indispensable. So we have produced an optimal Tg-clone pigs as diabetes model, by introducing mutant human hepatocyte nuclear factor 1α (HNF1α) gene, which is known to be causative gene for MODY3 (maturity-onset diabetes of the young). Intracytoplasmic sperm injection (ICSI) has been employed to obtain first TG piglet, of which the somatic cells were established to produce clone pigs. In this paper we report our trials to develop desirable transplantation sites, subsequently production of a new large animal diabetes model.

P-10.6

Biocompatible, 3-dimensional, cyclic RGD-modified, purified alginate scaffold designed to enhance islet viability, neovascularization, and efficacy of extrahepatic islet transplantation

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Background: Previously we had shown that alginate scaffolds impregnated with VEGF + PDGF had improved neovascularization of the transplantation bed and allowed for islet survival after intramuscular implantation. Now, we tested if enrichment of the scaffold with RGD peptide additionally enhanced neovascularization and viability of the mesenchymal stem cells (BM-MSC), which are known to promote islet survival upon co-transplantation. We also evaluated if advanced alginate purification may limit scaffold immunogenicity and pro-inflammatory properties.
Methods: Alginate was purified using customized protocol including charcoal treatment, hydrophobic polyvinilydene fluoride membrane filtration, dialysis, and ethanol precipitation. Next its immunogenicity was determined by residual mitogen contamination, splenocyte proliferation and by histology after intramuscular implantation in rodents ($n = 6$). Purified alginate was modified with cyclic RGDfK peptide. Viability of BM-MSC seeded into such scaffold was assessed after 1 week culture. Scaffold neovascularization was determined by immunostaining 2 months after in vivo implantation.
Results: Our purification reduced protein contamination by 71% (to 0.31%) and endotoxin content by $>90\%$ (<12.5 EU/g) ($p < 0.05$). Splenocyte proliferation was decreased by 69% and 31% compared to unpurified and to pharmaceutical grade alginates, respectively ($p < 0.05$). After purified scaffold implantation, fibrosis was minimal with 1.2 ± 0.7 multinucleated cells per high power field (HPF) vs 17.5 ± 4.2 in controls ($p < 0.05$). RGD-modified scaffolds increased BM-MSC viability by 63% compared to controls ($p < 0.05$). Factor VIII and α smooth muscle actin positive vessels per HPF increased respectively from 0.9 ± 0.1 and 1.2 ± 0.2 in unmodified scaffolds to 3.5 ± 1.5 and 4.9 ± 0.9 in RGD ones ($p < 0.05$).
Conclusions: Alginate advanced purification and conjugation with RGD peptide limits scaffold immunogenicity and enhances neovascularization after implantation. Such optimized scaffold has improved potential for extrahepatic site preconditioning and enhanced islets survival. It also improves BM-MSC viability when seeded into the scaffold which may be used for additional enhancement of islet engraftment.

P-10.7

Co-transplantation of rat endothelial progenitor cells to enhance the engraftment of pancreatic islets

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Aims: Islet transplantation is limited by extensive beta-cell death and poor revascularisation in the post-transplant period. Endothelial progenitor cells (EPC) represent a vascular 'tool kit' capable of stimulating repair of existing endothelium and the growth of new vessels. We characterised rat bone marrow-derived EPC and evaluated their potential to improve engraftment of transplanted islets.
Methods: Bone marrow was harvested from the femurs and tibias of albino Wistar rats, seeded onto fibronectin-coated flasks and cultured with heparin and growth factors (VEGF, FGF) for up to 14 day. At 48 h, non-adherent cells (termed 'EPC') were removed from adherent mature endothelial cells and monocytes (termed 'EC/monos') and reseeded. Flow cytometry was used to analyse adherent cells for expression of markers of stem cells (CD133, CD34), leukocytes (CD45) and monocytes (CD14), CXCR4 and VEGFR2. Endothelial phenotype was confirmed by expression of CD146, VCAM-1 and 3D tube formation in Matrigel. Pancreatic islets were isolated from albino Wistar rats and cultured overnight. Islets (1000–2000 IEQ) were transplanted under the kidney capsule of streptozotocin-treated diabetic NOD-SCID mice either alone, or after being combined with CFSE-labelled day six rat EPC. In homing experiments, labelled EPC were delivered via tail vein injection immediately following islet transplant surgery.
Results: Rat bone-marrow derived cells formed round clusters with adherent cell outgrowths by 48 h. In culture, 'EC/monos' formed monolayers, while 'EPC' produced colonies containing round and spindle-shaped cells, with strongly adherent perimeter cells. By day seven, a higher number of 'EC/monos' (30–40%) expressed CD45, CXCR4 and CD14 (representing hematopoietic/monocytic origin) compared to 7–10% of 'EPC'. Most 'EC/monos' were VCAM-1 + and expressed VEGFR2, indicating their endothelial nature, while 'EPC' expressed less VEGFR2 and were negative for VCAM-1, suggesting a more immature phenotype. Both populations were capable of forming tubes in Matrigel in 24 h. CFSE labelled EPC were observable within the islet graft at day six post transplant.
Conclusions: Rat EPC demonstrate similar phenotypic characteristics to murine EPC grown under the same conditions. In a rodent model of diabetes, EPC persist at the graft site, and may enhance revascularisation and improve islet function.

P-10.8

Islet transplant engraftment and function: a potential role for rosiglitazone

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Background: Human islet transplantation would offer a less invasive and more physiological alternative for the treatment of diabetes than either whole pancreas transplantation or insulin injections if islet graft survival can be improved. Factors contributing to graft failure include enzymatic disruption of the islet microenvironment during isolation, diabetogenic effects of immunosuppressants and metabolic stress resulting from slow revascularisation. This study investigated upregulation of vascular endothelial growth factor (VEGF) production in human islets by exposure to a thiazolidinedione (TZD, rosiglitazone) to enhance proliferation of endogenous islet endothelial cells.

Methods: Human islets were cultured in conventional static culture (SC) in medium199 (m199), RPMI1640, endothelial growth medium (EGM) and CMRL1066 in the presence and absence of 25 mM TZD. Additionally, islets were cultured in rotational culture (RC) in m199 ± TZD and in EGM. Insulin secretion was induced by static incubation with low glucose (1.67 mM), high glucose (16.7 mM) and high glucose with 10 mM theophylline (G+T). Both insulin and VEGF production were assessed by ELISA. Dual fluorescence staining was performed for insulin and VEGF. Islet morphology was documented photographically for SC and RC control groups at days two and five post isolation.
Results: Human islets cultured in m199 in SC and RC exhibited comparable preservation of morphology and insulin secretory profiles compared to islets maintained in the other media tested. All cultures showed significantly increased insulin secretion in response to 16.7 mM and G+T over basal; this was enhanced by RC and in certain instances further improved by addition of 25 mM TZD. TZD significantly increased VEGF production and release as determined by ELISA. Islets from all experimental groups stained positively for insulin and VEGF. RC islets showed enhanced insulin secretory capacity and improved morphological preservation when compared with SC.
Conclusions: RC supports human islet functional viability and structural integrity more effectively than SC. The addition of TZD occasionally further improves secretagogue induced insulin secretion and significantly upregulates VEGF production in islets as indicated by ELISA. Pre-treatment of islets in RC with TZD prior to transplant may improve the functional viability and revascularisation rate of islet grafts.

P-10.9

Fabrication of functional islet-cell sheet

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Background: The present study was designed to establish a novel tissue engineering approach for diabetes mellitus (DM) by fabricating a tissue sheet of pancreatic islet cells for the use of in vivo transplantation.
Methods: Single cell suspension of pancreatic islet cells was obtained from Lewis rats. Cells were then plated onto the laminin-5-coated thermo-responsive culture dishes. After the cells reached confluency, islet cells were harvested as a uniformly connected tissue sheet by lowering the culture temperature to 20°C for 20 min. The functionality of the harvested islet cell sheet was assessed by histological examination, culture studies, and transplantation in vivo.
Results: Histological examination revealed that the harvested islet cell sheet composed 76% and 22% of insulin- and glucagon-staining positive cells, respectively. When we replated the tissue sheet into a new culture dishes, we found positive response of the tissue sheet in the glucose-challenge test. In vivo existence of the islet cell sheet was confirmed in studies where tissue sheet was transplanted into the subcutaneous space of rats.
Conclusions: The present study describes an approach to create a functional sheet of pancreatic islet cells in vitro, which can be transplanted in vivo. The present study can serve a basis for the creation of novel type of cell-based therapy for DM without requiring cell infusing into the blood circulation.

P-10.10

Exendin-4 stimulates beta-cell replication in mouse adult islet grafts but not in human adult islet grafts in mice

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β cells of aged adult have extremely low rates of replication and that aging correlates with the decreased capacity for β-cell replication. In this study, we compared the effect of exendin-4 on stimulating β-cell replication in mouse adult islet grafts and in human adult islet graft in mice. Donor mouse islets were isolated from retired breeder C57BL/6 mice (over 36 weeks old). Total 100 islets were transplanted under the left kidney capsule of each streptozotocin induced-diabetic C57BL/6 mouse. Human islets with >80% purity and >90% viability, from three different adult donors (>46 years old), were obtained from the NIH and JDRF Islet Cell Resource Centers. Total 2000 IE human islets were transplanted under left kidney capsule of each streptozotocin induced-diabetic nude mice. These recipient mice were given bromodeoxyuridine (BrdU) at 100 mg/kg/day and with or without exendin-4 at 10 nM/kg/day. At 4 weeks posttransplantation, nephrectomy was performed to remove kidney bearing islet grafts for insulin and BrdU double immunofluorescence staining. With or without exendin-4 treatment, normoglycemia was achieved in all recipient C57BL/6 mice that received 100 syngeneic mouse islets. However, normoglycemia was achieved in significantly fewer days in exendin-4 treated C57BL/6 mice. The mean number of days required for achieving normoglycemia was 16 ± 5 days in untreated C57BL/6 mice (N=10) and 11 ± 6 days in exendin-4 treated C57BL/6 mice (N=11, $p<0.05$). At 4 weeks post-human islet transplantation, 10 of 14 untreated nude mice and 13 of 15 exendin-4 treated nude mice achieved normoglycemia. The mean number of days required for achieving normoglycemia was 11 ± 5 days in untreated nude mice (N=10) and 6 ± 4 days in exendin-4 treated nude mice (N=13, $p<0.05$). The mean percentage of BrdU+ β cells in mouse islet grafts was 3.2 ± 2.1% in untreated C57BL/6 mice and was 16.7 ± 5.8% in exendin-4 treated C57BL/6 mice ($p<0.01$). However, few BrdU+ β cells in human islet grafts were detected in untreated or exendin-4 treated nude mice. Our data demonstrated that exendin-4 treatment can stimulate β-cell replication in mouse adult islet grafts but not in human adult islet grafts and indicated that targeting GLP-1 receptors on human adult β cells by GLP-1 analogs is not sufficient to stimulate human β-cell replication.

P-10.11

Transgenic expression of insulin-like growth factor-II (IGF-II) in pancreatic islets to prevent apoptosis

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Aims: Pancreatic islet transplantation offers a potential cure for Type 1 Diabetes Mellitus (T1DM). However, due to a variety of extrinsic and intrinsic factors, greater than 60% of islets undergo significant apoptosis within the first 48-hours post-transplantation. Insulin-like growth factor II (IGF-II) is a potent regulator of apoptosis and physiological down-regulation of IGF-II in the neonatal pancreas correlates with beta-cell loss. Transgenic expression of IGF-II may protect transplanted islets. *Ex vivo* manipulation of islets prior to transplantation results in increased specificity, in addition to long-term and local transgene expression. The aim of this study was to infect human and rat pancreatic islets such that they express IGF-II and to determine whether IGF-II expression protects islets from a range of apoptotic assaults *in vitro*.
Methods: A replication deficient adenoviral construct encoding human IGF-II (AdV-IGF-II) was generated and used to transduce human and rat islets. Insulin release by transduced islets was measured by ELISA. The protective effect of IGF-II was determined by staining islets challenged with hydrogen peroxide for necrosis (7AAD) and apoptosis (TMRE). Pro-inflammatory cytokines known to induce islet apoptotic death (IL-1β and IFN-γ) were used to treat untransduced human and rat islets for 24 h. Assays for AnnexinV and caspase (three and seven) activity were used to detect early apoptosis by flow cytometry.
Results: Islets were successfully transduced with MOI 10–200. Transduction did not affect insulin secretion or cell viability. ELISA revealed transduced islets secreted 55 ng/ml IGF-II but did not show resistance to apoptosis following hydrogen peroxide challenge. An in vitro model of cytokine-induced apoptosis was established in isolated human islets, rat islets and rodent insulinoma cell lines (RINm and NIT-1).
Conclusion: Islets were successfully transduced without affecting function or viability. Further studies will determine the extent of IGF-II mediated protection on transduced islets following apoptotic assault. Transduction of islets with developmental genes is a novel strategy to improve islet viability.

P-10.12

IBMIR: *in vitro* study of role of rapamycine on macrophage activation during islet transplantation

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Human islets exposed to human blood trigged an ''instant blood mediated inflammatory reaction'', IBMIR, characterized by platelet consumption, and activation of the coagulation and complement systems. Less than 50% of infused islets in the portal vein survived after transplantation. Rapamycine is one of the three molecules that composed the immunosupressive treatment applied during pancreatic islets transplantation. However, its role on the blockage of IBMIR have to be determined. The aim of this work is to evaluate in vitro the role of the rapamycine in the prevention of inflammatory reaction by the study of macrophage activation. The cellular model used in this work was a primary culture of macrophage isolated from Rat. Effect of rapamycine on cellular viability was measured by Cell Titer® and apoptosis was studied by the cleavage of caspase 3 using western blotting. Macrophage migration was measured using a modified Boyden chamber and cytokine liberation was evaluated using RayBiotech® Cytokine Antibody Arrays. Finally, the study of molecular signaling pathway of macrophage activation during IBMIR was evaluated by the study of protein phosphorylation like p70-S6K for the mTOR (mammalian Target of Rapamycin) pathway and JAK2 (Janus Kinase 2) for the chemokine pathway. The results of viability test suggest that rapamycin decrease the macrophage viability by apoptosis. Indeed, the rapamycin toxicity is maximal after 48 h of incubation, the percentage of living cells is 26.22% ± 7.19 (*) and 28.61 ± 7.11 (*) for respectively 1 ng/ml and 100 pg/ml of rapamycine. The migration study shows a significant inhibition of chemotaxis induced by islet supernatant from 2.32 ± 0.3 to 1.31 ± 0.11($n=4$, $p<0.001$) with 100 pg/ml of rapamycin. The cytokine analysis shows an increase of IL-10 and IL-6 secretion when macrophages are exposed to islets supernatant. Moreover, this secretion is inhibited with rapamycin. Finally, study of molecular signaling pathway shows that IBMIR activates the chemokine pathway which is inhibited in presence of rapamycine These data suggest that rapamycin could modulate IBMIR throught the chemokines signaling pathway. Moreover, understanding the molecular signaling pathway of IBMIR could improve islet viability during transplantation.

P-10.13

Identification of new therapeutics targets to increase islet vascularization during transplantation

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Background: Rapid and adequate revascularization of transplanted islets are essential for islet survival and function during transplantation. Previous studies have shown that Deferoxamine (DFO), an iron chelator using in clinical treatment of iron overload, increases VEGF (Vascular Endothelial Growth Factor) expression in pancreatic islets by hypoxia and improve metabolic control of diabetic syngenic rats. However, molecular signalling pathways implicated in islets revascularization has to be elucidated. The aim of this work was to identify angiogenic factors implicated in vascularization of islets in order to develop new therapeutics targets.
Methods: β cell lines from rat insulinoma (RINm5f) were incubated with or without DFO (10 μM) during 1 and 3 days of culture. The identification of molecular pathway implicated in islets revascularization with DFO was realised using a Rat Angiogenesis RT^2 *ProfilerTM* PCR Array.
Results: DFO induces an overexpression of VEGF like demonstrated in our last study. However, analysis of PCR Array has shown that DFO also overexpress specificly the VEGFD. Moreover, 3 days after treatment, DFO overexpress several angiogenic factors: – Growths factors: IGF-1, FGF, PGF, PDGF, EGF, CTGF, Midkine and Epiregulin, – Constitutives proteins of extracellular matrix: Collagen type 18α1, PECAM-1, Fibronectin 1 and VE-cadherin, – Proteins implicated in the formation and in the maintenance of vessels: MMP19, MMP9, MMP3, TIMP3, BAI1 and angiopoietin2. Finally, we have observed an overexpression of leptin with DFO. Moreover, leptin induces an increase in VEGF/VEGFR2 levels.
Discussion: PCR Array represents an interesting tool to identify molecular signalling pathways of angiogenesis to stimulate pancreatic islets vascularization. Finally, this work could permit to test new pharmacological targets to improve islets viability during transplantation.

P-10.14

Rapamycin does not adversely affect intrahepatic islet engraftment in mice and improves early islet engraftment in humans

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Objective: In this study we examined the effect of rapamycin (RAPA), a key component of the immunosuppressive regimen in clinical islet transplantation, on islet engraftment and function *in vivo*.
Methods and Results: Diabetic C57BL/6 or BALB/C recipient mice were transplanted with 350 syngeneic islets through the portal vein (PV-Tx; C57BL/6 $n=60$; BALB/C $n=22$) and treated with once-daily oral RAPA (1 mg/kg) or vehicle. No differences in post-transplant blood glucose concentrations and glucose tolerance were observed between RAPA- and vehicle-treated mice. The impact of RAPA on human islet engraftment was assessed in 10 patients with type 1 diabetes treated with 0.1 mg/kg/day rapamycin before islet transplantation. Compared to non pre-treated islet transplant recipents ($n=12$), RAPA pre-treated patients had increased blood RAPA concentrations ($p=0.006$) and fasting C-peptide concentrations ($p=0.005$) in the two weeks post-transplant. RAPA pre-treatment was associated with a reduction in chemokines CCL2 and CCL3 concentrations pre-transplant ($p<0.01$), and a dampened chemokine response ($p=0.005$) post-transplant. Concordantly, in vitro RAPA inhibited the secretion of CCL2 and CCL3 by monocytes.
Conclusion: Rapamycin does not adversely affect intrahepatic islet engraftment in the mouse, and potentially improves islet engraftment in humans by an anti-inflammatory mechanism.

P-10.15

Role of CCL2/MCP-1 in islet transplantation

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Objective: High levels of donor-derived CCL2/MCP-1 have been associated with poor islet allograft outcome in patients with type 1 diabetes. The aim of our work was to determine whether CCL2/MCP-1 secreted by the islet has independent pro-inflammatory effects that influence engraftment and graft acceptance.
Research Design and Methods: A mouse model of syngeneic intraportal islet marginal mass transplantation in which $CCL2^{-/-}$ mice were used alternatively as islet donors or recipients was established to determine whether donor and/or recipient CCL2/MCP-1 affected islet function post transplant. In man, we analyzed the relationship of islet CCL2/MCP-1 and/or post islet transplant inflammation (CRP concentration) to islet function post transplant in 30 patients with type 1 diabetes who received a single islet infusion obtained from single donor.
Results: Both in mouse and human CCL2/MCP-1 is significantly positively associated with other cytokines/chemokines, in particular with the highly released ''pro inflammatory'' IL-6 and CXCL8/IL-8 or CXCL1/KC. Transplantation of $CCL2^{-/-}$ islet into syngenic recipients did not improve the transplant function. Transplantation of islet into $CCL2^{-/-}$ syngenic recipients led to a significant improvement of transplant function and partial abrogation of local hepatic inflammation. When evaluated in human islet CCL2/MCP-1 release was strongly related to the immediate local inflammatory response in the liver and impacted short-term human islet function dependently by the induced inflammatory response and independently by the immunosuppressive therapy.

Conclusions: The data showed that islet CCL2/MCP-1 release is a sign of ''inflamed'' islets without having a direct role in graft failure. On the other hands a causal effect for developing detrimental pro-inflammatory conditions after transplant was proved for recipient CCL2/MCP-1. Strategies to selectively decrease recipient, but not donor, CCL2/MCP-1 release may increase the success of islet transplantation.

P-10.16

The anterior chamber of the eye allows performing noninvasive, repeated live imaging of islet allograft rejection

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Background: Immune allograft rejection has traditionally been studied extrapolating from static snapshots of tissue sections retrieved from different animals. To study the cellular mechanisms of islet allografts rejection in vivo, we used a model of transplantation into the anterior chamber of the eye (ACE). Aims of the studies were (i) investigating whether islet allograft rejection occurs in the ACE and (ii) the feasibility of studying immune responses using noninvasive, live imaging in the ACE.
Methods: DBA/2 mouse islets were transplanted into diabetic C57BL/6 recipients either in the ACE or under the kidney capsule (KDN) without immunosuppression. Immune cell activity was monitored by live fluorescent microscopy using as islet transplant recipients C57BL/6(B6.129P2-Cxcr6tm1Litt/J) mice expressing green-fluorescent protein (GFP) in effector and memory T-lymphocytes.
Results: Graft loss (glycemia > 200mg/dl) occurred on postoperative day (POD) 14 ± 5 in the KDN (n = 7) and 32.7 ± 11.1 in the ACE (n = 9; $p < 0.002$). We repeatedly monitored intra-ACE grafts in individual mice at follow-up using live microscopy, which demonstrated increasing GFP+ lymphocyte infiltration that well correlated with loss of islet morphological integrity, reduction of volume and function. GFP+ T-lymphocytes began graft infiltration at POD + 3 without morphological changes. At POD + 14, T-lymphocyte numbers increased as they started invading the allograft; T-cells formed large clusters inside the islets that paralleled loss of islet mass and integrity. Overall T-cell behavior was evaluated by quantitative tracking of 4-dimensional recordings (20-min laps). T-cells traveled at 3μm/min track velocity both around and within the islets, suggesting that T-cells moved freely throughout the transplanted tissue. T-cells acquired a flattened morphology during the late phase of rejection extending large pseudopodia towards other T-cells. Dynamic analysis revealed extensive motility within very short distances.
Conclusions: Intraocular islet allografts are rejected via a T-cell-mediated destructive process resulting in hyperglycemia and that real-time, repeated, non-invasive live imaging of immune cell infiltration and islet morphology is feasible in the ACE. The ability of monitoring individual islets in the same animal over time is an invaluable advantage of this model over conventional transplant sites.

P-10.17

Prolonged allogeneic islet graft survival into a prevascularized, subcutaneous biohybrid device

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Background: Alternative implantation sites show great promise for islet grafts. Prevascularized, subcutaneous devices can sustain long-term function of syngeneic islet grafts in rats. Aim of the present study was to evaluate whether allogeneic islets could restore euglycemia if implanted into a subcutaneous, prevascularized biohybrid device designed to offer mechanic- but not immune isolation.
Methods: Chemically diabetic Lewis rats received fully mismatched allogeneic islets from Wistar Furth rat donors into a prevascularized, subcutaneous device under the umbrella of systemic immunosuppression.
Results: Combined treatment with sirolimus and tacrolimus prevented rejection (fasting glycemia < 200 mg/dl; well-preserved islet with minimal/absent mononuclear infiltrate at histopathology examination) for > 80 days, but was invariably associated with failure of achieving stable, nonfasting euglycemia (n = 4). We then developed a nondiabetogenic protocol based on anti-lymphocyte serum induction and chronic fingolimod (Novartis) treatment that resulted in euglycemia and long-term (> 60 days) islet allograft survival into a biohybrid device (n = 3). Additionally, well-preserved islets with minimal mononuclear cell infiltrate were observed in explanted devices.
Conclusions: Our data indicates that long-term islet allograft survival can be achieved in a prevascularized, subcutaneous device. Notably, the choice of immune intervention appears to be critically important to allow adequate engraftment and achievement of full function after islet implant.

P-10.18

Lack of cytoprotection of gabexate mesylate on pancreas cold preservation and islet cells

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Background: Activation of pancreatic enzymes during organ preservation and islet isolation may negatively impact organ quality and islet yields. The use of protease inhibitors in islet preservation solutions and/or at the time of pancreas processing has been proposed as a means to improve islet recovery after isolation. Clinical and experimental data support the cytoprotective effects of the protease-inhibitor Gabexate Mesylate (GM) for the treatment of acute pancreatitis. Aim of this study was to assess the impact of GM on pancreas cold ischemia and islet cells.
Methods: Donor rats were injected IV with 0.2 or 4 mg of GM and their pancreata were perfused with and stored in cold UW solution containing increasing concentrations of GM (0.0.2, and 0.4 mg/ml), respectively. Cold ischemia was carried on for ~18 h before islet isolation. Islet yields per pancreas, recovery after culture, glucose-stimulated insulin release (GSIR) and levels of NADPH-dependent oxidase (Nox; a measure of oxidative stress) were assessed. The impact of GM on islet engraftment was evaluated in a syngeneic model of intra-hepatic marginal mass transplantation (2200IEQ/Kg) in animals treated or not with intravenous GM (3 mg infusion over 1 h) at the time of transplant.
Results: After isolation, islet yield per pancreas was 559 ± 209 IEQ in controls, 752 ± 314 IEQ in GM-0.2 group and 786 ± 253 in GM-0.4 group (n = 5 isolations, 4–5 donors each), without reaching statistical significance. Recovery after overnight culture showed a loss of ~30% in control and experimental groups (p = NS). GSIR showed no statistically significant differences between islets obtained from ischemic glands treated or not with GM. Nox levels showed unremarkable differences between groups. Recipients of intra-hepatic marginal islet mass transplant and treated with GM reverted diabetes with tempos comparable to controls (median = 6 days) and showed unremarkable differences during intravenous glucose challenge.
Conclusions: Our data suggests that GM is not an effective cytoprotective agent for pancreas and islet cells, at least in the models and at the concentrations utilized in our studies.

P-10.19

Noninvasive, live imaging studies of human islet cell biology in the anterior chamber of the eye

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Background: Human islet cell biology is cumbersome to study in vivo and many assumptions on islet physiology have been derived by studying rodent and human islets in vitro, while in vivo data can only infer islet activity based on indirect measurements of plasma hormone concentrations. Notably, functional imaging of islets in a living organism remains challenging, since non- or minimally-invasive technologies remain not fully developed yet. The

anterior chamber of the eye (ACE) has been utilized as a valuable implantation site to study murine islet structure and function in vivo. In the present study, we explored the value of the ACE for the in vivo study of human islet biology.
Methods: Human islets (~500 islet equivalents per eye) were transplanted into the ACE of streptozotocin-treated athymic nude mice. Graft function was monitored by measuring nonfasting glycemic values and performing intraperitoneal glucose tolerance tests (IPGTT). Longitudinal imaging of human islet revascularization was performed by confocal laser scanning microscopy following intravenous injection of fluorescein dextran. Islets were visualized with reflected light.
Results: After transplantation, animals achieved and maintained normoglycemia for over 150 days. Human islets showed tight control of plasma glucose concentrations during IPGTT performed two months after transplantation. Islet neovascularization was completed within 30 days post-transplant. The time to diabetes reversal paralleled the progression of new vessel formation in islets.
Conclusions: Our data suggests that human islets transplanted into the ACE of immunodeficient mice engraft and are fully functional, allowing to achieve tight metabolic control. The ACE acts as a natural window allowing for real-time, repeated, noninvasive in vivo imaging of human islet cells in a living recipient. This can be performed on the very same islet(s) of individual animals multiple times during the follow-up period, which represents the uniqueness of this approach when compared to other in vivo models.

Islet graft characterization

P-11.1

Elevation of vascular endothelial growth factor production and its effect on revascularization and function of graft islets in diabetic rats

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Background: To determine whether the elevated vascular endothelial growth factor (VEGF) expression produced by the transfected vascular endothelial cells (VECs) could stimulate angiogenesis of the graft islets and its effect on the graft function.
Methods: The diabetic recipient rats were divided into three groups (n = 10 each). In the control group, each rat was transplanted under the capsule of the right kidney with 300 IEQ islets which were considered as marginal grafts. In the VEC group, each rat was transplanted with VEC together with the islets. In the VEGF group, each rat was transplanted with VEC transfected by pIRES2-EGFP/VEGF165 plasmid and the islets. Blood glucose and insulin were evaluated every other day after operation. The intravenous glucose tolerance test (IVGTT) was performed 10 days after transplant. Hematoxylin and eosin (Hand immunohistochemical staining of kidney tissues for insulin-6, VEGF and CD34 were used to evaluate the histology of graft islets, and the microvascular density (MVD) was calculated.
Results: Blood glucose and insulin levels of plasma of rats in the VEGF group restored to normal 3 day after transplantation. In contrast, diabetic rats receiving the same islets displayed moderate hyperglycemia and insulin, without significant difference between these two groups. IVGTT showed both the amplitude of blood glucose induction and the kinetics of the blood glucose of the VEGF group restored to normal after transplantation. HEand immunohistochemical staining showed that a large amount of graft islets were seen under the capsule of the kidney, which were positive stained by insulin-6 Ab and VEGF Ab in the VEGF group. In the cell masses, VECs positively stained by CD34 were observed. The similar masses were also seen in the other two groups, but with fewer positive cells. There were no VEGF positive cells appeared in theses groups. The MVD of VEGF group was much higher than those in the other two groups.
Conclusion: Elevated VEGF production by transfected vascular endothelial cells in the site of islet transplantation stimulates angiogenesis of the islet grafts. The accelerated islet revascularization in early stage could improve the outcome of islet transplantation, and reduce the mortality of the grafts

P-11.2

Comparison of adipocyte and bone marrow-derived mesenchymal stem cell as an immune modulator in pancreatic islet transplantation

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Background: Allogeneic pancreatic islet transplantation has the potential to cure type 1 diabetes. One of the barriers to islet transplantation is the alloreactive T-cell response between donors and recipients. Adult mesenchymal stem cells (MSCs) were recently found to suppress effector T cell responses and to have beneficial effects in various immune disorders. Previous data have proven autologous BM-derived MSCs (BM-MSCs) as an immune modulator to suppress T-cell and inflammatory responses and to induce generation of antigen-specific regulatory T cells in rodent allogeneic islet transplantation. As a source of these cells, the adipose tissue might be easier compared with bone marrow. However, the in vivo function of human adipose derived mesenchymal stem cells (hAD-MSCs) has never been compared with bone marrow-derived mesenchymal cells (MSC) in transplant model.
Methods: hAD-MSCs were isolated and characterised by adherence to plastic, appropriate expression of surface markers, and differentiation capability in vitro. The immunosuppressive properties of hAD-MSCs were determined in vitro in mixed lymphocyte reactions.
Results: The hAD-MSCs showed a homogenous population of cells with high expression levels of CD73 and CD90 and absence of CD31, CD34 and CD45. The stem cell potential of the hAD-MSCs was verified by differentiation into adipogenic, osteogenic, neurogenic, myogeneic and chondrogeneic cells. We compared the immunosuppressive properties of hAD-MSCs with the well-characterized immunosuppressive properties of BM-MSCs. hAD-MSCs did not provoke an in vitro xenoreactivity in MLR using rat splenocytes and, moreover, suppressed mixed lymphocyte reaction (MLR). In islet allotransplantation (Lewis to Fisher), hAD-MSCs injection alone did not prolong allograft survival. However, combined treatment of hAD-MSCs and CsA (5 mg/kg; for 2 weeks) significantly prolonged allograft survival compared with CsA alone (> 56.5 ± 21.9 days vs 9.2 ± 4.02 days).
Conclusions: These findings support that hAD-MSCs as well as BM-MSCs has immunosuppressive properties in islet transplantation model. From this result, we can deduce that hAD-MSCs could be a better alternative source for immune modulator than BM-MSCs in islet allograft. We are performing further study to dissect the mechanism of hAD-MSCs in prolongation of islet allograft survival.

P-11.3

The application of luciferase transgenic rats for experimental islet transplantation model

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Background: Luciferase-experssing Lewis transgenic (Luc-LEW Tg) rats has been developed by Dr. Kobayashi et al in Jichi Medical School. The aim of this study was bioluminescence image monitoring of islets isolated from Luc-LEW Tg rats and investigation of availability for experimental rat islet transplantation model.
Methods: Islets were isolated from Luc-LEW Tg rats using Liberase and 1000 islets were transplanted to STZ diabetic Lewis (LEW) rats via portal vein. After isolation, islets were incubated in culture medium including D-luciferin and in vitro luciferase imaging of islets was obtained by LAS3000mini™ (FUJIFILM). After transplantation, rats were injected D-luciferin via tail vein and also scanned by LAS3000mini to obtain in vivo bioluminescence image. Livers including grafted islets were resected, mashed and lysed by buffer to elute lusiferase. Eluted luciferase quantified by GloMax™96Microplate Luminometer (Promega) using ONE-Glo™ Luciferase Assay System (Promega).
Results: In vitro luciferase imaging of islets isolated from Luc-LEW Tg rats were detected by LAS3000mini™. Nevertheless, any in vitro lucifrerase imaging of grafted islets in the liver was not detected, although diabetes

induced by STZ was reversed. Livers including grafted islets were resected 0, 1, 7 days after transplantation and the amount of luminescence of eluted luciferase were 26.2 ± 1.2, 20.5 ± 1.7 and 8.6 ± 0.7 RLU/g, respectively. This fact that the amount of luminescence decreased to about one-third for 7 days after transplantation is compatible with what is being said that around 60–70% of injected islets in the liver would be destroyed after transplantation without allogenic rejection.
Conclusion: Islets grafted in the liver were semi-quantified by luminometer in LEW rat islet transplantation model using islets isolated from Luc-LEW Tg rats. These results suggest that Luc-LEW Tg rat is a useful tool for studies of islet transplantation.

P-11.4

Non-invasive imaging to monitor pancreatic islets function

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Dopamine 2-like receptors (D2/D3) are expressed in pancreatic beta cells and mediate inhibition of insulin secretion. We have developed a non-invasive method to monitor transplanted islets using 18F-fallypride (a selective dopamine D2/D3 receptor antagonist). In this study, our goal is to evaluate the ability of our new non-invasive method to monitor islets function. Isolated islets were incubated with 18F-Fallypride and autoradiographic images analyzed using Optiquant imaging system. Islets cultured at 37°C examined for their secretory responsiveness to glucose during a static incubation. Isolated rats islets were preserved at 4°C for 4 h in four different groups including: UW solution, UW supplemented with 5 mM glucose (UWG), UW with continuous bubbling of oxygen (UWO), and UW supplemented with both 5 mM glucose and continuous bubbling of oxygen (UWGO). Pre-oxygenation was done for 10 min with 100% oxygen at a flow rate of 2 l/min and continuous 100% oxygen bubbling in a flow rate of 0.2 l/min during 4 h of preservation. In vitro autoradiography revealed 18F-fallypride binds to isolated rat islets. Binding increased with the increment of the number of islets as well as the size of them. Dopamine (100 μM) competitively displaced 18F-fallypride bound to isolated rat islets. In Vitro glucose stimulation assay revealed it also inhibited insulin secretion of islets. Dopamine inhibitory effect was reversed by Sulpiride (a D2-like receptor antagonist). 18F-fallypride did not affect islets insulin release. In Vitro glucose stimulation assay revealed that oxygen treatment with or without glucose improved islet function. In vitro autoradiography revealed 18F-Fallypride binding increased with islet function improvement. Our results suggest that 18F-fallypride may be a suitable tracer for PET-based monitoring of islet function after transplantation. The in vivo experiment is on going.

P-11.5

Imaging of human islets vascularization using a dorsal window model

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Background: The islet of langerhans are micro-organs rich with blood vessels. The process of islet isolation and culture disrupts the vasculature structure of the islets. The reestablishment of an appropriate microvascular supply is an essential prerequisite for the long-term survival and function of the islet graft. In this study we examined the effect of coating the islets with fibrin on the process of neovasularization. Isolated islets were embedded in a three-dimensional fibrin or Matrigel matrix.
Methods: Human Islets (100 islet equivalent) were stained using the Dil dye and transplanted in a mouse dorsal window model to evaluate angiogenesis over a period of 17 days. Transplanted Islets were divided to two groups either free islet or islets coated with fibrin gel. Animals were imaged immediately, and at 3, 4, 8, 11 and 17 days post surgery using intravital microscope. The Dil dye caused the islet to become fluorescent and visible using Rodamine filter. FITC dextran was used to visualize the vasculature structure surrounding the islets.
Results: Slow appearance of the characteristics immature vessels by day four in the free islet transplant. Paucity of neovasculization expressed in low density/unit measure of new blood vessels persisted till the day eleven post free islet transplantation. In contradiction, the islet coated with fibrin demonstrated early (day three) appearance of network of immature blood vessels that produced significantly higher density/unit area for neovascularization by day 11 post transplantation.
Conclusion: Fibrin plays a role in early neovascularization and support sustaining new blood vessels development. Moreover, Fibrin forms a matrix that helps keep the three-dimensional structure of the islet intact and therefore reducing the environmental stress on the islets. The improvement of islet graft vascularization and the maintenance of adequate microvascular perfusion will contribute to the increased success of pancreatic islet transplantation.

Islet isolation

P-12.1

Islets purified by differential osmotic shock maintain normal distribution of cellular subpopulations and electrical coupling

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Background: pancreatic islet transplantation is considered an attractive therapy for a subset of unstable DM1 patients, but the clinical results have been disappointing. At present, the gold standard for purification of pancreatic islets is enzymatic digestion by the Ricordi system with purified collagenase; however, the enzyme itself induces damage to the islets, and that could explain the reduction in graft function. Moreover, the use of the enzyme greatly increases the cost associated with the entire process, limiting access to this therapy in developing countries. In this work we present the cellular and physiological characterization of islets isolated with a novel protocol without the use of enzymatic digestion, based on physiological properties of the beta cell.
Methods: we purified porcine islets with a non-enzymatic method, by initially exposing the pancreatic tissue to very high glucose concentrations and then washing with zero glucose solutions, to induce rapid swelling and necrosis of the exocrine cells. Then the islets were purified using a modification of the Ricordi system, and an islet enriched fraction was collected. The islets were fixed in 4% formaldehyde and evaluated for the distribution of the cellular subpopulations and for PDX-1 (+) cells. We also performed inmuno-histochemistry to identify active caspase-2. Finally we conducted micro-injection experiments with neurobiotin to evaluate the electric coupling of the islets specifically through connexin 36.
Results: With the described protocol we obtained 5357 islets per gram of pancreas, with over 90% purity. The cellular subpopulations show a normal distribution, with a dominant mass of beta cells. The cells maintain their electric coupling, with diffusion of the dye between the different subpopulations.
Conclusions: with the method proposed we can produce functional pancreatic islets with a high yield and over 90% purity. The cellular distribution conserves a near normal distribution of the endocrine subpopulations without a significant increase in apoptosis pathways. Also, the islets maintain electrical coupling, central for the glucose sensing mechanism.

P-12.2

Improvement of pancreatic islet cell isolation from NHBD: 15 cases report

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Background: Islet transplantation is a promising treatment for diabetes, but poor islet isolation efficiency is still a major challenge. We improved the islet

isolation method and used it to isolate functioning islets from non-heart-beating donors (NHBD).
Methods: Fifteen pancreases were procured from NHBD (age: 28–45, BMI: 18.5j5). The warm ischemia time was 2.5–4 min. The pancreases were stored in University of Wisconsin solution for 4–10 h. Each pancreas was cooled in situ via perfusion through only one cannula inserted into the duodenal orifice of the main pancreatic duct, and digestion was carried out using a modified Ricordi technique. Islets were purified on continuous Ficoll density gradients and identified by dithizone staining. Islet viability was assessed using acridine orange and ethidium bromide fluorescence staining, and islet function was assessed using the static glucose stimulation test.
Result: The islet isolation and purification procedure failed in one case (a pancreas from a donor with chronic pancreatitis) and succeeded in the other 14 cases. About 1597–2453 islet equivalents per gram of pancreas (average purity, 75%, activity, 79%, stimulation index, 3.2) were recovered.
Conclusion: the islet isolation method was improved by decreasing the amount of time between procurement and isolation and the use of only one cannula, inserted into the duodenal orifice of the main pancreatic duct.

P-12.3

Effect of cold ischemic time on rat islet yield

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Background: To investigate the effect of time of cryopreservation on quality and yield of islets isolated from rat pancreas.
Method: Pancreases from Wistar rats (n = 5 per group; one group for each period of storage) were stored 0, 3, 6, 9, 12, 15, 18, or 21 h in cold University of Wisconsin solution. Islets were isolated and purified using a modified University of Minnesota method. Yield and purity was determined using dithizone staining, viability using AO/EB staining, and islet function using the Insulin Secretion in Response to Glucose Challenge test.
Result: At 0 h of cryopreservation, the degree of purity and viability was 88% and 94%, respectively. The islet quality, viability, and in vitro function were similar at 0, 3, and 6 h. The quality and viability decreased after cryopreservation for more than 9 h. The yield and function of the islets decreased significantly from control levels during the period between 9 and 18 h _ Pà.01_ Pà.05 . At 9, 12, and 15 h, the structure of most islets was normal. Only a few islets had ruptured membranes with endocrine granule leakage. At 18 h, the purity, viability, and in vitro function (43%, 36%, and insulin release index = 1.5, respectively) failed to meet the clinical criteria required for transplantation. Moreover, at 18 and 21 h, the structure of most islets was abnormal.
Conclusion: The islet yield and function is reserved at least 6 h. After 18 h, the islet graft is no longer suitable for transplantation.

P-12.4

Effect of cold ischemic time on islet yield and function

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Objects: To investigate the effect of cold ischemic time on quality and yield of islets isolated from adult pancreas in UW solution.
Methods: Eight adult pancreases were selected according to the cold ischemic time: 4, 6, 8, 10, 12, 14, 20, and 24 h. Islets were isolated and purified according to modified Minnesota program. The quality and the degree of purity was defined as dithizone (DTZ, 140 mmol/l) staining. The viability was defined as AO/EB staining. Insulin Secretion in Response to Glucose Challenge in vitro defined the isolated islets function.
Results: The islet quality, viability and in vitro function showed no difference in 4, 6 and 8 h cases. The quality and viability decreased after preserved for more than 10 h. In 14 h case, the purity and the viability did not meet the clinical criteria. The in vitro function showed the same trend. The SI of 14, 20 and 14 h cases were less than 2.5, which means no reaction to glucose.
Conclusion: The islet yield and function was remained perfect within 8 h preservation. Long cold ischemic time reduced the yield and function, after 12 h preservation, the islet graft was not available for transplantation.

P-12.5

Establishing a clinical islet program with a quality management system in Australia

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Background: Moving from a research program in human islet isolation to clinical transplantation requires an exponential increase in islet quality, islet quantity, resources, staff and ultimately the financial resources to afford this change. There is also a corresponding need for clear documentation of all aspects of the isolation in the form of a Quality Management System, which adheres to the current Code of Good manufacturing Practice (cGMP). Our experience in this process of change is described here.
Method: In the initial stages (1997–2000) of our program, the documentation focus was on the isolation process and reagent preparation and this was documented by Word files. These formed the basis of our Production Standard Operating Procedures (SOPs). Assistance from an established source (2004) was crucial in establishing further areas of documentation for cGMP and the development of a quality management system. This documentation has included staff training, equipment management, inventory control and recording lot numbers for all reagents and consumables used during an islation.
Results: From our initial base of 10 Production SOPs, we now have developed 30 working SOPs, process records, data logs, labels and forms, forming a total of over 200 documents. We are now managing a cGMP system that includes Facility Management, Materiel Management and Control as well as Qualification (equipment, reagents, consumables), Personnel and Training, Process Development and Review including logging non-conformances, Audits, Process Control and Safety. A computerised document control system has been implemented and has assisted the quality manager in maintaining document quality management for our clinical islet isolation and transplantation program.
Conclusion: Moving to a cGMP document control process that conforms to regulatory requirements has taken many years and a dramatic increase in resources and funding. In moving from a research to a clinical islet program, planning for resources and personnel is required so as to adhere to cGMP.

P-12.6

Comparison of Australian donor, pancreas and preparation characteristics of transplanted and non-transplanted human islet preparations

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Background: We have successfully transplanted eight islet preparations into five diabetic recipients. Many factors influence the outcome of isolating islets, here we compare the characteristics of the donor, pancreas and islet preparations of transplantable isolations with isolations that failed to meet transplant criteria, including diabetic and non-heart beating donors (NHBD).
Methods: Pancreata were shipped on ice in UW preservative solution. While nearly all pancreata offered were accepted, pancreata from diabetic, NHBD or donors with malignancy or with positive viral serology were excluded from the non-transplant group or not processed. Islets were isolated using Serva Collagenase and Neutral Protease and a procedure based on the Ricordi Method.
Results: The transplantable group (Tx) include eight donor islet preps that were transplanted and an additional four islet preps that met transplant criteria but a matching recipient was unavailable. Average donor age of the Tx-group was 42 years compared to 50 years for non-Tx group (n = 34), 63 years for type 2 diabetics (n = 6) and 57 years for NHBD (n = 7). The Tx group donors were heavier 109 kg compared to 82 kg but not taller on average, both 172 cm. The Tx donor Body Mass Index was higher in the Tx group, 34 kg/cm^2 compared to the non-Tx group, 27 kg/cm^2 but had similar pancreas weight 80 gm vs 76 gm. Tx Islet yields were greater in the TX

group 404043 IEQ compared to 145546 IEQ, the yield difference as IEQ per gm of pancreas was 5014 for the Tx group compared to 2099 for the non-Tx group. While type 2 diabetic donors islet yields were not to dissimilar too the non-Tx group, 1861 IEQ per gm of pancreas, non-heart beating donors yield was much lower at 816 IEQ per gm of pancreas.
Conclusion: Using Serva Collagenase and Neutral Protease, transplantable islet preparations appear to come from younger, heavier donors with high BMI's. Clearly islet yields were best in the Transplanted Donors with little difference between non-Tx and Diabetic donors, while NHBD produced the worst islet yields. However, physical donor characteristics alone do not completely account for greater yields of islets obtained in the transplantable preparations.

P-12.7

Peri-insular laminin within pancreases of young human donors is not resistant to collagenase digestion

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Background: Islet isolation depends on collagenase digestion of the extracellular matrix (ECM) within the islet-exocrine interface of the pancreas. Islet yields from young donors (< 30 years) are usually insufficient for transplantation, although these islets potentially provide better physiological function than those from older donors. Our understanding of the action of collagenase to digest laminin, a basement membrane glycoproteins at the islet exocrine interface is incomplete. In this study, we used an in vitro assay to compare collagenase digestion of laminin at the interface in pancreatic specimens of young and old donors.
Methods: With appropriate consent and ethical approval, human pancreata were retrieved from five young (aged 19–28 years) and five old (aged 45–60 years) donors. Cold ischaemia time was < 10 h. Tissue blocks (~0.5 cm^3) were snap-frozen in liquid N2. Specimens were cryo-sectioned at 10–15-μM thickness and stored at -25°C. Sections were thawed and incubated ± Liberase at 1.4 mg/ml in HBSS for 5 min. at 37°C. Digestion of the ECM within the islet-exocrine interface was analysed by double immuno-labelling for insulin and laminin and semi-quantified by morphometry using image analysis (Zeiss KS-400), with 12 ± 5 islets assessed/section. Data were expressed as area of laminin/islet area ± SEM. Statistical analysis was by t-test.
Results: The mean laminin-islet area ratio in controls was 0.448 ± 0.060, compared to 0.246 ± 0.031 in Liberase treated sections ($p < 0.005$). No difference was seen between young and old donors (Control, HBSS alone 0.390 ± 0.063 vs 0.506 ± 0.106; Liberase treated 0.249 ± 0.041 vs 0.242 ± 0.047, respectively). Confocal microscopy showed dissolution of the double basement membrane surrounding islets following treatment with Liberase when compared to controls.
Conclusions: Laminin in the double basement membrane is digested by commercially available collagenase blends to the same extent observed with most collagen sub-types. Collagenase digestion of peri-insular laminin within the pancreas of young donors is not impaired. These results raise concerns that islets fragment during isolation following destruction of the glycoprotein framework. This could in part account for low islet yields.

P-12.8

The impact of ischemic stress on the quality of isolated pancreatic islets

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Background: Although the ischemic stress of the donated organs was revealed to have a strong negative effect on islet recovery, the impact on islet quality remains uncertain. In the present study, therefore, the influence of ischemic stress on the expression of inflammatory mediators on the isolated islets was examined. Furthermore, the impact of ischemic stress on the energy status of the pancreatic tissues was also evaluated.
Methods: The pancreatic islets were isolated from adult porcine pancreata subjected to 16 h cold ischemia times (CIT) in addition to 40 min warm ischemia times (WIT). The islet yield, the loss of islets during the first 24 h in culture, ADP/ATP ratio, ATP/DNA, glucose stimulated respiratory activity, in vivo bioassay, and the expression of inflammatory mediators (tissue factor (TF), MCP-1, macrophage migration inhibitory factor) on the isolated islets were evaluated. The ATP/DNA of the exocrine tissues during isolation procedures was also analyzed.
Results: The islet yield, survival rate during culture, and glucose stimulated respiratory activity were significantly lower in the case with 16 h CIT and 40 min WIT comparing to the control group ($p < 0.0001$, 0.0006, and 0.002, respectively). On the other hand, ADP/ATP ratio, TF expression, and MCP-1 expression on the isolated islets were higher in the ischemic group ($p = 0.005$, 0.16, and 0.005, respectively). During isolation procedures, the ATP/DNA of the exocrine tissues was extremely lower in the ischemic group compared to the control group ($p < 0.0001$). Notably, however, both ATP/DNA and ADP/ATP ratio of the isolated islets were well preserved even in the ischemic group ($p = 0.45$ and 0.40).
Conclusions: This data suggests that the ischemic stress during the preservation period negatively affects the energy status of the exocrine tissues, and the destruction of the exocrine tissues, in combination with warm ischemic stress during isolation procedures, subsequently decreases islet activity and induces the expression of inflammatory mediators on the isolated islets.

P-12.9

Comparison of Human islet isolations with or without the use of Desferrioxamine (DFO)

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Background: Islet transplantation has developed into a therapeutic treatment for select type 1 diabetic patients. Availability is however limited by the lack of donors and from losses during the islet isolation process. Manufacture of islets is a mixture of mechanical and enzymatic processes, exposing islets to biochemical and mechanical stresses. DFO, an antioxidant and iron chelator is known to inhibit oxidative stress-induced death. The use of DFO has also been shown to be beneficial to islets in rodent transplant models.
Aim: In this study we evaluate the benefits of DFO when used in the isolation of human islets.
Methods: All pancreata were shipped on ice in UW preservative solution. Islets were isolated using Serva Collagenase and Neutral Protease and a procedure based on the Ricordi Method. In isolations with DFO, DFO was added to solutions post the digestion step. This procedure was performed at both Australian Islet isolation facilities, St Vincent's Institute and Westmead Hospital.
Results: 13 pancreata that were isolated using DFO (grp:1) and 40 pancreata without DFO (grp:2). Group 1 and group 2 were similar in Donor Age, 49 years old and 50 years old, Body Mass Index, 29 kg/cm^2 and 30 kg/cm^2 and Cold Ischemia Time, 418 min and 3907 min respectively. Pancreas weights were similar between the two groups 74 gm and 77 gm respectively. Islet yields were higher in group 1 (3239 IEQ/gm pancreas) compared to group 2 (2679 IEQ/gm pancreas). In addition, 76.7% of islets were recovered post purification in group 1 compared to only 55% in group 2. Islets from DFO processed pancreata were more variable post culture with 61.4% surviving compared to 56%. In Group 1, 31% (4/13) isolations had transplantable yields (> 300000IEQ) compared to 15% (6/40) in group 2. Studies are ongoing to evaluate the benefit of DFO by dividing the post digest islets of individual pancreata into treated and untreated. Early results are encouraging, treated islets appear to have greater viability and beta cell content.
Conclusion: The use of DFO in the islet isolation process appearing to benefit islet survival and viability may result in more islet isolations being used for human transplantation.

P-12.10

Comparison of donor variables and islet isolation outcomes using liberase and serva enzymes

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Background: Successful treatment of individuals with type 1 diabetes has been achieved by intraportal transplantation of donor islets. The ability to consistently isolate large numbers of islets suitable for clinical transplantation is crucial if it is to become a widely accepted therapy. However, donor and enzyme characteristics that are predictive of a successful transplant outcome are yet to be determined. The aim of this study was to determine what donor characteristics were predictive of a transplantable islet preparation for both the Liberase HI and SERVA enzymes.
Method: Islets were isolated from donor pancreases with a modification of the Ricordi method using either Liberase HI or SERVA NB1 collagenase and NP (SERVA). Islet preparations were divided into groups (Transplanted vs Non-transplanted, Liberase vs SERVA) and for each isolation, total islet equivalents (IEQ) and IEQ/g pancreas were determined. Donor characteristics such as donor age, body mass index(BMI), pancreas weight and cold ischaemic time(CIT) were compared to type of enzyme used and analysed statistically. Groups were further stratified according to donor age into 10 year cohorts and analysed for total IEQ and IEQ/g pancreas.
Results: Transplanted islet preparations had a greater total IEQ and IEG/g pancreas compared to their non-transplanted counterparts. Donor characteristics predictive of a suitable islet preparation were shorter CIT(4.9 ± 0.5 h;$p = 0.002$), larger BMI(30.3 ± 1.0 kg/m^2;$p = 0.012$) and greater pancreas weight(98.1 ± 5.9 g;$p = 0.009$). Stratification of islet preparations according to whether they had been transplanted or not, and analysis according to type of enzyme used CIT($p = 0.001$), BMI($p = 0.02$) and pancreas weight($p = 0.024$) had a significant impact on total IEQ and IEQ/g pancreas in the Liberase HI, but not the SERVA group. When stratified for age of donor there were no significant differences between the Liberase or SERVA enzyme except in the 50–59 year cohort where preparations performed with Liberase achieved a significantly greater number of IEQ(496858.7 ± 73615.8 vs 305923.3 ± 40270.8;$p = 0.02$) and IEQ/g pancreas(6592.6 ± 850.1 vs 3617.9 ± 394.2;$p = 0.001$).
Conclusions: Donor factors predictive of a transplantable islet preparation were shorter CIT, higher BMI and larger pancreases. In the age group (50–59 years) enzyme type, but not CIT, BMI or pancreas weight, appear to predict better islet yields.

P-12.11

Comparative study on enzymatic activity and molecules stability of some commercial proteolytic enzymes used in pancreatic islet isolation

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In pancreatic islets isolation for cell therapy the major enzymes used are obtained from *Clostridium hystoliticum:* class I and class II collagenases. They are used in a defined tissue dissociation enzyme mixture together with neutral protease (Dispase) or thermolysin (Thermostable Neutral Protease). However, just to now, people working in islets production found variable outcomes in isolation procedures mainly due to large variability in enzymatic blend composition and efficacy. Using electrophoresis and gelatin zymography approaches together with densitometry evaluation assays we compared the composition, stability and auto-digestion processes of C. hystoliticum collagenases, Neutral protease and Thermolysin from Roches and Serva. Moreover, we have assessed: –the stability of enzymes when they are in solution at different temperatures (-20°C; 0°C and room temperature); –their digestive activity when applied at different working temperatures (25°C; 30°C; 37°C and 42°C). Our results shown a heterogeneous composition of the different enzymatic blend enzymes analyzed. Furthermore, heterogeneity is observed among different batch enzymes; we found several more proteins and/or fragments compare to HPLC profiles published by vendors. In gelatin zymographyes several digestive bands were catalytic, showing very high complex degradative patterns, in part active even in condition of calcium deprivation. Additionally, in neutral protease from Serva (and not in Thermolysin) contaminants with gelatinolytic activities were detected. An auto-digestive/inactivation processes of enzymes occurred at different working temperatures, reduced by lowering temperature up to 25°C. These data taken together strongly imply a not controlled digestive processes due to several contaminants in enzyme blends and to autocatalytic processes. Moreover, the presence of low molecular weight gelatine/degradative activities obstacles the possibility to control islet digestion due to aspecific catalytic activities. Generation of recombinant collagenases probably could be of help to overcome the variability in the extractive processes.

P-12.12

Glucose and Oxygen Supplements in UW Solution Improve Islet Isolation Outcome

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Current hypothermic pancreas preservation strategies have not adequately addressed issues of limited glucose and oxygen supply in the pancreas prior to islet isolation procedures. Our goal is to develop a preservation method to improve islet isolation outcome. Male Sprague-Dawley rats were used as the pancreas donors. Procured pancreata were preserved for 4 h in University of Wisconsin (UW); UW + 5 mM glucose (UWG); UW + 100% O_2 (continuo us) (UWO); UW + 5 mM glucose + 100% O_2 (UWGO). Tissue samples of pancreata were collected fresh and following 4 h cold storage. The following Parameters were assessed: cellular energetics [ATP, ADP, AMP]; anaerobic end product [lactate]; oxidative stress [glutathione (GSH)]; necrosis [lactate dehydrogenase (LDH) release]; and apoptosis [DNA fragmentation]. At 4 h of preservation, islet yield (Dithizone staining), membrane integrity (FDA/PI staining), and *in vitro* function (Stimulation Index) were determined after pancreata were digested with collagenase and islets were isolated and purified by Gradient Stock Solution-density centrifugation. Pig pancreata were procured and preserved for 4 h with continuous oxygenation (UWO) to test if oxygen diffusion is a limit in a large pancreas. Increasing the oxygen partial pressure in the oxygenated UW solutions improved pancreatic tissue metabolism as evidenced by higher ATP and energy charge and lower lactate levels. Although oxygen decreased intracellular anti-oxidant capacity (GSH), the 5 mM glucose supplement significantly reversed the oxygen side effect. Glucose and or oxygen supplement could prevent apoptosis in pancreatic tissue. However, glucose supplement alone (but not its combination with oxygen) induced necrosis. Post-purification islet yield and function was significantly improved with an oxygen/glucose supplement. The pig experiment revealed a significant ATP increase in peripheral tissue, that extended to tissue 3 mm deep, but not the tissue at the core (7.5 mm depth) of the pancreas as compared to the values prior to preservation. Mathematical equations revealed 3 mm oxygen diffusion improves intracellular ATP level in 64% of the pancreatic tissue volume. Continuous oxygenation of pancreas plus glucose supplement of UW solution could boost pancreatic tissue energy profile through aerobic metabolism and improve islet isolation outcome through prevention of apoptosis, necrosis, and improvement of intracellular antioxidant capacity.

P-12.13

MRI as a novel tool to develop new methods of pancreas distension to enable homogeneous enzyme distribution for successful islet isolation

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Background: A thorough, homogeneous enzyme distribution during pancreas distension is desirable to obtain maximum yields of isolated islets. It is thought that some regions of the pancreas do not consistently receive adequate enzyme to properly digest the extracellular matrix and free the islets from their attachments.
Aim: To develop a tool to investigate the homogeneity of classic and novel methods of enzyme delivery for porcine and human islet isolation.

Methods: Pancreata were procured from Landrace pigs via *en bloc* viscerectomy. The main pancreatic duct was then cannulated with an 18 g winged catheter and MRI performed at 1.5T. Images were collected before and after ductal infusion of chilled gadolinium-doped saline.
Results: It was observed that regions of the distal aspect of the splenic lobe and portions of the connecting lobe and bridge may exhibit reduced delivery of solution when traditional methods of distension are utilized.
Conclusion: Presently utilized methods of enzyme distension do not consistently deliver adequate enzyme uniformly to all regions of the pancreas. Novel methods of enzyme delivery such as selective re-cannulation and distension of identified problem regions should be investigated for improved enzyme distribution. MRI serves as a valuable tool to visualize and evaluate the efficacy of current and prospective methods of enzyme distension.

Pancreas procurement

P-13.1

Comparison of HTK and UW preservation solutions for clinical pancreas transplantation: an update of the Indiana University experience

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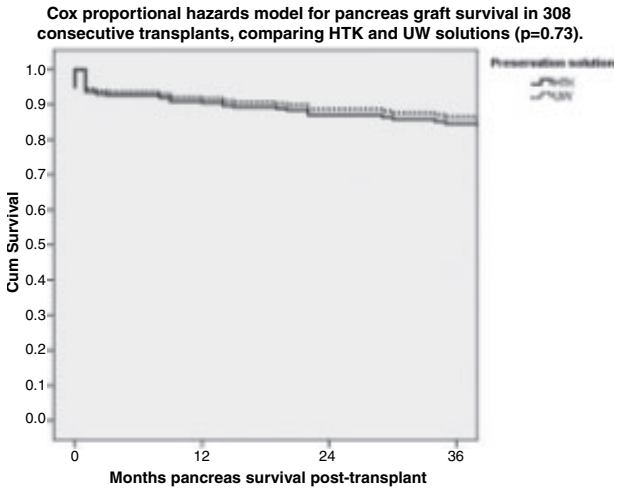
Objective: Histidine–Tryptophan ketoglutarate (HTK) solution has recently been scrutinized for use in pancreas transplantation (PTx). A recent single center study and a UNOS database review have suggested an increased incidence of allograft pancreatitis and graft loss when compared to University of Wisconsin (UW) solution. A recent randomized study failed to show any significant difference between HTK and UW for pancreas preservation. This study reviews our single center experience for pancreas allografts preserved with HTK or UW.
Methods: A retrospective review was performed of all PTx performed at IUH between 2003 and 2009 (n = 308). All local procurements used HTK as of 05/03. Our center routinely uses an HTK flush volume of 3–4 L. Data included recipient and donor demographics, 7d, 90d and 1 yr graft survival, peak 30 d serum amylase and lipase, length of stay (LOS), readmissions, HbA1C and c-peptide.
Results: Of the 308 PTx (HTK 258 (84%), UW 50(16%)), there were 172 SPK (56%), 74 PAK (24%), and 62 PTA (20%). There were more SPK compared to PAK and PTA in the HTK group (HTK: SPK 160 (62%), PAK 57 (22%), PTA 39 (15%); UW (SPK 11 (22%), PAK 17 (34%), PTA 22 (44%)), likely because the majority of SPK were from in-state donors flushed with HTK and the majority of UW group were isolated PTx (PAK or PTA) procured by other centers and imported . Otherwise, demographics were similar for donor and recipient age, race, gender, and BMI, and donor serum amylase and lipase. Median total ischemic times were longer for UW (HTK 8 h, UW 9 h, p = 0.01). Median follow-up was similar (HTK 38mo, UW 34mo). There was no significant difference in 7d, 90d or 1 yr graft survival [Figure 1], 30 day peak serum amylase (HTK 216, UW 163, p = 0.17) and lipase (HTK 156, UW 126, p = 0.68), LOS, readmissions, HbA1C or C-peptide.
Conclusion: No clinically significant difference between HTK and UW for pancreas allograft preservation was identified. Specifically, no increased incidence of allograft pancreatitis or graft loss was observed.



P-13.2

Continuous, real-time viability assessment of pancreata based on oxygen consuption

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Background: Quantitative information describing whole organ viability is limited using current donor organ quality assessment techniques. Visual and physical inspections by the transplant surgeon along with biopsy are the primary methods of determining organ quality, and are subject to interpretation and sampling variability. Consequently, there is a need to for a real-time technique that can offer quantitative information about whole organ health. This study develops a method for the measurement of whole organ oxygen consumption rate (OCR) normalized to tissue mass that can be used to quantitatively assess the metabolic activity, and thereby viability, of a porcine or human pancreas prior to islet isolation.
Methods: Porcine pancreata with preserved vasculature were procured from non-heart beating donors (n = 4). The celiac trunk and the superior mesenteric artery (SMA) were cannulated and coupled using a Y shaped splitter and then all leaks were identified and ligated. An oxygen sensor was placed in-line upstream of the arteries to measure the ('arterial') oxygen partial pressure (pO2) of the perfusion solution. The portal vein was also cannulated and another sensor was attached to measure the effluent ('venous') pO2. The temperature was maintained at 8°C and the organ was perfused. The arterial and venous pO2, the gross whole organ weight, and the measured flow rate were used to calculate the OCR per tissue. A slightly modified technique was also used to evaluate human research organs.
Discussion: The average OCR value for all four porcine organs measured at the time of arrival was 60 ± 84 nmol/min/g (range = 4–185). These findings reflect a large variability in organ quality prior to long term cold storage and demonstrate the sensitivity of the technique. Whole organ OCR may be a valuable research tool for the assessment of procurement and preservation techniques. More studies are required to develop baseline values for determination of donor organ viability.
Conclusions: This real-time technique could be used to assess whole organ quality during human and porcine pancreas procurement, during long term perfusion, before islet isolation, or before whole organ transplant.

Stem cells

P-14.1

***Ex-vivo* expansion of adult human pancreatic islet beta cells**

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Expansion of adult beta cells from the limited number of islet donors is an attractive prospect. However, while evidence supports the replicative capacity of both rodent and human beta cells *in vivo*, attempts at expanding these cells in tissue culture result in loss of beta-cell phenotype. Our recent lineage-tracing studies support the ability of human beta cells to survive and significantly replicate *in vitro*. Beta-cell delamination out of the normal epithelial structure, a process that results in dedifferentiation, seems to be required for significant *in-vitro* proliferation. Thus, ways must be found for inducing redifferentiation of the expanded cells *ex vivo*, or restoring their function upon transplantation. Elucidation of the signaling pathways altered

during beta-cell adaptation to growth in culture may provide clues for cell redifferentiation. We found that human beta-cell dedifferentiation and entrance into the cell cycle *in vitro* correlated with activation of the NOTCH pathway and downregulation of the cell cycle inhibitor p57. Inhibition of the NOTCH downstream target HES1 using short hairpin RNA reduced beta-cell dedifferentiation and replication, suggesting a potential target for inducing cell redifferentiation following expansion in culture for use in cell replacement therapy of diabetes.

P-14.2

Assessment of the differentiation potential of human adult pancreatic islets derived progenitor cells in mice

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Objective: The current study aims to assess the differentiation potential of progenitor cells derived from human adult pancreatic islets in mice.
Methods: Isolated adult human islets were cultured in media supplemented with 10% FBS, β-FGF and EGF. After the human islets progenitor cells (hIPCs) migrated out of islets and grew to confluence, they were passed by trypson-EDTA digestion, proliferated at least 2000-fold and then cultured in serum-free media for 4 days to form islet like cell clusters (ICCs). About 2 X106 hIPCs or ICCs were transplanted under the renal capsule of NOD scid mice.
Results: One month after transplantation, 19 of 28 ICCs and 4 of 14 hIPCs grafted mice were detected to secrete human C-Peptide at a low level. Only 7 of 19 and 2 of 4 C-peptide positive mice showed a glucose responsive manner.
Conclusion: Our results demonstrate that hIPCs have the ability to differentiate into insulin-secreting cells, but it's function is still far from satisfactory for diabetes cell therapy.

P-14.3

Report from Argentina of first three years follows up of autologous stem cells implant in diabetes type 1 (World Wide Pioneer experience)

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Background: The adults stem cells CD34 (+)CD38(-) have the capacity to differentiate in functional cells on endocrine pancreas We did the first implant of ASC in the world in diabetes 1 in 2005.
Objectives: To report the long time performance of Diabetic type 1 patients treated with Stems cells implant.
Method: Following first 3 year on cell therapy for diabetes 1 the conclusions are optimistic. In this study were observed the evolution of 38 Diabetic type 1 patients, 20 male, 18 female, average age 23.06 years old (±20.2). All the patients were under insulin therapy. For the transplantation were harvest bone marrow from iliac crest by aspiration, following the sample was processed using a density gradient separation method, obtained 120 ml (±95) of CD34(+)CD38(-) solution. For the implant was procedure a catheterization through Spleen Artery. No complications or further events were observed during or after the procedure. The patients were subjected to clinical and blood samples control during the 12 months following the implant. C-Peptide Basal (ng/ml), Basal 0.39, 6 Months 0.54, 36 Months 1.95, Increment 98.30%, C-Peptide after meal (ng/ml), Basal 0.46, 6 Months 0.56 36 Months 2.54, increment 151.63%, H1c Basal 8.4, 6 Months 7.39, 36 Months 6,54, Decrement 24.58%, Insulin Basal (MU/ml), Basal 5.09, 6 Months 12,25, 36 Months18.66, Increment23.25%, Insulin Doses (IU/day): Basal, 54,96, 6 Months 35,83, 36 Months 12.60, Decrement 65.44%.
Conclusions: The implant of mononuclear CD34+ CD38- (stem cells) from autologous bone marrow improve pancreatic function in patients with type 1 diabetes, in a safety form and is maintained after 3 years at least.

P-14.4

First report from Argentina of first three years follows up of autologous stem cells implant in diabetes type 2

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Objectives: To evaluate the long time performance of Stem cells implant in pancreas in Diabetes Tipe 2 patients The adults stem cells CD34(+)CD38(-) have demonstrate the capacity to differentiate in functional cells on endocrine pancreas.
Methods and Results: After 3 year on cell therapy for diabetes patients the conclusions are optimistic. In this study were observed the evolution of 58 patients Diabetic type 2, 37 male, 21 female, 29–71 years old. 29 patients were under insulin therapy, and 20 patients using Sulphonilureas + Biguanidas. For the transplantation were harvest bone marrow from iliac crest by aspiration, following the sample was processed using a density gradient separation method, obtained 120 ml (±95) of CD34(+)CD38(-) solution. For the implant was procedure a catheterization through Spleen Artery. No complications or further events were observed during or after the procedure. The patients were subjected to clinical and blood samples control during the 36 months following the implant the implant C-Peptide (ng/ml), Before Implante 1.18, 6 Months 1.17, 36 Months 2.19 8, Increment 48.42%, C-Peptide after meal (ng/ml), Before implant 2.22, 6 months 2.95, 36 months 4.40, Increment: 95.52% ($p = 0.0036$).HBC1: 9.14 basal, 8.25 at 6 months, at 36 months6.35, Decrement 21,25% ($p = 0.003$), Insulin Basal (MU/ml 12.33 6 months 15.27, 36 months 15.02 Increment 25.26%, Insulin After Meal (MU/ml) 19.11, 6 months 15.27, 36 months 34.7 Increment: 58,75% ($p = 0.016$) Pills per day 2,25 previously of implant after 36 months 0.33 (decrement of use 44.36% ($p = 0.0007$) Insulin dose (IU/day) basal 50.59, after 36 months 9.55 Decrement 89,03% ($p = 0.037$).
Conclusions: The implant of mononuclear CD34 + CD38- (stem cells) from autologous bone marrow improve pancreatic function in patients with type 2 diabetes, in a safety form and is maintained after 3 years at least

P-14.5

"Stemness" properties of in vitro long-term cultured human islet cell monolayers

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Background: We have examined long-term cultured, human islet-derived cell monolayers (CM). CM were easily expandable in culture, through several doublings, with no cell mass loss, and showed a prevailing β-cell functional identity, as assessed by mRNA and protein expression for endocrine cell markers. To evaluate whether long-term cultured CM would be associated with "stemness" capability we have examined CM versus whole human islets (HI), studying the expression of embryonic stem cell markers (Nanog, Sox-2, Oct-4).
Methods: HI were obtained by multienzymatic digestion of human pancreata, thereafter plated on tissue flasks, and allowed to adhere and expand for several passages. We performed imunofluorescence and real time PCR examination after transdifferentation into adipocytic, osteocytic and neuronal phenotypes. We then encapsulated CM prior to graft into diabetic or control NOD/SCID mice.
Results: Cell phenotype differed between HI and CM, during sub-culture doublings. The new condition resulted in either rearrangement of pancreatic hormone and key transcriptional factor expression, or decreased insulin secretion or declined glucose-stimulated insulin release. Moreover, in basal, when examined under immunofluorescence, CM unlike HI, showed intense co-localization of all pancreatic hormones. Both CM and HI were positive for Pdx-1, Islet-1, CD117, SCF, CD90, GK; although CM, unlike HI, were positive for NGN3. The protein and the messenger for the embryonic stem cell markers Sox-2, Nanog, Oct4 and ABCG2 were all expressed both in HI and CM. "Stemness" properties were confirmed by CM transdifferentation into adipocytic, and osteocytic and neuronal cell phenotypes, with the staining with Oil red O, Alizarin red S and

immunofluorescence analisys for neuronal marker (MAP2ab, TH, TUBβ3, Nestin, NeuN) confirming the differentiation potential. Quantification of upregulated expression for PPARγ, FABP4, Osteopontin and TUBβ3 was demonstrated by real-time PCR. Upon graft of encapsulated CM into normal or diabetic NOD/SCID mice, the retrieved cells were vital in all conditions at 60 days. Moreover, the encapsulated CM acquired an islet-like configuration both, in terms of morphology and function.
Conclusions: The obtained data seem to indicate that CM, while holding intrinsic stemness properties, may also expand and generate islet-like, insulin producing cell clusters.

P-14.6

Reprogramming spleen into pancreas: a novel study of the molecular mechanisms behind splenic mesenchyme-to-epithelial transition

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Background: A pluripotent islet source may overcome the current limitations of donor pancreas shortages for islet transplantation. We have previously shown that spleen can differentiate into insulin-producing cell-clusters through mesenchyme-to-epithelial transition (MET). However, understanding the molecular mechanisms controlling MET is essential for harnessing this for future therapy. The aim of this study was to investigate whether the spleen is truly re-programmed to no longer express the splenic marker Tlx-1, but instead express the pancreatic master gene Pdx-1.
Methods: Embryonic quail spleens (E4.5) and chick pancreatic epithelia (E4) were micro-dissected, recombined as chimaeras, and cultured for 7 days in a 3D rotator culture system. These underwent *in situ* hybridisation with Tlx-1 (n = 9) or Pdx-1 (n = 9), followed by sectioning (10mm). A peroxidase reaction was performed to detect the quail-specific antigen QCPN. Micro-dissected quail spleen controls (E4.5) also underwent *in situ* hybridisation with Tlx-1 or Pdx-1 respectively (n = 22). Expression was assessed by 2 independent observers.
Results: All quail spleen controls were positive for Tlx-1 (n = 11) and negative for Pdx-1 (n = 11), confirming clean separation prior to recombination. However, no recombined chimaera showed Tlx-1 expression in the splenic mesenchyme, indicating 100% down-regulation of this gene (Tlx-1 is normally expressed at this gestation). 5/9 (56%) chimaeras had co-localisation of QCPN and Pdx-1 indicating up-egulation of Pdx-1.
Conclusions: Splenic mesenchyme underwent dramatic down-regulation of Tlx-1 (100%) in the chimaeras and up-regulation of Pdx-1 in over half of chimaeras (56%). This indicates that combining splenic mesenchyme with pancreatic epithelium results in genetic re-programming from a splenic identity to a pancreatic epithelial fate. This study demonstrates for the first time the genetic control of islet MET in the avian spleen and is particularly significant, as Pdx-1 expression is considered essential for normal beta-cell function.

Others

P-15.1

Correction of diabetes in mice following transplantation of an insulin-secreting human liver cell line

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Somatic gene therapy is one strategy being considered to correct patient blood glucose concentrations in Type I diabetes. It has been previously shown that the stable transfection of the full length insulin cDNA contained in a plasmid vector into the human liver cell line HuH7 resulted in synthesis, storage and regulated release of insulin to the physiological stimulus glucose (Huh7ins cells), with reversal of diabetes. However, glucose tolerance was not entirely normal. In this study we introduced furin-cleavable human insulin (INS–FUR) into Huh7 cells using a lentiviral vector (HMD) that has been previously shown to elicit high levels of insulin expression when delivered directly to rat and mouse livers, with permanent reversal of type 1 diabetes. The HMD lentiviral vector also expresses the enhanced green fluorescent protein (EGFP). The resulting cell line Huh7/HMDins-FUR was sorted by flow cytometry and a cell population with EGFP expression was isolated and expanded in culture with EGFP, tested for insulin secretion in vitro by radioimmunoassay and RT-PCR. The Huh7/HMDins-FUR cells were subsequently transplanted subcutaneously into streptozotocin-diabetic NOD/ SCID mice and the animal's blood glucose and body weights were monitored and an intraperitoneal glucose tolerance test (IPGTT) was performed. The cells secreted human insulin into the supernatant (16.8 ± 3.4 pmol/106 cells). Insulin was detected in cells by RT-PCR as were transcription factors NeuroD and PDX-1 that are also endogenously expressed in the parent cell line, Huh7. Subcutaneous grafts were visible 1–2 weeks after being transplanted. The blood glucose levels of all six diabetic mice that were transplanted became normal 6.1 mM ± 3.0, in 17–29 days after the cells were implanted. Blood glucose levels continued to decline reaching subnormal levels, 2.8 mM ± 0.7, within a week of blood glucose levels normalization. Removal of the grafts at this time resulted in a prompt increase in the blood glucose levels to hyperglycemic values, 27.8mM ± 1.7. Following an IPGTT animals blood glucose levels peaked at 30 min, 22.3 mM ± 9 and returned to normal levels at 120 min, 4.3 mM ± 1.2. The IPGTT curve was not significantly different from normal animals. This study indicates that liver cells are an appropriate cell type for the gene therapy of diabetes.

P-15.2

Regression of diabetic complications by singenically transplanted rat pancreatic islets

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Type 1 diabetes is a chronic disease often leading to several complications, such as peripheral neuropathies, nephropathy and cardiovascular disease. However, clinical and experimental studies have reported that a strict metabolic control by endogenous insulin does not prevent the development of chronic diabetic lesions. Pancreatic islets transplantation is extensively investigated as a strategy for the cure of type 1 diabetes, allowing a more efficient and physiological metabolic control. We investigated the effects of islet transplantation by immunoisolation on diabetic complications in a model of streptozotocin-induced diabetic rats. To this end we investigated 3 groups of Lewis rats: healthy control rats, untreated diabetic rats and diabetic rats transplanted with microencapsulated islets into the peritoneal cavity 2 months after diabetes induction. Following transplantation, hyperglycemic rats became normoglycemic in few days and this was accompanied by a rapid rose in body weight, reaching values similar to control rats. Meanwhile, thermal (hot plate test) and mechanical sensitivity (Randal–Selitto pan withdrawal test measured with an analgesymeter) were increased and decreased by 180 and 40–60% in transplanted rats, respectively. In addition, the density of footpad intraepidermal nerve fibers was reduced by 20% in diabetic group and islet transplantation restored normal skin innervations. Other parameters of peripheral neuropathy, the nerve conduction velocity and the Na+, K+-ATPase activity in the sciatic nerve, both reduced by about 25% in diabetic rats were normalized by islet transplantation. In kidney of diabetic rats, mild tubular dilatation and tubular cast formation were found. These changes were absent in the rats with transplanted islets. Morphological changes of cardiac tissue were also observed in diabetic rats. Myocyte loss (-34%) and reactive hypertrophy of the remaining viable myocytes were observed in rats with diabetes, while islet transplantation reduced cardiomyocyte death. In conclusion, our data showed that in a model of type 1 diabetes, transplantation of microencapsulated pancreatic islets, beside controlling glycemia, reversed neuropathy and was able to restore all the diabetic-induced alterations within the 2 month follow-up period after transplantation.

P-15.3

Effects of pancreas transplantation on oxidative stress in pulmonary tissue from alloxan-induced diabetic rats

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Background: There is considerable evidence that cellular oxidative stress caused by hyperglycemia plays an important role in the genesis and

evolution of chronic diabetic lesions. In this study we evaluate the effectiveness of pancreas transplantation (PT) in preventing the imbalance caused by excessive production of reactive oxygen species over antioxidant defenses in lungs of rats rendered diabetic by alloxan injection.
Methods: Sixty inbred male Lewis rats weighing 250–280 g were randomly assigned to 3 experimental groups: NC-20 non-diabetic control rats; DC-20 untreated diabetic control rats; PT-20 diabetic rats that received syngeneic pancreas transplantation from normal donor Lewis rats. Each group was further divided into 2 subgroups of 10 rats, which were killed after 4 and 12 weeks of follow-up or PT, respectively. Glucose, glycosylated hemoglobin and insulin were determined in plasma for all rats. Lipid hyproperoxide (LPO) concentrations and enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were measured in pulmonary tissue of all rats.
Results: DC rats showed elevated blood sugar and glycosylated hemoglobin levels, with insulin blood levels significantly lower than NC ($p < 0.001$). These rats also showed significantly increased LPO concentrations in the lungs ($p < 0.01$) after 4 and 12 weeks follow-up. In contrast, SOD, CAT, and GSH-Px antioxidant activities were reduced in these periods, significantly ($p < 0.01$) 12 weeks after diabetes induction. Otherwise, successful PT corrected all clinical and metabolic changes in the diabetic rats, with sustained normoglycemia throughout the study. Excessive lung LPO production and low SOD, CAT, and GSH-Px antioxidant activities were already back to normal 4 weeks after PT.
Conclusions: We conclude that PT can control oxidative stress in pulmonary tissue of diabetic rats. It may be the basis for preventing chronic diabetic lesions in lungs (Research supported by Fapesp).

P-15.4

Pancreas transplantation prevents morphological and ultrastructural changes in pulmonary parenchyma of alloxan-induced diabetic rats

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Background: Few studies have examined the effects of diabetes mellitus on the pulmonary system and whether lung lesions can be prevented by pancreas transplantation (PT). The purpose of this study was to establish the efficacy of PT in controlling the course of histopathologic changes in the alveolar structure of rats rendered diabetic by alloxan injection.
Methods: Sixty inbred male Lewis rats weighing 250–280 g were randomly assigned to 3 experimental groups: NC-20 non-diabetic control rats; DC-20 untreated diabetic control rats; PT-20 diabetic rats that received syngeneic pancreas transplantation from normal donor Lewis rats. Each group was further divided into 2 subgroups of 10 rats each, which were killed after 4 and 12 weeks of follow-up or PT, respectively. Clinical and laboratory parameters, fresh and fixed lung weights, and fixed lung volume were recorded for all rats. The left lungs were used for scanning light microscopy and the right for transmission electron microscopy. Morphometric studies were performed using digital images, KS 300, and Leica Quin Lite 3.1 software. Total number of alveoli, alveolar surface area, alveoli perimeter, and alveolar epithelial (AE) and endothelial capillary (EC) basal laminae thickening were randomly measured in 10 rats from each experimental group (5per subgroup).
Results: DC rats showed elevated blood sugar and glycosylated hemoglobin levels, with insulin blood levels significantly lower than NC ($p < 0.001$). Fresh and fixed lung weight and volume were significantly reduced in these rats. The total number of alveoli per lung in diabetic rats was higher than controls, while alveolar perimeter and surface area were significantly diminished ($p < 0.01$). AE and EC basal alveoli laminae were significantly thicker in DC than NC ($p < 0.01$). Successful PT corrected all clinical and metabolic changes in diabetic rats, with sustained normoglycemia throughout the study. Morphological and morphometric changes observed in diabetic lungs were completely prevented in PT rats from 4 weeks after transplant.
Conclusions: We conclude that PT can control morphological and ultrastructural changes in pulmonary parenchyma, opening up a good perspective for preventing other chronic diabetic lesions (Research supported by Fapesp).

P-15.5

Effective treatment of metastatic insulinoma using multiple treatments with systemic rna interference targeting mouse pdx-1 in a mouse insulinoma model

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Background: The purpose of this study was to determine whether multiple treatments with iv liposome-delivered small hairpin-RNA targeting mouse PDX-1 (L-mPDX-1 shRNA) could serve as PDX-1-targeting therapy for metastatic insulinoma in a SCID mouse model.
Methods: The efficiency of silencing mPDX-1 gene expression and the ability of inhibitory cell proliferation *in vitro* was determined by western blot and MTS assay, respectively, after mPDX-1 shRNA transfection of mouse insulinoma βTC-6 cells. The mice bearing IP βTC-6 tumor were grouped to receive 1st :one treatment cycle, 2nd : two cycles and 3rd : three cycles of biweekly, iv L-mPDX-1 shRNA or corresponding L-pSUPER vector as control via tail vein injection, ($n = 15$ mice/group). On day 7 after each gene delivery, serum glucose and insulin levels were measured, immunohistochemistry and TUNEL assay on pancreas were performed. Survival was assessed by Kaplan–Meier in SPSS statistical software ($p < 0.05$).
Results: *In vitro* knock down of PDX-1 expression resulted in 2 fold and 2.5 fold reduction of cell proliferation as compared to control at 48 and 72 h after shRNA transfection, respectively($p < 0.05$). All 3 *in vivo* treatment cycles of iv L-mPDX-1 shRNA reduced insulin levels and increased glucose levels in the βTC-6 SCID mice compared to controls ($p < 0.05$ for 2nd and 3rd cycles). Both insulin and glucose levels returned to normal in survival mice at 6 months after treatment. All treated mice had prolonged survival compared to controls (53.0 ± 1.5d), however, the survival time in 3rd group mice (180 ± 39.4d; all mice in this group survived and were sacrificed) was longer than that of 2nd (123.0 ± 13.1d) and 1st group (129 ± 38.5d) ($p < 0.05$). Islet histology revealed decreased PDX-1, insulin and PP expression and increased SST expression. Islet cell apoptosis was increased in all three treatment groups.
Conclusion: Multiple treatments of iv L-PDX-1 shRNA were effective against metastatic insulinoma in a SCID mouse model. The therapy prevented hyperinsulinemic death, however resulted in a reversible mild form of type 3 diabetes. This study demonstrates that PDX-1 is a therapeutic target for insulinoma in mice.

P-15.6

Photochemical pathogen activation of human serum enables its large-scale application in clinical islet transplantation

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Background: Human serum is regarded as the preferred supplement throughout the process of clinical culture and transplantation. However, for safety andregulatory purposes, mainly related to the risk of transferring infections to the recipient, many centres involved in clinical cell transplant programs are required to use human albumin instead. In many European countries, the Intercept technology is routinely used for pathogen inactivation of platelets and plasma for clinical use. Intercept uses small molecules of psoralen which can pass through cell membranes and capsids binding to the helical regions of the nucleic acid. When UV-A light is applied, the psoralens crosslink to the DNA and RNA, both free and in the genome, thus blocks both transcription and replication. The aim for this study was to evaluate this method on human serum used for culture and transplantation of human islets of Langerhans.
Method: Pathogen inactivated human serum (PI-HS) compared to human control serum (C-HS) was examined on human islets of Langerhans, cultured with PI-HS or C-HS for 3–4 days under standard conditions (37°C and 5% CO2). The functional capacity was assessed by measurements of intracellular insulin content, expression of inflammatory mediators (IL-6, IL-8, TF and MCP-1), ADP/ATP ratio (energy content), insulin release in response to a dynamic glucose stimulated perifusion and finally after transplantation to STZ-diabetic athymic (nu/nu) mice.
Results: There was no difference between islets cultured in PI-HS or C-HS in terms of insulin stimulation index, the ADP/ATP-ratio and the capacity to cure STZ-diabetic mice. Likewise, no difference was found in intracellular insulin content, expression of IL-6, Tissue Factor or MCP-1 but there seemed to be a slightly higher expression of IL-8.

Conclusion: The presented technique for pathogen inactivation of human serum provides a solution to an important safety and regulatory problem in modern cell therapy. PI exerts no negative impact on human islets of Langerhans. INTERCEPT® treatment of human serum allows the routine use of human serum in clinical cell transplantation. A major advantage of PI is that it, due to its mechanism of action, eliminates also unknown emerging pathogens of RNA and DNA origin for which tests do not yet exist.

P-15.7

RNA interference targeting mouse pdx-1 gene corrects hypoglycemia in somatostatin receptor 1 and 5 knockout mice

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Background: Previous studies have demonstrated that knockout of somatostatin receptor 1 and 5 (SSTR1/5ko) resulted in severe hypoglycemia in aging mice. PDX-1, a crucial transcription factor in regulation of insulin gene expression, has been recently shown to be associated with cell islet proliferation. The purpose of this study is to investigate if PDX-1 could serve as a molecular target to treat islet neoplasia and hypoglycemia.
Methods: SSTR1/5ko mice at age of more than 6 were used for hypoglycemia animal model. Multiple cycles of liposomal mPDX-1 shRNA (L-PDX-1 shRNA) treatment were employed on these mice via tail vein injection at dose of 35ug per mouse, once biweekly and three deliveries in total. At one week after each treatment and 4 months after initial treatment, Serum was collected for evaluation of glucose and insulin levels. Necropsy was performed and pancreas was removed for immunohistochemisty, western blot, TUNEL studies. IPGTT was performed after treatment.
Results: During treatments, serum glucose levels increased and reached maximum of 218.8 ± 26.7mg/dl after second L-mPDX-1 treatment, accordingly, insulin levels continuously decreased and down to the minimum of 0.4 ± 0.1ug/ml on second treatment. However, the basal glucose and insulin levels were back to original levels at 4 months after initial treatments, showing no significant difference as compared to that of control of SSTR1/5ko mice. Interestingly, IPGTT assay showed similar pattern of glucose tolerance in the period of treatment compared with at 4 months after treatments. L-PDX-1 shRNA treatments resulted in reduction of PDX-1 and insulin expression in islets, reducing PCNA expression associated with down regulated Cyclin E and Cdk4, increasing islet cell apoptosis. No significant toxic effect was found during and after treatments.
Conclusion: Our studies indicate that multiple cycles of L-PDX-1 shRNA is new therapeutic strategy for the treatment of hypoglycemia related disorders, such as islet neoplasia. Inhibition of PDX-1 expression in islets not only reduces insulin expression and secretion, but also affects islet cell proliferation and apoptosis.

P-15.8

Fluid intake, volume overload, hypertension and body composition in indian renal transplant patients

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Background: After transplant urine output settles down to 5–7 l/day. Indian patients drink 6–7 l/day of water (X4.97 ± 1.62) to maintain high urine output. Glomerular hyperfiltration and intrarenal hemodynamics are important causes of physiopathology of CAN.
Objectives: Evaluate volume expansion, relationship of hypertension with body water, body composition and dry weight in post transplant patients using bioelectrical impedance analysis (BIA). 100 well nourished clinically non edematous transplant patients (TP) were compared with 60 controls. BIA parameters included dry weight, total body (TBW), extracellular (ECW), intracellular water (ICW), extracellular fluid (ECF), plasma, interstitial fluid (IF), fat free mass (FFM), fat mass (FM), body cell mass (BCM), muscle mass (MM), protein.
Results: Compared to controls TP had low body weight, BMI and fat but higher ECF, plasma, IF and FFM, systolic (120.8 ± 13.0 /116.2 ± 6.6) and diastolic (80.5 ± 8.0/77.0 ± 3.0) blood pressure. TP: Significant difference ($p = 0.000$) in water intake of patients on 1 antihypertensive drugs (AHD) (3.12 ± 0.86 litres) and on ≥2 AHD (5.80 ± 1.06). Significant correlation between water intake and TBW (.000), ECW (.005), ICW (.045), Dry weight (.011), Plasma (.005), Inst Flud (.005). Significant correlation observed between water intake, TBW (.023), ECW/ICW (.049) and Dry weight (.021) in patients on ≥2 AHD.Dry weight derived from BIA was lower than actual weight($p = 0.000$).TP groups based on GFR: low GFR < 40 ml/min; High GFR > 40 ml/min.Patients with low GFR had low body weight (53.0/61.5), dry weight(50.1/58.7), BCM(21.3/23.1), FFM(42.8/48.3), TBW(30.0/34.4), ICW(46.7/51.3), MM(19.8/23.4), protein(9.1/10.1) and ECS(4.36/5.01) compared to patients with higher GFR. Patients with low GFR had higher ECW. When grouped according to blood pressure (controlled BP < 120/80, High BP > 120/80 mmHg), patients with controlled blood pressure had higher GFR (47.2 ml/min $p = 0.001$) than those with high BP (40.80 ml/min). Based on AHD patients were divided into two groups (one AHD, > 1 AHD). Patients on one AHD had significantly lower TBW, ECW ICW, plasma and IF than those on > 1 AHD.
Conclusion: Fluid intake should be monitored in non-edematous transplant patients requiring > 2 AHD. Evaluation of expanded volume is necessary for better control of blood pressure. Control of blood pressure is a must for good GFR.Water intake should be thirst driven.

P-15.9

Near normoglycemia in rats achieved by immune protected and oxygenated islets of langerhans

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The current treatments for insulin dependent patients require frequent blood glucose testing and insulin injections by syringes, pens or pumps. Many times this treatment fails to achieve full glucose control. Islet transplantation, although sucessesfull, requires life-long therapy with immunosuppressive agents. Beta O2 Technologies had developed the βAir, a bioartificial pancreas device aimed to be implanted under the skin. The device contains three compartments: Immune-protected donor islets of Langerhans, gas tank and a remote septum. The gas tank is separated from the islet compartment by a gas-permeable membrane and is daily refueled by injecting O2 via the septum. βAir device transplanted into STZ induced diabetic rats, achieved near-normal glycemic control for a period of 60–90 days. Upon retrieval of the device, blood glucose always increased to diabetic levels. In iso-type transplantations (Lewis-to-Lewis), experiments terminated after 90 days. In allo-type transplantations (Sprague Dawlley-to-Lewis) – after 60 days. HbA1C levels of normal and diabetic rats are 4.4% and 12%, respectively. At the termination of the experiment HbA1C levels found to be 5.4% in iso-type transplanted rats and 6.5% in allo-type transplanted animals. Our data suggests that the βAir device is capable of maintaining blood sugar at near-normoglycemic levels.

P-15.10

Post-transplant processing of superparamagnetic iron nanoparticles used for islet labeling and MR detection

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Background: Labeling of isolated pancreatic islets with spio nanoparticles enables to detect the islet graft by magnetic resonance for as long as 6 months after transplantation (Tx). Although during the in vitro culture the particles are incorporated into all major types of islet cells, little is known about their further processing *in vivo*. We studied morphology of labeled islets after Tx and tried to identify the type of cells retaining the particles.
Methods: Isolated rat islets were incubated with ferucarbotran (Resovist®) for 48 h. Iron labeled islets were transplanted under the kidney capsule of syngeneic animals. The kidneys were removed 1, 7, 30 days and 3 months after Tx. Samples were fixed for TEM and frozen for immunohistochemistry. Immunodetection of endocrine cells, macrophages (antigen ED1), fibroblasts (antigen S100A4) and collagen was combined with Fe3 + detection.

Results: *In vitro*, ferucarbotran gradually incorporated into all types of islet cells and macrophages. First day after Tx, some particles were still in islet cell cytoplasm; however, many free iron particles were among the components released from the procedure-related destructed tissue. Components of dead cells including iron particles were engulfed by phagocytic cells. One week after Tx, ferucarbotran was rarely found in the endocrine cells, although the mechanism of iron release from beta-cells was not evident. Iron particles were stored in macrophages and in large fibroblast-resembling cells, which, however, were not positive for a fibroblast marker S100A4. One month after Tx, large amount of iron was concentrated in the islet surrounding macrophages and in the cells enclosed by collagen producing fibroblasts. No iron particles were found in beta cells. Three months after Tx the iron particles were still detected in the same cell types and in similar amount with increased amount of collagen around.
Conclusions: Although the nanoparticles were readily incorporated into the endocrine cells *in vitro*, after islet Tx under the renal capsule they were gradually eliminated. Iron was accumulated and persisted in macrophages and other fibroblast-like cells in their proximity for at least 3 months. Our findings could be relevant for the interpretation of islet transplant magnetic resonance imaging in the clinical setting as well. Supported by grant 2B06175.MSMT, CR.

P-15.11

Transplantation of annular pancreas grafts. report of two successful cases
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Background: Annular pancreas is a rare malformation, and two-thirds of these patients will remain asymptomatic for life. Symptoms related to duodenal obstruction or pancreatitis can occur in one third of these patients, and can be treated by division of the constricting ring of pancreatic tissue and endoscopic sphincterotomy, respectively. To our knowledge, only two grafts with annular pancreas have been used for transplant. We present here two successful cases.
Methods: From Mars 1995 to September 2008, we performed 118 pancreas transplants. Two of thse patients underwent simultaneous pancreas-kidney transplantation using annular pancreas grafts. First case. A 19-year-old male, dead from a head trauma was approved as a multiorgan donor after evaluation (ICU stay of 24 hrs, no cardiac arrest, use of norepinephrine, serum amylase, 799 IU/l, normal serum glucose and creatinine). The graft was transplanted into a 45-year-old male recipient, and porto-caval and bladder-drainage were performed. Second case. A 30-year-old male, dead because of head trauma, was accepted for multiorgan procurement after evaluation (no cardiac arrest, norepinephrine use, normal values of serum creatinine and glucose). The recipient was a 37-year-old male, treated with peritoneal dialysis. Graft implantation was performed in right iliac fossa by porto-caval venous anastomosis, and enteric drainage.
Results: First case: At 8th day duodenal-bladder fistula was diagnosed and reoperation for reconversion to Roux-en Y duodeno-ileal diversion was carried out. Two more reoperations were needed for resolve the fistula. After 4.5 years later the pancreas and kidney show normal function. Second case: The patient develops systemic inflammatory response syndrome with satisfactory evolution, and 6 months after pancreas transplant the patient maintains graft normal function.
Conclusions: Annular pancreas grafts can be safely used for pancreas transplantation.

Thursday, October 15, 2009 and Friday, October 16, 2009

Islet Xenotransplantation II

These abstracts are also listed under the Tuesday abstract session from 9.1 to 9.7

P-16.1

The age of porcine Sertoli cells is critical for providing immune protection of porcine islets xenografted into rats and mice
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Islet transplantation is a viable cell replacement therapy with great potential to effectively cure type 1 diabetes mellitus (T1DM). The supply of high quality donor pancreata and harsh immunosuppressive drug regimes are two major obstacles that need to be overcome to progress the field. Several limitations have prevented islet transplantation from emerging as a standard of care for T1DM. Sertoli cells can provide an immune privilege to the islets of Langerhans when co-transplanted, thus bypassing the need for immunosuppression. Research to-date has focused on the use of porcine neonatal Sertoli cells and has neglected the potential for the use of Sertoli cells of adult porcine origin. Thus, we set out to investigate whether it was an advantage to using Sertoli cells of adult origin as opposed to neonatal. We isolated Sertoli cells of varying ages from pigs ranging from 8-days to 5-years of age and screened for various markers. We observed based on Real-Time PCR, protein expression profiles and functional in vitro assays that there is an ''ideal'' age bracket where adult Sertoli cells can impart their immune-modulatory functions. In addition, FasL mRNA levels as determined by Real-Time PCR were 12- to 15-fold higher in expression when comparing the different age brackets ($p < 0.001$). To investigate functions in vivo, we then xenotransplanted adult Sertoli cells under the kidney capsule of diabetic nude BALB/c mice and immune-competent FVB/n mice. In parallel, we extended these studies by xenotransplanting labelled adult Sertoli cells into polypropylene chambers previously transplanted subcutaneously in STZ-induced diabetic Lewis rats. We concluded that adult Sertoli cells did not affect the functioning of the co-transplanted islet cells as assessed by the presence of porcine insulin production. Also, our novel labelling technique was useful to monitor transplanted adult immune-modulatory pig Sertoli cells in these and other cell therapeutic applications.

P-16.2

Xenotransplantation of microencapsulated porcine islet cells in diabetic rats
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Background: Xenotransplantation of microencapsulated porcine islet cells might be a possibility to overcome the shortage of human donor organs. Several materials for microencapsulation of cells are described in literature which all show severe disadvantages. NaCS is easy to produce, does not show any cytotoxicity and cell lines survive for a nearly unlimited time-spam after microencapsulation. However, this material has not been tested for microencapsulation and xenotransplantation of porcine islet cells.
Methods: Porcine islet cell isolation and purification was performed according to a newly modified Ricordi method. Porcine islet cells were microencapsulated with NaCS. Diabetes was induced in Sprague Dawley rats by intraperitoneal injection of STZ. Only rats that showed polydipsia, polyuria and blood sugar levels higher than 400 mg/dl over a time period of 14 days were used for the experiments. Microencapsulated porcine islet cells

were transplanted under the kidney capsule of the animals. Blood sugar levels were monitored on a weekly basis, porcine C-Peptide levels and insulin levels were measured using ELISA. Intravenous glucose tolerance testing was performed once a month. After 4 months, the animals were sacrificed, the kidney containing the microencapsulated porcine islet cells was retrieved and processed for histological and immunohistochemical examination.
Results: After xenotransplantation of microencapsulated porcine islet cells diabetes was reversed in rats. Animals stayed normoglycaemic up to 4 months. Functionality of transplanted porcine islet cells was detected by insulin measurement and detection of C-Peptide. After scarification, histological and immunohistochemical evaluation showed no signs of fibrosis or inflammation in the surrounding tissue. Viability of microencapsulated porcine islet cells after explantation was proven by immunohistochemical viability stains.
Conclusion: It is feasible to reverse diabetes in rats by transplanting porcine islet cells microencapsulated in NaCS. Rats stayed normoglycaemic until the end of the study period. No signs of fibrosis could be detected in the surrounding tissue. NaCS seems to be a promising material for microencapsulation of porcine islet cells in order to treat diabetes. Further studies have to be carried out to show long term survival of transplanted porcine islet cells microencapsulated in NaCS in diabetic rats.

P-16.3

Creating a prevascularized site for islet transplantation using a V.A.C.®–GranuFoamTM and HBO in rats

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Background: Naturally, islet cells are highly vascularized in the pancreas. This physiological vessel structure is damaged during the isolation process. Therefore, isolated islet cells dependent on diffusion of oxygen and nutrients from the surrounding tissue. After transplantation a lot of freshly isolated islet cells become apoptotic because of hypoxia. Thus, insulin independency can not be achieved because of graft dysfunction. The aim of the study was to show, that it is feasible to create a prevascularized site in rats, using a V.A.C.® (Vacuum Assisted Closure) GranuFoamTM, that is normally used in wound healing and HBO (hyperbaric oxygenation) to induce angiogenesis.
Methods: Forty Sprague–Dawley rats are divided in five groups and the V.A.C.®–GranuFoamTM is implanted in the subcutaneous tissue. According to the V.A.C.®–Therapy, a drainage is used to suck the secretion and to accelerate wound healing and vascularization of the V.A.C.®–GranuFoamTM. HBO is administered to the different groups at different timepoints for at least 1 week after implantation to a maximum 1 week prior and 3 weeks after implantation. After the experiments, the blood flow is measured using Szintigraphy. Moreover the V.A.C.®–GranuFoamTM is explanted and processed for histology and immunohistochemistry to assess angiogenesis.
Results: It is feasible to create a prevascularized site in the subcutaneous fatty tissue of rats, using a V.A.C.®–GranuFoamTM and HBO. Angiogenesis is not induced without HBO within one month after implantation of V.A.C.®–GranuFoamTM but within 2 weeks after implantation of the system and HBO therapy. Vessels are not only distributed in the outer parts of the V.A.C.®–GranuFoamTM, the whole sponge-like V.A.C.®–GranuFoamTM is pervaded by new vessels. HBO therapy prior to the implantation does not have a significant influence on vessel growth.
Conclusion: As ischemically damaged islets are likely to undergo cell death or loose functionality due to hypoxia, the use of the V.A.C.®–GranuFoamTM and HBO might be a promising method to create a prevascularized site to achieve better results in islet transplantation.

P-16.4

In vivo increase of alpha cells in pig islets by low dose of STZ improves insulin secretion

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Background: Human and pig islets differ by their structures (60%/25% vs 90%/8% for ß/α cells, respectively) and functions (stimulation index at ~12 vs 2 for a G1–15 mM, respectively). An increase of intracellular cAMP is required to improve insulin release by pig ß cells. Glucagon may play a crucial role by stimulating ß cell. Therefore, we investigated the possibility to modify in vivo the pig islet structure (in native pancreas) in order to increase the proportion of α cells per islet and then to possibly improve insulin production by isolated islets.
Methods: Selected doses (0, 30, 50, 75, 100 mg/kg) of streptozotocin (STZ), were injected in 27 young pigs to assess the effect of STZ on α cells into native pancreatic islets. Pancreatic insulin/glucagon contents were measured by hormonal extraction/radioimmunoassay and the islets remodelling was quantified by histomorphometry for α/ß cells proportion at 3 months post-transplantation. After the selection of STZ-dose increasing α cells by 20% in islets, nine additional pig pancreas (STZ-treated and Ctrl) were procured and digested (by Liberase PI). Isolated islets were tested in vitro for glucose stimulation.
Results: A significant correlation was found between the dose of STZ and (i) the pancreatic content of insulin ($p < 0.05$, $R = -0.86$) as well as (ii) the proportion of ß cells inside islets ($p < 0.05$, $R = -0.84$). A maximum of 50 mg/kg STZ was required to obtain the optimal remodelling with a significant destruction of ß cells (74% vs 51% of ß cells/islet for STZ < 50 vs STZ > 50 mg/kg; $p < 0.05$) and a concomitant increase of the proportion of α cells/islet in native pig pancreas (26% vs 48% of α cells/islet for STZ < 50 vs STZ > 50 mg/kg; $p < 0.05$). This remodeling was essentially found in small islets (50–200 μm). At 3 months post-STZ treatment ((30 mg/kg, $n = 6$) and (50 mg/kg, $n = 3$)), pig islets were isolated and compared to normal isolated islets ($n = 3$). A higher proportion of α cells was obtained in STZ-modified islets than Ctrl ($p < 0.05$). After in vitro stimulation, isolated STZ-pig islets demonstrated a significant higher glucagon content (65.4 ng/ml vs 21.02 ng/ml, $p < 0.005$) and insulin release (144 μU/ml vs 59 μU/ml, $p < 0.05$) without cAMP raising agent such as Fsk than Ctrl animals, respectively.
Conclusions: Streptozotocin low dose (< 50 mg/kg) can modified in vivo the pig islets structure and improve their functions after isolation.

P-16.5

The effects of glucagon-like peptide 1 (GLP-1) and gastrin on the proliferation and differentiation of neonatal pig pancreatic cell clusters (NPCCs)

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Islet transplantation is a promising therapy for cure of type 1 diabetes, however, donor shortage hampers wide application of this treatment option. Neonatal pig pancreatic cell clusters (NPCCs) is one of the good candidates for alternative sources due to the technical easiness of isolation, maintainability in tissue culture and growth potentials in the recipient. One major drawback using NPCCs is that long time (6–12 weeks) is needed for correction of hyperglycemia of diabetic animals upon transplantation. Therefore, promotion of NPCCs differentiation toward mature phenotypes during culture period would obviate glucotoxicity to implanted NPCCs and obviously facilitate early functioning. In this study, we examined the effect of glucagon-like peptide 1 (GLP-1) and gastrin either alone or in combination on NPCCs proliferation and differentiation using RT-PCR and immunocytochemical methods. Although either GLP-1 or gastrin treatment increased the progenitor ductal cells proliferation and early differentiation of endocrine cells compared with untreated control, in particular at high concentration (100 nM), the combination of GLP-1 and gastrin (10 nM each) potently enhanced those processes as revealed by increased Ki67+ cells in ductal cells and early expression of endocrine cell markers such as insulin, glucagon, GLUT2, and PDX-1. Therefore, treatment of appropriate factors such as GLP-1 and gastrin during NPCCs culture period could

promote their differentiation toward mature phenotypes and expand endocrine pools by increasing proliferation of progenitor duct cells.

P-16.6

Long-term culture of neonatal islet cell clusters demonstrates better outcomes for reversal of diabetes

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Background: Porcine neonatal islet-like cell clusters (NICC) have the potential to be a limitless source of beta-cells for replacement therapies in type 1 diabetes. However, after transplantation there is a lag time before they develop and secrete insulin in response to glucose.
Aim: To determine the optimal time point for NICC culture that produces the best in vivo functional outcomes.
Methods: NICCs were isolated from 1–3 days old pig pancreases, and cultured for up to 4 weeks. The following parameters were determined at weekly intervals during culture: number (IEQ), FACs analysis of percentage beta cells and percentage beta cell viability, stimulation index, Insulin: DNA ratio, ATP activity, ethidium bromide and acridine orange viability staining, and gene expression levels of GLP1-R, insulin, glucagon, Caspase 3, and tissue factor (TF). At weekly timepoints during culture, NICC were transplanted under the renal capsule of streptozotocin induced diabetic SCID mice.
Results: NICCs cultured for ≥2 weeks achieved normal blood glucose levels within a mean of 37 days in all transplanted diabetic mice. NICC cultured for 1 week achieved normoglycemia in only 50% of animals and took a mean of 53 days to reach this objective. As a result of longer culture time there was however a significant loss of IEQ over time. Preliminary gene expression data indicate an increase in the level of insulin and a decrease in level of TF gene expression over time in culture, whilst Caspase 3, GLP1-R and glucagon expression remained constant.
Conclusion: Culture of NICCs for at least 2 weeks provided the best in vivo functional outcome for transplantation.

P-16.7

Comparison of the portal vein and hepatic artery as sites for pig islet xenotransplantation in non-human primates

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Aims: Pig islets offers a potential solution to the limited human islet transplantation for type 1 diabetes. The intra-portal infusion has been commonly used for islet transplantation. However, potential risk exists in the intraportal islet infusion. In this study, we compared the portal vein and hepatic artery as sites for pig islet xenotransplantation in rhesus.
Methods: Immunosuppressed streptozotocin (STZ)-induced diabetic rhesus were transplanted intraportally (PV; n = 8) and intrahepatic arterially (HA; n = 6), respectively with 50,000 neonatal porcine islets (NPIs)/kg. Graft survival and function were determined by blood glucose monitoring, and examination of porcine C-peptide and liver biopsy post transplantation.
Results: One and four HA and PV animals, respectively, died during and after transplantation procedure. All and three of the remaining PV and HA animals, respectively, became insulin-free from days 70 to 110 after transplantation for more than 120 days with insulin positive NPIs in their liver biopsy samples. In addition, another two remaining HA animals demonstrated partially NPI function. Porcine C-peptide and no PERV infection were detected in all the recipients.
Conclusions: The intra-portal and -hepatic artery NPI xenotransplantation achieved similar function outcomes in diabetic rhesus recipients. However, the intra-portal infusion procedure may cause unnecessary mortality.

Animal Models

P-17.1

Ultrasonography to quantify renal perfusion of hDAF pigs xenografts transplanted to Macaca cyclopis monkeys

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Purpose: An attempt to develop a novel system to monitor hyperacute rejection of xenograft was made. Using power Doppler of ultrasonography, acquisition of vascular index (power Doppler vascular index (PDVI); mean of color weighted power Doppler vascular index (WPDVImean); renal vascular perfusion index (RVPI)) was done to quantify perfusion changes in renal xenotransplantation of hDAF transgenic pigs' kidneys to Macaca cyclopis.
Background: Xenotransplantation is considered as a potential solution for shortage of organ, provides xenograft rejection can be managed and controlled. Doppler ultrasonography has proposed to quantify renal perfusion in canine model and is expected to be useful to detect xenograft rejection. The complement activation of Macaca cyclopis showed a greater cytotoxicity than that of human, and this primate may serve as a more conservative model to study xeno-rejection.
Methods: Macaca cyclopis use was approved in accordance with the Wildlife Conservation Act of ROC (Taiwan). Care of animals was in accordance with the Guide for the Use and Care of Laboratory Animals prepared by the IACUC. Pigs of hDAF transgene (n = 5, 6.0–14.7 kg) were used as the kidney donors and Macaca cyclopis (n = 6, SPF kept, 6.6–10.6 kg) were used as renal recipients. The surgical procedures of life-supporting kidney xenotransplantation are similar to those of clinical kidney allotransplantation.
Results: Only one monkey survived surgery up to 104 hours, others expired within 24 hours post-operation. The cause of death was referred to disseminated intravascular coagulation (DIC). The power Doppler ultrasonography showed that calculated index values (PDVI, WPDVImean, and RVPI), which could quantify the renal interlobular perfusion, slightly decreased after xenotransplantation and they were maintained until development of DIC. Paraffin sections (HEstain) revealed that hDAF transgene exerted some protection against hyperacute rejection in four of five xenografts, a result agreed with ultrasonographic indexes obtained.
Conclusion: A renal xenotransplantation model of hDAF transgenic pigs' kidneys to Macaca cyclopis has successfully established and by using ultrasonography to quantify renal perfusion, a real-time monitoring of hyperacute rejection of xenograft is presented.

P-17.2

Ex vivo porcine renal xenotransplantation model using a pulsatile machine preservation system

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Background: Experimental models to investigate interventions in xenotransplantation require complex techniques and animal utilization. The aim of our study was to develop a reliable technique to test interventions to ameliorate hyperacute rejection.
Methods: Three anesthetized Miniature Swine (30 kg) being utilized for a nonsurvival study were used as donor animals. Autologous blood was collected in a citrated bag prior to sacrifice. A 10Fr aortic cannula was place in the infrarenal aorta and kidneys were then flushed with 1 l of HTK solution after a suprarenal clamp was applied. Kidneys were rapidly

explanted and chilled to 4°C. Kidneys were randomized to be the Xenograft Kidney (XK) (n = 3) and the mate was used as a control (CK) (n = 3). Kidneys were cannulated and placed in a cassette on two separate Waters RM3 perfusion devices (Waters Medical, Rochester, MN, USA). Kidneys were left for 30 minutes at room temperature to recapitulate anastomotic/rewarming time. A normothermic, oxygenated modified Waters RM3 system (Waters Medical) was utilized. Systolic perfusion pressure were maintained at 100 mmHg at a rate of 60 cycles per minute. Real-time perfusate analysis were used to titrate P02 > 100 mmHg and glucose levels > 80 mg/dl to ensure homeostatic conditions. CKs were reperfused with the autologous blood collected at procurement. XKs were reperfused using fresh whole human type 'B' blood. Physical characteristics and urine output were recorded. Cortical biopsies were taken at 15, 30 and 60 minute intervals in the XK group and 15, 60 minutes and end of perfusion for CK group. HEstained slides were examined by a blinded pathologist for evidence of antibody mediated rejection. Results:

XKs demonstrated homogenous reperfusion which rapidly became patchy at 5–7 minutes. XK kidneys had become complete black and thrombosed by 60–70 minutes. XKs at 15 minutes had only minor histologic changes but 30-minute biopsies demonstrated peritubular capillaritis and endothelial denudation consistent with antibody attack. CK kidneys demonstrated homogenous reperfusion and urine production and demonstrated nonspecific inflammation.

Conclusions: Our ex vivo porcine xenotransplant model shows early promise as a tool to study xeno-associated hyperacute rejection. Further immunofluorescence and molecular studies are warranted to validate these findings. This ex vivo model maximizes animal and resource utilization

P-17.3

Using Macaca cyclopis as a primate model to study xenotransplantation of porcine kidney with hDAF transgene

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Purpose: To test primate model of Macaca cyclopis (MC) in xenotransplantation study by revealing changes of blood clinical measurements of MC after receiving kidney xeno-graft of hDAF transgenic pigs.

Background: Short-lived outcome of MC with xeno-graft of hDAF transgenic pig's kidney, suggested that MC may serve as a conservative animal model to study xeno-rejection of hDAF transgenic pigs. This is supported by a finding that MC serum exerted a more noxious effect to porcine aortic endothelial cells than that of human in vitro. After actual xenotransplantation, the changes of blood clinical profile of MC received kidney graft obtained from hDAF transgenic pigs would more effectively to reveal the protection of transgene against hyper-acute rejection.

Methods: A total of six hDAF transgenic pigs (6.0–14.7 kg) were used as kidney donors and six MC (SPF kept, 6.6–10.6 kg) were exercised as xeno-graft recipients. All the animal uses were approved by related authorities. The surgical procedures of life-supporting kidney xenotransplantation, were similar to those of clinical kidney allotransplantation, except primates had been sedated throughout the observation period. Blood samples were taken from a jugular catheter. Measures of blood clinical chemistry were conducted either by analyzers designed for veterinary use or by commercial ELISA-kit measurements.

Results: All the recipients died within 24 hours after starting of xeno-graft perfusion except one that lasted 104 hours. There was a sudden drop of systolic pressure around 2 hours prior to death, while diastolic pressure steadily declined throughout observation period. Blood type cross matching tests (PBMC and monkey serum) were all positive to highly positive. Hematological readings were all significantly altered and a drop of 88% in platelet counts was noted. Blood clinical chemistry profile revealed that all the major items of metabolites and enzymes were changed with a 250% increase in creatinine. However, plasma IgG and IgM levels were maintained. Pathological evaluations (congestion, interstitial hemorrhage and glomerular thrombus) suggested that hyperacute rejection of xeno-graft was not evident in four recipients.

Conclusion: Primate model of MC is proven capable to study xenotransplantation and hDAF transgene in pig's kidney successfully exercised its function in this model.

P-17.4

Surgical and non surgical complications of a pig to baboon xenotransplantation model

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A pig to baboon heterotopic cardiac transplantation model at the NIH using a modified immunosuppressive regimen has been developed. Graft survival has been prolonged, but despite this, our recipients have succumbed due to various surgical or non surgical complications. In this abstract, different complications and management strategies are described. The most common complication was hypercoagulability (HC) after transplantation, causing thrombosis of both small and large vasculature and ultimately leading to graft loss. The Consumptive Coagulopathy (CC) was encountered in some recipient baboons and was not able to be reversed by stopping anti coagulation and transfusing blood multiple times. A solid state left ventricular telemetry probe is placed into in the transplanted heart, induction of hypocoagulable states by continuous heparin infusion led to uncontrollable intra-abdominal bleeding in one baboon from the ventricular site. One instance of cardiac rupture originating from an lateral wall infarction site occurred. Earlier studies have shown infections to be uniformly fatal in this transplant model.

However due to the telemetry placement, infections may be identified early by temperature spikes and can be treated with prompt administration of antibiotics. We had several cases of wound dehiscence due to recipients picking at sutures. These were promptly resolved by either re-suturing the wound or finding distractions for the baboon. A few of the most common problems we faced in our earlier experiments were related to the jacket, tether and the infusion pumps. It was difficult to keep the jackets on some of the baboons and tether had to be modified several times before long term success was assured. Infusion catheter replacement resulted in transplant heart venous obstruction and thrombosis from a right common femoral venous line. Conclusions: Homeostatic perturbations such as HC and CC and baboon-induced wound complications comprised most of the complications encountered. Exsanguination due to telemetry implantation and infarct rupture did occur in two baboons. Despite the variety of complications, significant graft prolongation in this model was achieved.

Innate Immunity, Immune Responses and others

P-18.1

Pig xenogenic adipose-derived mesenchymal stem cells for bone reconstruction

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Background: The potential of human adipose-derived mesenchymal stem cells (AMSCs) was recently demonstrated for bone reconstruction. However, a delay of 2–3 months is required between the original adipose tissue procurement/isolation and the implantation of osteogenic-differentiated stem cells. AMSCs could constitute an indefinite origin for cell banking and rapid delivering. This study investigated the in vitro and in vivo potential of pig AMSCs for xenotransplantation of osteogenic-derived stem cells.

Methods: AMSCs cells were isolated from the subcutaneous fatty tissue of transgenic "Green Fluorescence Protein" (GFP, n = 3) and Belgium

Landrace (n = 7) pigs. Osteogenic differentiation was performed by supplementing the standard media with dexamethasone/sodium ascorbate/sodium dihydrophosphate. Osteogenic differentiation was determined by staining for calcium phosphate deposition, osteocalcin/ von Kossa stainings. The immunophenotype of AMSCs was performed for CD90 cell surface phenotype (by Flow Cytometry) and for immunomodulation (by MLR). The Galactosyl epitope (Gal) expression was also characterized on both non-/differentiated AMSCs. Osteoblastic differentiated GFP-pig AMSCs were seeded on cancellous bone and implanted in the para-vertebral musculature of nude rats (two implants/recipient for bone alone vs Bone + AMSC, n = 10). After 30/60 days, implants were analyzed by microCT-Scan and explanted for osteocalcin and GFP monoclonal antibody expression.
Results: AMSCs maintained a constant expression of CD90 marker and a capacity to down-regulate T-cell response up to sub-culture passage 10th. Pig AMSCs, totalling ~34 population doublings by passage 10th, demonstrated the capacity of bone nodule formation with collagen synthesis, calcium deposition, osteocalcin expression and mineralization. A significant decrease of Gal epitope expression was found in differentiated tissue in comparison to non-differentiated AMSCs (28 ± 12% vs 41 ± 12% of surface implant,respectively, p = 0.011). After 30/60 days post-implantation in nude rats, a significant higher mineralization process (by micro CT-Scan) was found in composite grafts made of Bone + osteogenic GFP-AMSCs. Differentiated cells with osteocalcin expression were found in 100% of explanted composite implants made of Bone + AMSC in contrast to bone alone.
Conclusions: Pig AMSCs constitutes a potential source for cellular reconstruction of bone defect.

P-18.2

Airways homing of human amniotic fluid stem cells and repair in newborn rats exposed to hyperoxia

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Background: Alveolarization and angiogenesis play the main role in lung growth and consequently in the pathogenesis of bronchopulmonary dysplasia (BPD). Human amniotic fluid stem cells (hAFSC) may represent a cell lineage suitable for lung cell xenotransplantation. The purpose of this study was, therefore, to test the effects of postnatal hAFSC intratracheal administration in a newborn rat model of BPD.
Methods: A newborn rat model of BPD obtained following a 2 week exposure to 60% oxygen was administrated intratracheally with hAFSC previously transfected with a LacZ Adenovirus. Pups were postoperatively immunosuppressed with cyclosporine A (Novartis). Homing was evaluated by human specific mitochondrial and ribosomal duplex PCR. Furthermore, HEspecimens of the lung were stained for the identification of beta-galactosidase activity in transfected hAFSC. Lung morphometric, histological and terminal-deoxynucleotidyl transferase labelling (TUNEL) analysis were performed 3 weeks after hAFSC administration. Vascular endothelial growth factor (VEGF), angiogenesis and gas exchange were also assessed.
Results: Homing accounted for an average of 1.43% (ranging from 1.07 to 3.21%) of the lung alveolar cells. The lungs of the hAFSC-administered animals presented increased radial alveolar counts (RAC) and alveolar numbers if compared to the oxygen exposed and vehicle-treated groups. VEGF and the alveolar capillary network increased due to hAFSC transplantation.
Conclusions: These experiments indicate that hAFSC postnatal lung cell xenotransplantation have a favourable respiratory impact. HAFSC enhance pulmonary growth and decrease the hyperoxic arrest of lung development. In this model, the benefit deriving from postnatal treatment with hAFSC is likely to be a consequence of their homing which results into both alveolar growth and angiogenesis.

P-18.3

The in situ Langendorff model: a new method for AAV2/9-mediated gene transfer into myocytes in heterotopic heart transplantation models

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Objectives: Adeno-Associated Virus (AAV) vectors have become a widely used means to mediate gene transfer. However, application time, tissue temperature during transfection and volume of vector needed are limiting factors for a successful transfection of solid organs. The aim of this study was the development of a novel method to overcome these drawbacks, enabling an effective transfection of porcine hearts with AAV vectors. Eventually this method may facilitate the production of triple-transgenic pigs by utilizing hearts from gal-KO/hCRP-double transgenic pigs.
Methods: We established an in situ Langendorff recirculation model (without the need for cardiac arrest) for AAV vector transfection under normothermia and normoxia. After canulation and distal clamping of the ascending aorta and the pulmonary artery coronary perfusion was maintained via an extracorporeal circuit. The inferior and superior caval veins were ligated, thus the perfusion volume consisting of autologous blood could be reduced to approximately 500 ml. Hearts were perfused in the Langendorff mode for 45 minutes with 10^{13} AAV 2/9 LacZ vectors and 100 µg VEGF (n = 3). Then cardioplegia was applied and allogeneic heterotopic heart transplantation into a recipient pig was performed. Immunsuppression of the recipients consisted of a single dose of methylprednisolone (1 mg/kg) and tacrolimus (0.3 mg/kg body weight) for 8 days. Thereafter immunosuppresion was stopped and graft survival was assessed daily. After a maximum of 2 months hearts were explanted for immunohistology and gene expression analysis.
Results: All grafts maintained stable sinus rhythm during Langendorff perfusion. One animal died on day 9 after transplantation due to acute renal failure and one recipient died on day 24 with pneumonia. In the remaining animal graft function was maintained for the whole study period. Immunohistology showed mild to moderate signs of rejection. High LacZ expression was confirmed with fluorescence microscopy in all 3 animals (day 9, 24 and 61 after transfection).
Conclusion: Our novel in situ Langendorff model enables transfection of porcine myocytes in the intact cardiac graft with AAV2/9 vectors under normothermic conditions for 45 minutes. This leads to local application of high vector titers in a solid organ, resulting in an excellent transgene expression.

P-18.4

Potential role of complement activation on rejection of xenogeneic pig chondrocytes

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Xenotransplantation of genetically engineered pig chondrocytes could provide a solution to the pressing need of good therapies for cartilage repair. However, xenogeneic cartilage is rejected by a not-well-understood process that comprises humoral and cellular mechanisms. In particular, the complement system may contribute to rejection through opsonisation, anaphylatoxic and cytolytic activities. In fact, we previously demonstrated deposition of antibody and complement components C3 and C4 after incubating porcine articular chondrocytes (PAC) with 20% and 40% human serum. In this work, we investigated the potential role of complement on death and activation of pig chondrocytes. First, we incubated PAC for various times with 20%, 40% and 80% human serum and the corresponding heat-inactivated controls and measured total cell death by propidium iodide staining and flow cytometry. We verified that the serum batches used caused substantial lysis of porcine aortic endothelial cells after 1-hour incubation. Notably, this incubation time did not suffice to cause major PAC death even with 80% serum (12–14% over background, 6–7% at 20% serum). Longer exposures such as 24 hours were needed to observe significant cell death. Even then, it was relatively low (about 10–15% at 20% serum). We next determined the amount of apoptosis that contributed to cell death after 24-hour incubation by measuring the % of hypodiploid cells by flow cytometry. PAC consistently showed a low level of

apoptotic cell death (3–6%) at high serum concentration (>40%), but not at 20% serum. To assess other complement effects, we measured and confirmed a robust release of pig IL-6 and IL-8 by ELISA after incubation of PAC with human serum for 8 and 24 hours (20% serum being sufficient for this effect). In summary, these results indicate that pig chondrocytes are highly resistant to human serum-mediated cytolysis, but show complement-dependent cell activation as demonstrated by the release of pig cytokines. Thus, complement deposition may play a role in rejection of xenogeneic cartilage by promoting the cellular immune response.

P-18.5

The free radical scavenger S-PBN reduces human leukocyte migration across porcine endothelial cells

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Background: In solid organ xenotransplantation the first interactions between the recipient immune system and the graft occur at the endothelium. Reactive oxygen species activate the endothelium, leading to modulation of endothelial phenotype and permeability. This process may be inhibited by nitrone radical scavengers, such as the low toxic nitrone S-PBN (2-sulfophenyl-N-tertbutyl-nitrone). Own preliminary results showed that addition of S-PBN to 15-deoxyspergualin increases mouse heart xenograft survival in rats. Here we established an in vitro model to study the influence of S-PBN on adhesion and transendothelial migration (TEM) of human peripheral blood mononuclear cells (PBMC) on porcine endothelial cells (pEC).
Methods: TEM and adhesion of human PBMC were analyzed using permeable inserts precultured with pEC lacking Galα1,3Gal. Human PBMC were preincubated with S-PBN, added to the inserts and allowed to migrate for 4 hours. In order to differentially study the effect of S-PBN on adhesion and TEM, both total adhered and fully migrated out of total adhered (FM/TA ratio) PBMC were analyzed.
Results: Preincubation of human PBMC with increasing concentrations of S-PBN decreased both the adhesion and the FM/TA ratio of the monocyte subpopulation, reaching a 60% and 21% reduction, respectively, at 50 mM S-PBN as compared to control. For the lymphocyte population incubation with 50 mM S-PBN decreased the FM/TA ratio by 41% but not the adhesion. Whereas S-PBN alone did not directly affect the adhesion receptor expression on pEC, the expression intensity of VCAM-1 on pEC cultured for 4 hours with PBMC preincubated with 50 mM S-PBN was 30% lower as compared with pEC cultured with untreated PBMC. Finally, incubation of PBMC with S-PBN induced a significant downregulation of CD62L in the monocyte subpopulation.
Conclusion: S-PBN treatment reduces the adhesion and TEM of human PBMC on pEC. The reduction may in part be due to a decreased adhesion receptor expression on PBMC, and on a weaker induction of adhesion receptors on pEC by S-PBN-treated PBMC. Thus, S-PBN may inhibit xenograft infiltration by attenuating human leukocyte interactions with the porcine endothelium.

P-18.6

Role of HMGB1 in lung xenograft injury and cytokine production

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Background: Lung injury after xenotransplantation occurs despite use of organs lacking the α-galactosyl (Gal) antigen. High mobility group box 1 (HMGB1), which is released from activated neutrophils, macrophages, platelets, and endothelia cells, amplifies systemic inflammation through binding to RAGE, TLR2 and TLR4. In separate studies, we showed increased levels of HMGB1 after xenogenic Gal-independent lung perfusion. Here we tested whether HMGB1 mediates lung xenograft injury and cytokine production.
Methods: Wild-type (WT) C3HOuJ mouse lungs were perfused ex vivo with human blood containing Nextran 1285 (Gal antigen control) n = 5 or human blood with Nextran + HMGB1 inhibitor (Glycyrrhizin 0.3 mg/ml) n = 5. Lung survival, PVR, complement and platelet activation, CBC, mouse and human plasma (Luminex) and tissue (qRT-PCR) cytokine levels were measured.
Results: All lungs survived for the time of study and perfusion was electively terminated at 240 minutes. The pulmonary vascular resistance (PVR) rose moderately above normal levels similarly in both groups, not meeting rejection criteria. Complement (measured as plasma C3a) and platelet (β-thromboglobulin, expression of CD62P) activation were not different between groups. Thrombosis and hemorrhage were evident after 4 hours of perfusion, and not prevented by HMGB1 blockade. Mouse IL-6 and KC as well as human IL-8 and TNFα were consistently increased at 4 hours with or without HMGB1 blockade. Tissue expression of mouse IL-6, IP-10 and TNFα transcripts was significantly up-regulated at 4 hours of perfusion, but not influenced by HMGB1 blockade.
Conclusions: The physiological and molecular phenotype of lung HAR appears essentially independent of the pro-inflammatory mediator HMGB1. While a role for HMGB1 as an amplifier of inflammation in late injury cannot be excluded, control of early blood cell activation, lung injury and NFkB-dependent gene expression will require alternative therapeutic strategies.

P-18.7

Characterization of human and mouse cytokine profiles in xenogeneic lung injury

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Background: Lung injury after xenotransplantation occures despite control of α-galactosyl (Gal) natural antibodies. Current strategies targeting complement and coagulation cascade activation delay but do not completely prevent lung injury and blood cell activation. Pro-inflammatory cytokines, if produced, may stimutale or amplify rejection mechanisms. Cytokine profile changes during xenogenic lung injury have not yet been systematically studied. Here we characterize the production of pro-inflammatory cytokines at the protein and transcriptional level in an effort to understand Gal-independent related xenogenic lung injury.
Methods: Wild-type (WT) C3HOuJ mouse lungs were perfused ex vivo with human blood containing Nextran 1285 (Gal antigen control, n = 5). Mouse and human cytokines were measured in the plasma by Luminex at 60 and 240 minutes mouse cytokine transcripts were assessed in the lung tissue by qRT-PCR at 240 minutes and expressed as fold increase over pre-perfusion expression levels.
Results: All lungs survived until elective termination at 240 minutes. However, lung pulmonary vascular resistance rose above normal levels and features of lung rejection (thrombosis, hemorrhage) were detectable at 240 minutes. At the protein level, human IL-8 and TNFα (240 minutes) and mouse IL-6 (240 minutes) and KC (60 and 240 minutes) showed the greatest changes (see table). Human IL-1β, mouse IL-1β, IP-10 and TNFα remained unchanged. At the transcriptional level, tissue expression of mouse IL-6 was significantly increased (58 ± 14-fold), while mouse TNFα (8 ± 3) and IP-10 (6 ± 3) were moderately increased at 240 minutes. Mouse IL-1b mRNA level was essentially unchanged (1.7 ± 0.9).
Conclusions: Hyperacute lung injury is associated with prolific production of pro-inflammatory cytokines at the protein and transcriptional levels. Our data suggest that human IL-8, TNFα or mouse IL-6, KC are potential therapeutic targets to prevent lung injury and sequestration of leukocytes. Alternatively, approaches modulating blood and endothelial cell activation may have a beneficial effect.

P-18.8

Imaging heterotopic cardiac transplants in rodents

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Background: Abdominal heterotopic heart transplantation in rodents is an established experimental procedure that is used routinely to test new immunosuppressive regimens in xenotransplantation. Most commonly graft survival is determined by manual palpation of the transplanted heart. However, this approach is subjective, non-quantitative, and its diagnostic

accuracy has never been objectively assessed. Here we evaluate the accuracy of manual palpation to determine graft survival time, using transabdominal ultrasonographic echocardiography (TUE).
Methods: In 26 heterotopic abdominal heart transplanted mice (20–30 g BW) daily graft palpation and weekly TUE (n = 126 exams) were performed. For TUE mice were anesthetized via mask with isoflurane (1.5–2%), shaved and fixed with tape on a heated platform. The Visual Sonics® Vevo 770 Imaging System was used with the RMV 716 ScanHead. Left and right ventricular function, wall thickness, chamber diameters, and thrombus formation were measured and recorded. Presence or absence of contractility was compared to independent, blinded palpation measurement done on the day of TUE.
Results: TUE took ~5 minutes to perform, and was able to visualize the graft in all recipients. At 90.5% of the investigation time points (n = 114), TUE corroborated presence of heart contractility as diagnosed by manual palpation. However, in 9.5% (n = 12), results were discordant: contraction of the graft by palpation was not confirmed on TUE. three additional mice exhibited LV thrombus in association with poor or absent left ventricular function which progressed to complete graft failure within 3–7 days. Furthermore the ultrasound revealed that occasionally (5/126 exams) right ventricular contraction can still be present and also be detected by palpation when left ventricle contractility had ceased. The sensitivity to detect graft function by palpation was 90%.
Conclusions: We conclude that TUE improves detection of graft dysfunction and failure in a heterotopic heart transplant model, permits objective documentation of study outcomes, and is an important adjunctive technique to monitor xenograft function, especially for long-term follow-up. Although quantitative TUE as performed here required sedation of the recipient, in principle the technique could be adapted for routine use in non-sedated experimental subjects to confirm presence or absence of graft contractility.

P-18.9

Chronic immunosuppression with tacrolimus is associated with an increased risk of drug-induced diarrhea post islet xenotransplant in cynomolgus macaques

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Background: Chronic immunosuppression is associated with gastrointestinal complications including diarrhea. When conventional anti-diarrheal therapy fails often tapering, substitution, or cessation of immunosuppressive medications is required. Many drugs show a low absorption upon oral administration so that high dose levels are required. Often, the window between immunopharmacologic activity and gastrointestinal side effect is small, necessitating careful selection of dose levels.
Methods: We studied diarrhea incidence in 54 cynomolgus monkeys in our preclinical islet transplant program. Two recipients were treated with injectable or oral tacrolimus (TAC), 15 with injectable everolimus (EVL), 18 with oral sirolimus (SRL), two co-treated with EVL and TAC, and 17 co-treated with SRL and TAC for maintenance immunosuppression.
Results: Diarrhea occurred in all groups (Fig. 1). Monkeys receiving combination maintenance manifested a significantly higher risk of diarrhea than monkeys receiving monotherapy (RR = 5.15, 95%CI [1.3–19.8]; p = 0.003). Diarrhea episodes increased in linear manner consistent with the duration of administration.
Interpretation and conclusions: Oral administration of tacrolimus to cynomolgus monkeys, especially in combination with sirolimus or everolimus are associated with a particularly high incidence of diarrhea, varying between 15 and 25 episodes per 100 subject days. On the one hand this can be explained by the high dose levels required to achieve sufficient absorption and exposure in the pharmacologically active range, on the other hand with the composition of the monkey intestine, which renders the animal prone to development of diarrhea. These results suggest an additive effect of immunosuppression and, particularly tacrolimus, and duration of administration with respect to mucosal injury.

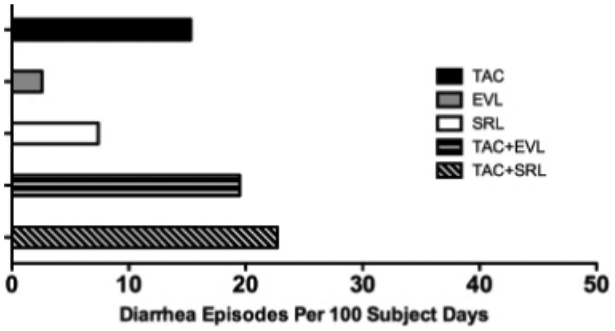

Figure 1. Diarrhea episodes rate per 100 subject days. The diarrhea episode rate was 15.3 per 100 days in the TAC group, 2.6 per 100 days in the EVL group, 7.4 per 100 days in the SRL group, 19.5 per 100 days in the TAC+EVL group, and 22.5 per 100 days in the TAC+SRL group.

P-18.10

Human platelet lysate expanded mesenchymal stem cells promote rapid commitment of CD34+ progenitor cells

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Background: Human mesenchymal stromal cells (MSCs) are promising candidates for the treatment of steroid resistant GvHD as well as promoting engraftement after HSCT. Translation of laboratory experiments into clinical applications has been limited by the dependence of MSC propagation on fetal calf serum (FCS) which is a potential source of infections. This has lead to the development of therapeutic protocols based on the non-transfusional use of hemocomponents, including human platelet lysate (HPL)
Objective: Two expansion protocols for MSCs had been compared starting from diagnostic BM aspirates. Unmanipulated and mononuclear cells (MNC) obtained by density gradient centrifugation and cultured in standard conditions (10% FCS) were compared to those obtained with the same culture conditions to which 10% HPL preparation substituted FCS. After the fifth passage; time of expansion, number of cells, morphology and cell surface markers were evaluated. One of the important functional capacities of MSC's which allows their clinical use in HSCT is their capacity to support &maintain the growth of CD34+ progenitors. In an attempt to test this function; differently expanded MSC's were subplated upon which cord blood CD34selected hematopoietic progenitors were added to evaluate their effects. No growth factors were added.
Results: MSC's were efficiently generated from all culture conditions including unmanipulated BM samples. Cells met all MSC criteria, e.g. plastic adherence, spindle-shaped morphology, surface marker expression; positive for CD73, CD90, CD105, CD106, CD146 and HLA-ABC while being negative for CD34, CD45 MSCs expanded in either FCS or HPL displayed comparable morphology and immunophenotyping but HPL-MSCs were superior in terms of clonogenic efficiency and proliferative capacity; the highest cell numbers were obtained with HPL-MSC's and the expansion time was the shortest. As compared with MSCs-HPL, MSCs-FCS maintained CD34+ HPC in culture for longer periods. Progenitors co-cultured with MSCs-HPL failed to express the CD34 marker after 10 days.
Conclusions: Unmanipulated BM can be used to efficiently initiate MSC cultures without the need for cell separation. HPL contains very high levels of growth and chemotactic factors which alter the functional capacity of MSCs. This should be taken in consideration during the co-transplantation of MSC and HSC for promoting engraftment.

P-18.11

Continuous, real-time viability assessment of kidneys based on oxygen consumption

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Background: The current state-of-the-art in donor organ quality assessment offers limited quantitative information. Organ quality is primarily

determined with visual and tactile inspection by the transplant surgeon and by histological analysis requiring biopsy. These techniques can be subject to interpretation and sampling variability. Consequently, there is a need for real-time and quantitative techniques for the assessment of whole organ health. This report describes a method for the measurement of whole organ oxygen consumption rate (OCR) normalized to tissue mass that can be used to quantitatively assess the metabolic activity, and thereby viability, of a porcine or human kidney.
Methods: Porcine kidneys (n = 5) were procured from non-heart beating donors. The pig was administered a bolus of heparin (100,000 U) 5 minutes prior to sacrifice using a fatal injection of sodium pentobarbital into the jugular vein. After confirmation of death, the pig was exsanguinated, the viscera were removed by *en bloc* viscerectomy, and the renal arteries (RA) were quickly cannulated and flushed with 4 l of cold lactated Ringer's solution (LRS). After flushing the vasculature, the kidneys were removed and the RA and renal veins (RV) were kept intact. The RA and RV were all cannulated and attached to in-line fiber optic oxygen sensors. The kidneys were then connected to a peristaltic cold perfusion system, and perfused with LRS. The flow rate, the gross organ mass, and the RA and RV oxygen partial pressure (pO_2) were measured to determine the whole kidney OCR per tissue weight.
Discussion: A significant pO_2 drop was measured between the RA and the RV in every organ studied. The average OCR value measured was 130 ± 80 nmol/minute/g (range = 22–229). The OCR per tissue of a dead organ was measured as 17 nmol/minute/g. Estimating that the OCR value for a healthy organ is 200 nmol/minute/g, these results demonstrate a large variability in organ viability suggesting the need for more quantitative assessment methods. This technique could be used to quantitatively study the quality of a donor organ during long-term cold perfusion or immediately prior to transplant.
Conclusions: Whole organ OCR shows promise as a clinical tool in monitoring kidney quality during the pre-transplant period. This technique could also provide significant research utility in evaluating the efficacy of organ procurement and preservation techniques.

Xenoantigens and Antibodies

P-19.1

Lewis, Duffy and Kidd system prevalence in baboon subspecies and pigs
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Background: Baboon has become specie of interest as receptor for xenotransplantation. In many transplant studies using pigs and baboons these antigens remain an unknown and untested variable. The influence of Kidd, Lewis and Duffy system antigens, in allorejection, has been demonstrated recently. We have tested the presence of these antigens in pigs and baboons using microtyping cards.
Methods: Sera samples of olive baboon (*Papio anubis*, n = 48), Guinea baboon (*P. papio*, n = 14), chacma baboon (*P. ursinus*, n = 9) and (Duroc x (Landrace x Large white)) conventional pigs (*Sus scrofa*, n = 8) were incubated in microtyping cards for Lewis (Lea and Leb), Duffy (Fya and Fyb) and Kidd (Jka y Jkb) detection. A five degrees score (0–4) was used to evaluate the results, depending on the hemagglutination reaction.
Results: Leb (92, 100 and 78% of samples from olive, Guinea and chacma baboons, repectivelly), Fyb (85, 100 and 89%, respectivelly) and Jka (100% all species) were found, Lea was only found in olive baboon (21%). Pigs showed Fya (100%) and Fyb (100%). To analyze pig's data it should be taken into account that phenotyping was done using human reagents that could content anti α-gal antibodies, but the hemagglutination reaction means that the presence of anti-Fy in baboon sera could react against pig tissues.
Conclusions: Blood antigens other than ABO should be investigated in xenotransplantation to prevent possible immunological rejections (host versus organ and organ versus host) and to manage adequately the blood bank needed in this surgery. The microtyping cards offer a quick and easy tool to assess these antigens.

P-19.2

ABO blood group phenotyping in pigs using microtyping cards
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Background: The pig is known to have a blood group AO system comparable to the ABO blood group system of humans and it is widely in experimental surgery. In fact is used as donor species in xenotransplantations to humans and non-human primates recipients. The complete concordance of ABO antigens should be assessed in such surgical experiences, as in allotransplantation. This work describes the use of a quick and simple microtyping cards to assess the ABO group in pigs.
Methods: ID-Microtyping cards ABO and RhD with human antibodies (ABOh; Diamed, Switzerland) and Dia-Clon ABO and RhD with monoclonal antibodies (ABOm; Diamed) were tested, using RBCs from 19 conventional pigs. The blood was 5% diluted with ID-Dilutant I (Diamed), containing Bromelin and incubated 10 minutes at room temperature. Twelve microlitres were disposed into each well, incubated 10 minutes at 37°C and centrifugated 10 minutes at 1,500 g. A reaction was considered positive when agglutinated RBC forming a red line on the surface of the gel.
Results: Using the ABOh cards, 8 A positive and 11 B positive were found on the basis of hemagglutination. However, using the ABOm, 8 A and 11 O pigs were found. The B group has not been described in pigs, and only A and O exists. Using the ABO cards there is an interference with α-gal antigens due to the molecular similarity between B and α-gal residues and producing a cross-reaction, whilst the ABOm avoid this interference and are able to detect the A and O animals (Figs 1 and 2).

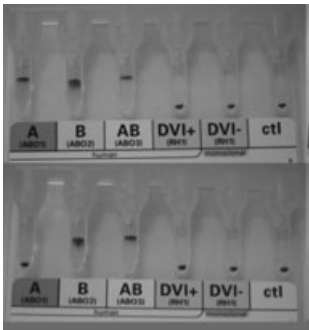

Figure 1: ABO phenotyping of A and O pigs using human reagents

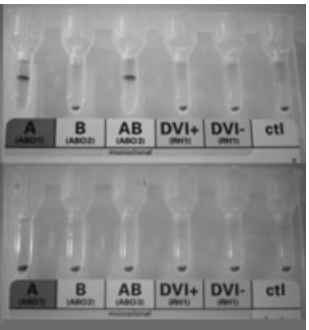

Figure 2: ABO phenotyping of A and O pigs using monoclonal reagents

Conclusions: The ABO blood group of pigs, used in xenotransplantation experiences, can be assessed by means of ABOm cards but not with ABOh cards. This system allows a very quick and easy system to phenotype blood of pigs involved in research.

P-19.3

Pig anti-baboon antibodies tested at different pig's ages
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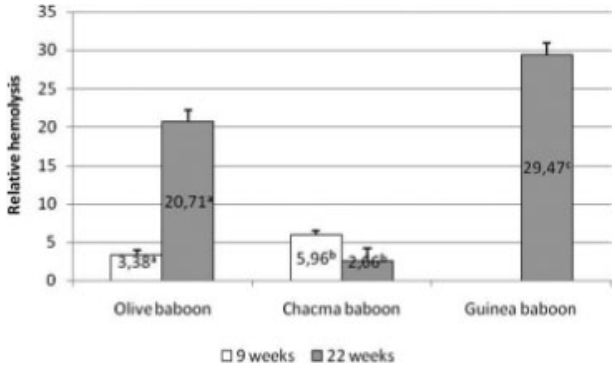
Background: There is a large number of studies about human and non-human primates anti-pig antibodies, but there is very few information regarding the presence of pig anti-baboon antibodies. This work has assessed the presence of pig anti-baboon antibodies in 9 and 22 weeks old pig's sera by complement mediated hemolysis (CMH) and hemagglutination.
Methods: Sera samples were obtained from 9 weeks old (n = 5) and 22 weeks old (n = 8) pigs. We have selected these ages because the former is used in pig-to-baboon xenotransplantation model and 22th weeks old (80 kg approx) should be the size for donation to humans. Blood samples were

obtained from olive baboon (n = 48), Guinea baboon (n = 14) and chacma baboon (n = 9). An anti-baboon exogenous complement mediated test (for all samples) and a hemagglutination (for olive and chacma baboons' samples) test using liss-combs microcards were performed. Hemolysis results were expressed as relative percentage and hemagglutination was scored 0–4. Finally, 659 reactions for each test were performed. The difference for hemolysis were done using an ANOVA test and for hemagglutination a Mann–Withney's U test.

Results: Results for hemolysis are shown in Fig. 1. There were significant difference comparing the 9th and 22th weeks old hemolysis in olive baboon ($p < 0.001$) but not in chacma baboon. There were differences comparing the hemolysis degree produced by 22th weeks old pigs comparing all the three species ($p < 0.001$). Hemagglutination was observed for 62% and 94% of olive and chacma samples with 9th weeks old pigs sera and 89%, 100% and 100% of olive, chacma and Guinea baboons with 22th weeks old pigs sera.



Conclusion: The pig-to-primate complement-mediated hemolysis has been previously reported, but we have found differences depending on the baboon specie and the pig's age. Chacma baboons are those suffering lower relative CMH and Guinea baboon the higher. The hemagglutination score was high in all species. These data should be taken into account to analyze the influence of graft-versus-host disease in xenotransplantation.

Coagulation and thrombosis

P-20.1

Xenotransplantation and coagulation disorders: different levels of human activated protein C in human and porcine endothelial cells

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Background: Microvascular thrombosis, following the activation of the coagulation cascade after a xenotransplant from pig to primate, is a central point in the process of graft rejection. Protein C (PC) activation plays a critical role in the negative regulation of blood coagulation. PC is activated on endothelium cell surface by the thrombin-thrombomodulin (TM)-endothelium PC receptor (EPCR) complex. Microvascular thrombosis, which causes loss of the graft, is probably associated to incompatibility between primate coagulation proteins (thrombin and PC) and receptors expressed by porcine endothelium (TM and EPCR).

Aim of the Study: To set up an in vitro model for the evaluation of human PC activation on different cell lines: HUVEC (Human Umbilical Vein Cells), HCAEC (Human Coronary Aortic Endothelial Cells) and PAEC (Porcine Aortic Endothelial Cells). To confirm the contribution of EPCR and TM in the APC generation, we inhibited the PC activation in HCAEC with antibodies specific to TM and EPCR.

Methods: Confluent cells were incubated with human thrombin and human purified PC with or without anti-EPCR and anti-TM antibodies. After 60, 90, 120, 180, and 240 minutes a sample was taken and added to hirudin. The APC formed was measured using the chromogenic substrate S2366. The concentration of APC was calculated using a standard curve of purified APC.

Results: After 240 minutes, the generation of APC on HUVEC, HCAEC and PAEC was 4.71 ± 0.03 μg/ml, 9.58 ± 0.47 μg/ml and 0.87 ± 0.02 μg/ml, respectively. Without cells the generation of APC was 0.72 ± 0.56 μg/ml. The APC levels on HCAEC were significantly higher than on HUVEC ($p < 0.001$) and PAEC ($p < 0.001$). After 120 minutes, APC formation on HCAEC was decreased in the presence of antibody to EPCR or to TM (3.87 ± 0.10 μg/ml and 0.84 ± 0.01 μg/ml, respectively; 6.01 ± 0.45 μg/ml without antibodies) while PC activation was completely inhibited after treatment with both antibodies.

Conclusions: PC was highly activated on HCAEC, mildly on HUVEC and not activated on PAEC. In fact, HCAEC, originating from small vessels, express high levels of TM as compared to large vessels, indicating that the amount of human TM expressed on endothelial cell surface is crucial for PC activation. PAEC require the expression of human TM and EPCR in order to activate anticoagulant PC pathway and prevent clot formation.

P-20.2

Correlation between xenoreactive antibodies and xenogenic activation of the coagulation system in xenoperfused pocine kidneys

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Background: Xenoreactive antibodies (XRA) can bind to pig endothelium leading to an activation of the recipient's complement system and eventually resulting in graft loss. Even if complement activation is successfully inhibited, the acute vascular rejection (AVR) can occur. During the AVR an activation of the recipient's coagulation system can be observed resulting in derangement of coagulation and consecutive graft dysfunction or death of the recipient. It is still unclear if the derangement of coagulation is independent from AVR or just an epiphenomenon of acute or chronic humoral xenograft rejection. In this study we examined the effect of XAR titers on the grade of the derangement of coagulation using an *ex-vivo* perfusion system.

Methods: An *ex vivo* perfusion circuit based on C1-Inhibitor (C1-Inh) and low-dose heparin was used to asses activation of coagulation and perfusion time in the different experimental groups. Porcine kidneys were perfused with freshly drawn pooled human AB blood. The experiments were performed in five different groups: kidneys from wild-type pigs with addition of C1-Inh, but without any further pharmacological intervention (group B, n = 5); kidney perfusions without C1-Inh (group C, n = 3); kidney perfusions with addition of C1-Inh and antithrombin (group D, n = 5) or activated protein C (group E, n = 5). Perfusion with porcine blood served as a control group (group A, n = 5). Donor sera were collected and XRA pre-perfusion titers were measured by flowcytometry.

Results: Mean kidney survival in group B was 126 ± 78 min. Mean XRA titer in this group was 1:2049, mean d Dimer level was 18116 ± 4730 μg/l. Depletion of C1-Inh in group C resulted in reduced perfusion time (mean 42 ± 26 min) with a mean XRA titer of 1:2389. Pharmacological intervention with either antithrombin (group D) or APC (group D) resulted in a prolonged mean perfusion time of 240 ± 0 min. XRA titers were 1:2901 in group C and 1:4096 in group D, while mean d Dimer levels were 1255 ± 1070 μg/l in group C and 788 ± 390 μg/l in group D.

Conclusion: No correlation between donor pre-perfusion XRA titers and perfusion time was found. More precisely high XRA titers did not lead to reduced perfusion time and vice versa. Furthermore, XRA titers did not correlate with the grade of the derangement of coagulation observed in the perfusion circuit.

P-20.3

Towards the generation of human TFPI transgenic pigs with improved features for xenotransplantation

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Introduction: There is a shortage of organs for transplantation. The clinical application of pig- to- human xenotransplantation has the potential to solve

this problem. However, the immunological rejection of discordant xenografts poses a major obstacle. Part of this rejection mechanism is caused by molecular incompatibilities between regulators of coagulation on the xenograft endothelium and their soluble targets in the recipient circulation. Tissue factor pathway inhibitor (TFPI) is the primary regulator of the initiation phase of the coagulation pathway. Here, we embarked on a study to express human TFPI (hTFPI) on endothelial cells of transgenic pigs employing recombinant DNA technologies and somatic cell nuclear transfer (SCNT).

Methods: Vectors containing the promoter of human EF-1α or of porcine ICAM-2 were used to express both isoforms of hTFPI. The constructs were transfected into WT porcine fetal fibroblasts (PFF) and transgene integration was confirmed by PCR. For each construct, two or more positive clones were used in SCNT. After birth, DNA and mRNA from different tissues and cell lines were analysed by PCR and RT-PCR. An immortalized porcine endothelial cell line (PEDSV.15) was also transfected and then checked by PCR. In addition, mRNA was analysed by RT-PCR.

Results: We obtained pigs that had integrated the DNA for hTFPIβ under the hEF-1α promoter (SCNT efficiency of 4.5%). Unfortunately, mRNA specific for hTFPI could only be found in fibroblasts derived from ear punctures (irrelevant for xenotransplantation). In the experiments using the porcine ICAM-2 promoter, two of the cell clones that were hTFPI-positive in PCR screening, were used as donor cells for SCNT. One hundred reconstructed embryos were transferred to two synchronized recipient sows. One of the sows from the hTFPIβ experiment is pregnant and expected to give birth in August 2009. The recipients from the hTFPIα experiment still have to be checked for pregnancy. The transfected PEDSV.15 cells were positive in PCR and RT-PCR.

The production of human protein is currently being tested.

Planned Experiments: Tissue-specific expression of hTFPI isoforms and their biological function in cloned piglets will be assessed by *in vitro* test assays and perfusion systems.

P-20.4

Comparison of procoagulant phospholipids levels between human and cynomolgus monkeys

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Background: Clotting cascade activation is central to the rejection process that occurs when pig organs are transplanted into primates. Indeed, intravascular coagulation is a feature of all solid organ of xenograft rejection and represents an intrinsic barrier in xenotrasplantation. Little is known on the overall coagulative process which leads to clot formation in primates. Human procoagulant phospholipids (PPL) properties depend mainly on the phosphatidylserine (PPS) externalization and exposure on the membrane lipid bilayer which enable PPS to promote the activation of coagulative cascade and subsequently thrombus formation. Elevation of plasma PPL levels particularly reflects cellular injury and now appears as a marker of vascular dysfunction.

Aim of the Study: To evaluate the levels of PPL in cynomolgus monkeys and compare with PPL levels present in human plasma from healthy donors.

Methods: Blood samples were obtained from 40 healthy human subjects and from 20 cynomolgus monkeys. PPL test (Diagnostica Stago, Gennevilliers, France), consists in the measurement of clotting time (in second), uses a system in which the addition of sample phospholipids in depleted plasma makes the test dependent on the level of PPL. A reduction of clotting time of the sample compared with usual values means an increase in PPL.

Results: PPL depending clotting time (mean ± SD) was shorter in cynomolgus monkeys than in healthy subjects (43.9 ± 15 sec and 55.97 ± 12.55 sec, respectively), the difference was statistically significant ($p < 0.0008$).

Conclusions: Cynomolgus monkeys present an increase in circulating procoagulant PPL levels compared to healthy humans. The observed association underscores the possibility that in cynomolgus monkeys generated PPL initiate coagulation which may lead to thrombus formation.

P-20.5

Human thrombomodulin plays an important role in coagulation control: engine braking is essential for physiological regulation

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Background: Human plasma is clotted more rapidly in porcine endothelial cells (PAEC) than in human endothelial cells (HAEC). We have reported that human thrombomodulin (hTM) expression in PAEC might be a promising strategy for overcoming the problem of molecular incompatibility relevant to activated protein C (APC) production. Furthermore, hTM expression has the potential to prevent direct prothrombinase activity which can directly produce thrombin in PAEC. Considering the production of genetically engineered pigs or pharmacological administration of anticoagulants in recipients, we are bound to be anxious about overexpression- or overdose-related adverse effects such as bleeding tendency. It is important whether anti-coagulation strategy would be under physiological control.

Methods: (1) Clotting and thrombin generation assay were conducted in PAEC under various situations. The effect of (i) inhibition of antibody binding, (ii) inhibition of complement activation and (iii) expression of hTM on human blood coagulation in relation to PAEC was examined. (2) Concentration-effect relationship of clinically used anticoagulants (argatroban: ARG, fondaparinux: FOND and soluble hTM) was examined in clotting assay.

Results: (1) Suppression of antibody binding or complement activation decreased thrombin generation, but did not changed clotting time effectively. hTM expression significantly reduced thrombin generation and improved the shortening of clotting time. (2) For the correction of clotting time in PAEC, effective concentration ranges of ARG, FOND and soluble hTM were 0.1–0.5 ug/ml, 0.1–0.5 ug/ml and 0.5–20 ug/ml, respectively. High concentration of ARG and FOND caused extreme prolongation of clotting time. In contrast, soluble hTM was moderately suppressed even in high concentration.

Conclusion: Inhibition of antibody binding and complement activation alone was ineffective for the correction of clotting time of human blood in PAEC. Additional anti-coagulation therapy is essential. Only after thrombin is formed, hTM can produce APC, which would not reach excessive levels. Thus, hTM was considered to exert a role as a physiological regulator of coagulation unlike other anticoagulant agents with narrow therapeutic range. Genetically engineered pig expressing hTM (on vascular endothelial cells) showed no bleeding tendency.

P-20.6

Activation of human protein C by α1,3-galactosyltransferase gene-knockout porcine aortic endothelial cells expressing human thrombomodulin

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Background: Activation of the clotting cascade, fibrin deposition and thrombosis are recognized as important elements of humoral rejection, which remains the major barrier to the long term survival when pig organs are transplanted into primates. The formation of microvascular thrombosis is probably related by the inability of pig thrombomodulin (TM) and protein C receptor (EPCR) to activate the host circulating protein C (PC). Activated PC (APC) is critical for the anticoagulation on endothelial surface. At present, in order to prevent acute humoral xenograft rejection, a1,3-galactosyltransferase gene knockout (GalT-KO) pigs are used. All graft, from these transgenic pigs, undergoing rejection exhibited thrombotic microangiopathy with platelets-rich fibrin thrombi in the microvasculature.

Aim of the Study: *In vitro* study of activation of human PC by GalT-KO porcine aortic endothelial cells (GalT-KO-PAEC) and by GalT-KO-PAEC expressing human TM (GalT-KO-PAEC-hTM). As a control, we used

human coronary artery endothelial cells (HCAEC) expressing very high levels of TM.
Materials and Methods: GalT-KO cells were transfected with the gene encoding for human TM. All cells were tested by immunofluorescence (IF) technique for the expression of pig and human TM. Cells were incubated with human PC and thrombin. Generated APC was assessed using a chromogenic substrate. The same experiments were carried out after treatment of cells with anti-human TM antibody.
Results: IF assay confirms the expression of human TM by GalT-KO-PAEC-TM cells. GalT-KO-PAEC cells did not activate human PC. The generation of APC was significantly higher on GaT-KO-PAEC-TM than on HCAEC (15.89 ± 0.50 μg/ml and 9.58 ± 0.47 μg/ml, respectively; $p < 0.001$). The thrombin-TM dependence of the human PC activation by GalT-KO-PAEC-TM cells was confirmed by the significant ($p < 0.001$) inhibitory effect of the anti-human TM antibody, known to block binding of thrombin to TM.
Conclusion: These preliminary results demonstrated higher activation of human PC by GalT-KO-PAEC-TM than HCAEC. Further studies in these cells co-expressing EPCR/TM are necessary to investigate PC coagulation system. Genetic modification of the donor organ expressing inhibitors of coagulation pathway maybe have overcame the problem of coagulation dysregulation between species.

Genetic Engineering of the Donor Species

P-21.1

Analysis of transgene expression in transfected somatic pig cell to be used as donor in nuclear transfer

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Background: Somatic cell nuclear transfer is the method of choice to generate transgenic large animals. Success largely depends on a high expression level of the target protein.

Selection of cell clones with the desired expression level is of paramount importance before nuclear transfer. In this work we compare different methods that can be used on a small number of cells and their predictive value.
Methods: Two expression vectors for CD55 and CD39 were separately transfected to PK15 cell line. Following selection, five of the best growing clones resulting from each transfection were expanded and subjected to RT-PCR and Immunohistochemistry (IHC) analyses. For IHC analyses the mAB IA10 (BD-Pharmingen) – for CD55 – and the mAB BU61 (Ancell) – for CD39 – were used. The same antibodies were also used in Western blot (WB) analyses performed on samples subjected to non-reducing SDS–PAGE and electroblotted on PVDF membrane. The presence of the target transcripts was confirmed by Northern blot (NB) analyses using DIG-labeled probes (Roche). The proteins expression was also analysed by FACS conducted on chosen clones. Fibroblasts and PAECs deriving from one CD55–CD39 stillborn cloned piglet were subjected to IHC, NB and FACS analyses.
Results: Three out of five (#24, #2 and #15) PK15–CD55 clones were positive to RT-PCR but only clone #24 was positive to IHC. Clone #24 was further analysed by NB, WB and FACS that confirmed the high expression level. Clone #2 revealed a low expression level by FACS not detected by IHC. All five PK15–CD39 clones were positive to IHC and RT-PCR analysis. Clone #10 was further analysed and confirmed positive by NB and WB. The IHC, NB and FACS data obtained on fibroblasts and PAECs of cloned piglet confirmed the donor cell lines CD39 expression detected by IHC. This was not the case with CD55 expression since the positivity detected by IHC was not confirmed with FACS and NB analyses.
Conclusion: IHC is the method of choice when few cells for each clone are available, being more accurate than RT-PCR. Nevertheless, since this technique is not accurately quantitative, it needs to be complemented with alternative methods (Western Blotting or Real time PCR) to obtain a more complete evaluation of the expression pattern of the transgene. This study was supported by EU grant n° LSHB-CT-2006-037377.

P-21.2

An episomal vector containing the ecto-5′-nucleotidase gene

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The most serious barriers to xenotransplantation are hyperacute rejection (HAR), acute humoral xenograft rejection (AHXR) and cell-mediated xenograft rejection. HAR has been largely overcome in pigs lacking α1,3Gal or overexpressing human regulators of complement such as hDAF, but the latter two problems still remain. An endothelial surface enzyme, ecto-5-nucleotidase (E5′N) controls conversion of pro-inflammatory extracellular nucleotides into adenosine, that exhibits cytoprotective, immunosuppressive and antiinflammatory effects. It has been established that expression of human E5′N (hE5′N) in pig EC attenuates hNK cell-mediated toxicity against these cells. The activity of E5′N is much lower in pig as opposed to human EC and heart, which can create problems in the utilization of the pig for xenotransplantation. We have cloned the hE5′N gene into a vector in the intent of adding this gene to pigs genetically modified for the control of HAR. Episomal vectors that replicate autonomously in host cells are the vectors of choice in xenotransplantation, since they will not cause genetic damage to donor organs. pEPI-GFP is a mitotically stable mammalian episomal vector that allows for efficient expression of transgenes. Pigs genetically modified by pEPI-eGFP have been produced in which the plasmid was shown to be episomal and to express GFP in the different tissues of the animals. We have shown that the hE5′N gene cloned in pEPI-eGFP (pEPI-hE5′N) has a sequence identical to that contained in the NCBI database, and that all pEPI-eGFP sequences are present in pEPI-hE5′N. pEPI-hE5′N was transfected into PIEC pig cells and was subjected to RT-PCR analysis, showing that hE5′N was expressed efficiently. Plasmid DNA was extracted from transformed PIEC cells and used to transform *Escherichia coli*: bacterial clones were obtained, showing that the plasmid was episomal. Restriction analysis of the resident plasmids showed that pEPI-hE5′N in pig cells had not been structurally altered. The introduction of this plasmid into pigs that have been genetically modified to overcome the problems caused by HAR will be likely to provide an important contribution for facing the remaining aspects of rejection and for other therapeutic approaches that require efficient immunosuppression in substitution of medical therapy.

P-21.3

Double transgenic Gal$^{-/-}$ piglets over-expressing hCD39

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Over-expression of human CD39 in transgenic pigs is a potential strategy to bypass acute vascular rejection in xenotransplantation. The aim of this work is the production of transgenic cloned pigs using a Gal$^{-/-}$ CD55/CD39 cell line. A neonatal pig Gal$^{-/-}$ fibroblast line cultured in DMEM/M199 1:1 + 10% FCS + 5 ng/ml bFGF was co-transfected by nucleofection with two ubiquitous expression vectors, the first carrying hCD55 under Elongation Factor promoter and a HygroR cassette; the second carrying hCD39 under pCAGGS promoter and a 3′ MAR region. After nucleofection, cells were plated in Petri dishes and selected with Hygromycin B for 8 days. Drug resistant colonies were isolated and expanded for transgene expression analysis. We used immunohistochemistry (IHC) to detect the expression of the proteins. For hCD55 we used IA10 and for hCD39 BU61. Cells co-expressing CD55–CD39 were serum starved for 24 h before being fused to enucleated oocytes. Following electric activation, embryos were grown in vitro up to compact morula/blastocyst and all ($n = 144$) were transplanted

in two synchronized sows. PAEC and fibroblasts derived from delivered piglets were analysed with FACS, using the following antibodies: BRIC110, IH4, 2G2, and MEM-118 for hCD55 and TU66 for hCD39 detection respectively. Using a double transgenic CD55/CD39 $Gal^{-/-}$ colony in a Somatic Cell Nuclear Transfer (SCNT) experiment we have obtained 35.4% compacted morula/blastocyst development. One of two sows resulted in a pregnancy. At day 117 of gestation, this sow was induced to farrowing and delivered two stillborn piglets that were probably too immature and died from respiratory failure. Nevertheless, IHC analysis performed on PAEC and fibroblasts derived from these piglets showed strong expression of CD55–CD39, as in the original colony. FACS analysis confirmed robust human CD39 expression but showed a very low level of hCD55 expression. Two $Gal^{-/-}$ CD55/CD39 piglets were obtained. Over-expression of hCD39 seems to be compatible with normal pig fetus development. Efficency of SCNT using the double transgenic $Gal^{-/-}$ line was slightly lower than that observed using the $Gal^{-/-}$ line itself, from which we obtained 40.7% (n = 2583) blastocyst development in previous experiments. This cell line will be used for generation of other cloned piglets and for in vitro test analysis. This study was supported by EU grant n° LSHB-CT-2006-037377 and by Fond Banca Pop Cr.

P-21.4

Significant differences of some physiological parameters in humans and pigs

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Purpose: The incompatibilities between porcine and human physiologies may have dramatic influences on the outcome of a patient after xenotransplantion. In this study, we reported the reference values of clinical chemistry, hematology and coagulation parameters of pigs to give insight into the evaluation of liver functional compatibility and the graft monitoring in pig to human liver or hepatocytes xenotransplantation.
Methods: Blood were collected from 27 healthy Chinese Guizhou Minipigs of both sexes. Values of the routine parameters from pigs were compared with that of healthy humans.
Results: (1) The bilirubin, albumin, uric acid, and cholesterol of pigs were significantly lower than those in humans, whereas, their serum enzyme levels were obviously higher than that of humans. (2) Red blood cell count, platelet count, and white blood cell count of pigs were significantly higher than those of humans. Particularly, lymphocyte count and its percentage were obviously higher. (3) Coagulation activities of porcine factor VII was much higher than that of humans. In contrast, the prothrombin times and activated partial thromboplastin times were a little longer in pigs. Thrombelastograph showed that pigs were in relative hypercoagulable condition compared with humans.

Statistical significances were lower than 0.05 ($p < 0.05$) in all of the above parameters in the two species.

Reference values of chemistry and hematology parameters in humans and pigs

Parameter (unit)	Healthy humans (n=30)	Pigs (n=27)
Tbil (¼mol/l)	13.68±4.61	1.96±0.69
TBA (¼mol/l)	5.76±8.49	20.49±17.79
ALT (IU/l)	24.77±11.57	68.30±37.50
AST (IU/l)	21.63±4.88	100.04±55.64
Alb (g/l)	45.50±2.75	38.23±6.32
Uric (¼mol/l)	300.59±62.42	0.26±2.79
Chol (mmol/l)	4.13±0.62	1.99±0.36
ALP (IU/l)	68.40±19.25	128.67±60.54
GGT (IU/l)	20.40±12.22	105.77±64.07
CK (IU/l)	78.53±37.49	662.33±275.87
LDH (IU/l)	157.28±43.30	678.22±120.33
HBDH (IU/l)	25.49±4.65	489.74±107.15
RBC (1012/l)	4.82±0.35	8.50±0.86
HB (g/dl)	144.35±10.44	150.85±19.84
PLT (109/l)	180.73±48.31	313.08±149.09
WBC (109/l)	5.94±1.33	15.96±3.92
PT	12.99±0.59	14.35±0.69
APTT (sec)	37.52±3.20	48.93±8.77
CFVII (%)	(55—–170) 112.5	191.17±49.60
Statistical significances were lower than 0.05 (P<0.05) in all of the above parameters in the two species.		

Conclusion: The significant differences of many parameters in pigs and humans suggested that liver functional incompatibility may happen after liver xenotransplantation, particular in term of metabolism and coagulation functions.

P-21.5

Production of transgenic mini-pigs with membrane-bound human FasL by somatic cell nuclear transfer

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Objectives: The cell-mediated xenograft rejection, including NK cells and CD8+ CTL, is a major obstacle for successful pig to human xenotransplantation. Human CD8+ CTL is highly cytotoxic against pig endothelial cells (PEC) and the overexpression of membrane-bound human FasL (mFasL) is effective in preventing this CTL-xenocytotoxicity (*Transplantation* 2006:81). In this study, we produced transgenic mini-pig haboring mFasL by somatic cell nuclear transfer (SCNT).
Methods: cDNA of mFasL carrying the deletion at the cleavage site with metalloproteinase and lacking the death domain in its cytoplasmic tail was subcloned into pcDNA3.1 expression vector driven by the CMV promoter containing neomycine-resistance cassette. The mFasL expression vector was transfected into mini-pig fetal fibroblasts by lipofection method. G418-resistant cell clones were screened by PCR. The surface expression of mFasL was confirmed by FACS and Confocal imaging system with the mouse anti-human FasL antibody. One clone was used as a donor cells for SCNT. The SCNT embryos were surgically transferred to the oviduct of naturally cycling sow on the first day of standing estrus.
Results: Five cell clones were successfully established following G418 selection and confirmed by PCR, FACS and Confocal analysis. The reconstructed embryos were produced with these clonal cells and transferred to nine surrogate sows. Ultrasound examination of recipient surrogates on days 35 after embryo transfer confirmed established pregnancies in three recipients. One recipient delivered piglets with normal birth weight. PCR analysis revealed that transgene vector was integrated in the offspring genome. Transgene-expression analysis and CTL assay are currently underway. The other recipients are going to term to produce transgenic piglets.
Discussion: The present results show that transgenic pigs were produced with mFasL cDNA for controlling cell-mediated rejection. CTL assay will be conducted *in vitro* to test if the transgene expression level is sufficient to protect cells from CD8+ CTL.

Ethics and Regulations

P-22.1

Study of the attitude of Scottish citizens toward xenotransplantation in the South East of Spain

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Background: In xenotransplantation it is important to find out about social acceptance, especially in populations where there are preclinical projects and a possibility of xenotransplantation to humans. In the native population of south-eastern Spain this programme is well-known but in recent years there has been a considerable social change due to significant immigration into the area, especially of Scottish citizens. The objective of this study is to analyse the attitude of Scottish citizens residing in south-eastern Spain towards xenotransplantation and to determine the variables that affect this attitude.

Methods: A random sample (n = 350) of the population of Murcia (South East Spain) that was born in Scotland. The instrument used to measure attitude was a questionnaire with different categories validated in our local area. The survey was self-administered and completed anonymously between November 2005 and March 2006. The control group was a random sample of the native population (n = 250). Student's T-test and Fisher's exact test were applied in the statistical analysis.
Results: The questionnaire completion rate was 93% (n = 325). Most respondents (67%) were in favor, 8% were against and 25% were undecided, assuming that the animal organs functioned as well as human ones. Attitude was similar to that of the control group ($p = 0.0794$). With regard to animal organ donation for humans, assuming the organs functioned worse than human donor organs, 26% (n = 83) would be in favor, 55% (n = 178) undecided and the remaining 19% (n = 62) would be against. A favourable attitude is related to the following factors: being a male ($p < 0.001$), marital status ($p = 0.008$), level of education ($p = 0.041$), a partner's favourable attitude toward transplantation ($p < 0.001$) and a favourable attitude toward human donation, both deceased ($p = 0.001$) and living ($p < 0.001$).
Conclusions: The attitude of Scottish residents in southeastern Spain is similar to that of the native Spanish population and is determined by many psycho-social factors that are mainly related to prior attitude towards the different types of human organ donation.

P-22.2

Refinement of a primate xenotransplantation model: application of a subcutaneously implanted port as a means of gastric nutrition

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Background: The basis of the ethical approach to animal research is anchored in the globally acknowledged principles of the Three Rs (Replacement, Reduction and Refinement). In particular, Refinement means the employment of methods to ensure that any possible harm and sufferance during the course of the experiments, are kept to a minimum, as well as improving the care, treatment and living conditions of the animals in order to enhance their well-being. The main goal of our study was to allow percutaneous access to the stomach, in order to obtain nonstressful intragastric administration of solids and fluids in xenografted primates.
Methods: Six adult female nonhuman primates (nhps) (*Macaca fascicularis*), mean weight 3.370 kg, were implanted with a surgically placed gastrostomy (SPG). The device, supplied by BARD Access Systems, consists of two primary components: an injection port with a self-sealing silicone septum and a radiopaque silicon catheter. In particular, a Groshong* valve, positioned at the end of the catheter, ensures security against gastric juice reflux into the port/catheter system. The catheter was inserted into the gastric lumen, by surgical approach through the abdominal wall, and anchored to the gastric wall with a tobacco-pouch suture. The port was placed subcutaneously on the anterior chest wall, for stable fixation when using the port needle.
Results: No surgical complication occurred during the positioning of the port. In one case, after 41 injections, the port had to be removed as a consequence of a subcutaneous infection. Five nhps continue to receive drugs and nutritional supplements twice a day. To date, no infections at the subcutaneous port have been detected in these animals and no obstruction of the system has been experienced throughout the observation period.
Conclusion: Our data suggest that a subcutaneously implanted port is a safe and effective device for primates that undergo xenotransplantation. Indeed, it permits long term administration of oral treatments without the need for repeated handling of the animals. In this light, the system represents an effective refinement tool that fully complies with the Three Rs tenet.

P-22.3

Improvement of analgesic protocols in renal xenotransplantation procedures

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Background: Ischemia and reperfusion (IR) injury is an unavoidable insult occurring to the graft as a consequence of transplantation. Specifically following kidney xeno- and allo-transplantation, proximal tubular cells are particularly susceptible to apoptosis or necrosis induced by ischemia and ATP depletion. Opioids are the most common and effective analgesics used in humans and animals. However only few reports evaluate kidney responses to these drugs during ischemia. The aim of this study is to evaluate the *in vitro* effect of the clinically used opioids morphine, fentanyl and buprenorphine on tubular cells expressing κopioid receptors prior and after marked ATP depletion.
Methods: OK3 cells (opossum kidney tubular cells) underwent 2 h of simulated ischemia (SI) by treatment with 10 μM Antimycin A and 2 mM 2-Deoxy-D-Glucose. Cells were exposed to morphine, fentanyl or buprenorphine (0.1 nM, 10 nM, 10 μM) 1 h before and following the ischemic treatment. Cell viability, ATP content, caspase-3 and -7 activity were evaluated immediately at the end of the SI and at 12 and 24 h following ischemic treatment. Apoptosis/necrosis were also evaluated by annexin-V/propidium iodide staining and flow cytometry analysis.
Results: Immediately after SI, ATP content decreased by 85% compared to untreated control cells. An increase in percentage of apoptotic and necrotic cells was also observed. At this time point, ATP levels and apoptotic/necrotic cells were not influenced by opioid treatment. On the other hand opioid treatment counteracts the increase in caspase activation observed following ATP depletion. This effect was maintained even at 12 h post treatment with both opioid exposure and was not observed following treatment with buprenorphine a κopioid antagonist. At 12 h post treatment fentanyl 10 nM – 10 μM seems to preserve ATP content of cells compared to untreated ischemic cells and decrease the percentage of apoptotic/necrotic cells at 12 h post ischemic treatment. Morphine exposure had the same effects as fentanyl but was detected only at 24 h post SI. In contrast exposure to buprenorphine did not preserve ATP content.
Conclusion: Morphine and fentanyl preserve intracellular ATP content, thus influencing the effects of ischemia on apoptosis. Current data suggest that these drugs should be preferred in xenotransplantation procedures to control intraoperative and postoperative pain.

Xenozoonoses

P-23.1

Xenovirology monitoring: tasks and issues

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Every successful xenotransplantation program must have an elaborate microbiology monitoring strategy. At the early stages of program development significant effort should be given to such tasks as elucidating the microbiological status of the pig population in the local geographical area, the identification of the microorganisms relevant to xenotransplantation and the development of a monitoring schedule for source herd identification and maintenance. The following criteria should be considered essential for the establishment of a donor herd: the absence of xenotransplantation important pathogens and favourable PERV characteristics (absence of an infectious PERV, low PERV copy number). This includes the establishment of reliable and sensitive assays for the detection of porcine infectious agents as well as the development of strategies for donor safety evaluation, source herd maintenance and recipient follow-up. Diagnostic assays for recipient follow-up must also be developed. The second stage of the xenovirology program is the conversion of the research laboratory to a medical diagnostic laboratory and its certification. The unique characteristic of a xenovirology laboratory is the synergy of knowledge and skills from veterinarian and human medical diagnostics as well as the ongoing search for new infectious agents and improvement of modern techniques for donor and recipient surveillance.

P-23.2

Animal model sysyem of PERVs transmission in xenotransplantation using pig's organ

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Xenotransplantation has been presented as a possible solution to the lack of human donor organs. Pig organs are good alternative organs to human organs for the xenotransplantation. Porcine endogenous retrovirus (PERV) is existed in the pig's genome, and PERV have become a major transmissible pathogen in xenotransplantation. PERV has infectivity to human cell in vitro, but, *in vivo* activation of PERV has not been examined. We focused on the possibility of the PERV transmission to human in the humanized pig which is reduced rejection and it was constructed the CsA treated immune-competent mouse model system using murinized PERV (PERV produced from the murine cells). PERV producing mouse cell line, NIH3T3/PERV, was constructed. The NIH3T3 cells and NIH3T3/PERV cells were transplanted to the CsA treated or non-treated mouse using S.C. injection or kidney capsule injection. After 6 weeks of transplantation, each organ was extracted from the mouse, and the PERV transmission was identified in the RNA level and DNA level. When the NIH3T3/PERV cells were transplantated to the mouse, the PERV gene was detected in several organs under the RNA or DNA levels, but not normal NIH3T3 cells.

In the CsA non treated groups, the PERV detection frequency was decreased compared to CsA treated groups. Through the immunohistochemistry assay using the spleen, murinized PERV transmitted to the spleen and were expressed the env proteins.

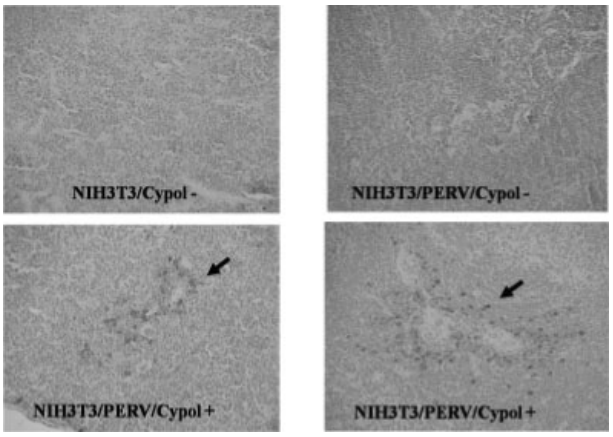

Figure for P-23.2.

Groups	Organs	PCR (gDNA)	RT-PCR (RNA)	No. PERV detected mice	Groups	Organs	PCR (gDNA)	RT-PCR (RNA)	No. PERV detected mice
	Brain	0/5	0/5			Brain	1/5	0/5	
	Liver	0/5	0/5			Liver	2/5	1/5	
	Lung	0/5	0/5	0/5		Lung	0/5	0/5	2/5
	Heart	0/5	0/5			Heart	0/5	0/5	
	Kidney	0/5	0/5			Kidney	0/5	0/5	
	Spleen	0/5	0/5			Spleen	0/5	0/5	
	Brain	0/5	0/5			Brain	0/5	0/5	
	Liver	0/5	0/5			Liver	0/5	0/5	
	Lung	0/5	0/5	0/5		Lung	0/5	0/5	0/5
	Heart	0/5	0/5			Heart	0/5	0/5	
	Kidney	0/5	0/5			Kidney	0/5	0/5	
	Spleen	0/5	0/5			Spleen	0/5	0/5	
	Brain	0/5	1/5			Brain	0/5	0/5	
	Liver	2/5	1/5			Liver	0/5	0/5	
	Lung	1/5	2/5	4/5		Lung	0/5	0/5	0/5
	Heart	1/5	1/5			Heart	0/5	0/5	
	Kidney	3/5	2/5			Kidney	0/5	0/5	
	Spleen	3/5	1/5			Spleen	0/5	0/5	
	Brain	1/6	1/6			Brain	0/5	0/5	
	Liver	1/6	1/6			Liver	1/5	0/5	
	Lung	0/6	2/6	5/6		Lung	0/5	0/5	2/5
	Heart	2/6	2/6			Heart	1/5	0/5	
	Kidney	4/6	1/6			Kidney	0/5	0/5	
	Spleen	4/6	2/6			Spleen	1/5	1/5	
	Brain	0/5	1/5			Brain	0/4	0/4	
	Liver	1/5	0/5			Liver	1/4	0/4	
	Lung	0/5	0/5	2/5		Lung	0/4	1/4	3/4
	Heart	1/5	0/5			Heart	0/4	0/4	
	Kidney	0/5	0/5			Kidney	0/4	0/4	
	Spleen	1/5	1/5			Spleen	0/4	0/4	

Table for P-23.2.

In our mouse model system, the possibility of PERV transmission to human was identified, and there is a risk of PERV transmission in xenotransplantation using the organs of pig. If the pig used for the organs source of xenotransplantation to human, PERV will be important barrier and it have to remove the PERV from the pig's genome.

P-23.3

Assessment of the potential risk of infection associated with *clostridium difficile* from porcine xenografts

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Background: There are numerous concerns over the potential for transfer of pathogens between species during clinical xenotransplantation, and although current clinical application is limited, porcine xenografts have been previously used to treat patients with severe burns. Donor animals providing the xenografts are sourced from a healthy commercial herd, however, as pigs are a known source of zoonotic agents, a number of diseases are required to be excluded from pigs used for xenotransplantation purposes. Many studies have indicated the relevance of viral zoonoses, however, little has been done with regard to the potential for transfer of non-viral pathogens related to healthcare associated infections.
Methods: *Clostridium difficile (C. difficile)* is a major cause of neonatal enteritis in pigs and an important feature of this organism is that pigs can be asymptomatic carriers. The aim of the study was to assess the prevalence and ribotype of *C. difficile* present in healthy donor animals, the antimicrobial susceptibility of any isolates and make a preliminary assessement of the potential risk for *C. difficile* infection to immune-suppressed patients receiving this treatment.
Results: Animals were found to have toxin B+ human ribotype 017 present in the faecal matter, however, no *C. difficile* was isolated from skin samples. In addition, the antimicrobial susceptibility of the *C. difficile* isolates was found to be similar to that identified for human isolates.
Conclusion: There is increasing evidence of porcine *C. difficile* genotypes which are indistinguishable from human isolates and the potential for cross species infection is deemed high. In addition, due to the sporogenic nature of *C. difficile*, there is a potential to survive antibiotic treatment of porcine xenografts prior to use.

However, this data would indicate that subsequent infections could effectively be treated with vancomycin or metronidazole. These findings suggest that screening of donor pigs should include examination for *C. difficile*.

P-23.4

Hepatitis E virus: a potential risk for xenotransplantation?

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Background: Hepatitis E Virus (HEV) has been found to be prevalent in many pig herds worldwide and has caused acute disease in humans in contrast to the mild symptoms observed in pigs. HEV infection carries significant morbidity and carries an adverse prognosis in pregnant women, in individuals with pre-existing chronic hepatitis and in immunocompromised patients including transplant recipients. The incidence of locally-acquired HEV infection is becoming increasingly recognised in the developed world and is thought to be due to zoonotic transmission from pigs to humans; either from close contact or from the consumption of undercooked meat. There is also evidence to suggest transmission of this virus via blood donors and allo-transplantation. As such, the prevalence of this exogenous virus in animals used for Xenotransplantation and the potential for transmission should be determined in order to remove the risk.
Methods: The HEV status of animals to be used for xenotransplantation purposes is unclear. To our knowledge, no epidemiological data is available regarding the HEV status of specific pathogen free (SPF) pigs in the UK and Europe as routine testing for this virus is not widely implemented. The aim of this study was to assess the prevalence of HEV in pig herds to be used for xenotransplantation and identify potential modes of transmission.
Results: HEV sero-prevalence was analysed in pigs from SPF (n = 3), barrier (n = 53) and commercial (n = 33) sources. Animals were aged from 2 to 8 months. Sero-prevalence ranged from 33% in animals kept under SPF conditions to 70% in commercial animals. Animals were also indentified that were positive for HEV RNA and sequencing confirmed the virus as genotype 3. In addition, baboons that had received xenografts from HEV positive pigs are being examined for the presence of viral RNA post transplant.
Conclusion: HEV is seroprevalent in herds used for xenotransplantation, this data suggests that monitoring of herds for HEV should be a requirement to prevent the risk of transmission to human recipients.

P-23.5

Suppressor of cytokine signaling transcription in human cytomegalovirus-infected porcine endothelial cells

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Background: Xenograft damage is partly caused by the action of strong pro-inflammatory mediators such as IFN-γ and IL-6 which are released during graft rejection and infection. Negative regulation of inflammation by suppressors of cytokine signaling proteins (SOCS) 1 and 3, two important regulators of IFN-γ and IL-6, might be crucial for graft survival and function. The aim of this study was to analyze the expression of SOCS in porcine endothelial cells (pEC).
Methods: Due to the high nucleotide homology between porcine and human SOCS, different primers and probes were evaluated for their species specificity. SOCS mRNA expression was then quantified by RT PCR in porcine endothelial cells devoid of alpha-Galactosidase (PAEC-KO) after human cytomegalovirus (HCMV) infection and after coculture with human PBMC. For infection, both HCMV strain AD169 and TB40/E were used in a MOI of 1. Cells were harvested 1 and 2 days after infection.
Results: By choosing primer and probes in regions of discrepancy between the porcine and human sequences we were able to specifically detect and quantify porcine SOCS mRNA. SOCS1, but not SOCS3 expression, was strongly upregulated in HCMV infected PAEC-KO. In contrast, coculture with human PBMC did not significantly alter SOCS1 and SOCS3 mRNA expression levels in PAEC-KO.
Conclusions: SOCS1 and three transcription was demonstrated in porcine endothelial cells and was, at least in part, differentially regulated during infection and xenogeneic endothelial cell activation. Therefore, further understanding of SOCS mediated control of inflammatory responses might allow to develop novel approaches to reduce xenograft damage.

Immunobiology and Tolerance

P-24.1

Isolation of human CD4+ lymphocytes from spleens of pigs with minimal chimerism

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Background: In previous experiments, tolerance with prolonged xenograft survival was achieved by growing lymphocytes from the recipient within fetal donor pigs. The splenocytes from the chimeric pigs were infused into the recipient. This procedure was effective for pig heart xenografts in sheep with modest post-transplant immune suppression and pig islet tissue in monkeys with no immune suppression. The chimeric lymphocytes are

CD4+CD25+foxP3+ T regulatory cells. However, the transfusion of unfractionated splenocytes had drawbacks. The pig cells often caused a transfusion reaction and could sensitize the recipient. With minimal chimerism, the number of T regulatory cells may be insufficient for tolerance. These problems could be overcome by isolating the chimeric recipient cells and expanding T regulatory cells in culture with human interleukin 2 (rhuIL2).
Methods: Human marrow was infused into fetal pigs as described previously. Chimerism was assessed with PCR and flow cytometry. Spleens were procured from three pigs with minimal chimerism (PCR+, undetectable human CD45+ PBL by flow cytometry). A suspension of splenocytes was produced. The lymphocytes were incubated with mouse anti-human CD4 and CD45 antibodies. Human CD45 and CD4 positive lymphocytes were enriched by positive selection using ferretin labeled rat anti-mouse microbeads and Miltenyi LS columns. The enriched cells were assessed for human CD45+ and CD4+ lymphocytes by flow cytometry. This suspension was then sorted with the Becton–Dickenson FACS Aria II cell sorter for human CD4 and CD45. The methodology was developed with normal pigs spiked with human PBL.
Results: Similar to the peripheral blood, human chimerism in the isolated splenocytes was minimal or limited, with 0.2%, 0.08%, and undetectable chimerism. After positive selection with Miltenyi columns, human lymphocytes were enriched to 25%, 12%, and 0.13% respectively. After cell sorting, 3x10^4 to 1.3x10^6 CD4+CD45+ human lymphocytes were isolated. Reanalysis of one preparation demonstrated 99.4% human CD45+CD4+ lymphocytes.
Conclusions: Using a combination of positive selection and fluorescence cell sorting, relatively pure human CD4+ lymphocytes can be produced from pigs with minimal chimerism. This should greatly reduce the risk of transfusion reaction and sensitization. The purified suspensions could be expanded by culture with rhuIL2.

P-24.2

Porcine MIC2 does not trigger human NKG2D-dependent cytotoxicity

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Background: Results of pig to primate transplantation using genetically modified pig organ donors suggest that hyperacute rejection can be overcome, but the cellular immune response remains problematic. NK cell-mediated mechanisms are an important component of the human anti-pig cellular response. Activating receptors responsible for triggering human NK cytotoxicity against pig targets include NKG2D. pMIC2 and pULBP1 have been identified as porcine homologues of the human MIC and ULBP families of NKG2D ligands. pULBP1 is a functional ligand for human NKG2D. However, NKG2D-dependent cytotoxicity persists after cleavage of pULBP1, strongly suggesting the presence of at least one additional ligand. The most obvious candidate is pMIC2.
Aims and Methods: The aim of this project was to evaluate the ability of pMIC2 to trigger killing by human NK cells. Because full-length transcripts for pMIC2 have not been cloned, we created a chimeric molecule between the extracellular portion of pMIC2 and the transmembrane and intracellular tail of human MICA, fused to GFP. We examined the killing of p815 target cells expressing either MICA-GFP, pMIC2-GFP or GFP alone by the NKG2D-expressing NKL cell line. Untransfected p815 cells are not susceptible to killing by NKL. Expression of pMIC2 was determined using a polyclonal rabbit Ab we generated against pMIC2. Binding of NKG2D to transfectants was assessed using an Ig-fusion protein of HuNKG2D (NKG2D-Fc).
Results: p815 transfected with GFP alone did not express pMIC2, MICA, or any other ligand able to bind to human NKG2D-Fc. For both MICA-GFP and pMIC2-GFP, the level of GFP expression correlated with the level of pig NKG2D ligand expression. However, whilst there was a clear correlation between expression of MICA and binding of human NKG2D-Fc, only cells with the highest expression of pMIC2 bound NKG2D-Fc. Cytotoxicity assay clearly demonstrated killing of p815 cells expressing MICA, but not untransfected p815 cells or those expressing GFP alone. Cell populations with a broad range of pMIC2 expression did not trigger NKL cell-mediated killing.
Conclusions: Some binding of NKG2D to cells expressing high levels of pMIC2 was demonstrated, but pMIC2 expression did not promote NKG2D-dependent killing of p815 targets. pMIC2 is unlikely to be the additional ligand responsible for the persistence of killing after enzymatic cleavage of pULBP1 from target cells.

P-24.3

Successful induction of mixed chimerism and tolerance with non-myeloablative conditioning in mice presensitized with fully MHC-mismatched skin grafts

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Many patients do not qualify for organ transplantation because they have been presensitized to HLA antigens, and are likely to have inferior graft outcomes if transplanted. The ability to induce donor-specific tolerance in presensitized recipients would broaden the pool of eligible transplant recipients. While lethal irradiation has been shown to allow engraftment of high doses of allogeneic bone marrow in presensitized mice, such regimens would be neither appropriate nor effective in the clinical setting. We used presensitized B cell-deficient μMT B6 mice to assess the ability of non-myeloablative bone marrow transplantation [BMT] regimens to tolerize presensitized T cells. Twelve or 40 weeks after skin grafting either μMT B6 [H-2b] or wild-type B6 [H-2b] mice, respectively, with fully MHC-disparate B10.A [H-2a] tail skin, we administered anti-OX40L, anti-CD40L, anti-CD4, anti-CD8, and anti-NK1.1 monoclonal antibodies [mAbs] along with low dose [3Gy] total body irradiation [TBI] and high-dose BMT. Mixed chimerism and donor-specific skin graft tolerance were achieved in both groups. We further refined this regimen to include only CD4 and CD8 T cell-depleting mAbs and anti-CD40L along with 3Gy TBI and high-dose BMT. This simplified regimen established multilineage mixed chimerism in 8 of 8 presensitized μMT B6 mice, and donor-specific skin graft tolerance in seven of seven chimeras, while third party skin grafts were rejected at a median of 12 days. The full regimen also permitted successful allogeneic marrow engraftment in 4 of 10 presensitized wild-type mice. Our results indicate that mixed chimerism and tolerance can be established in presensitized hosts without the use of high dose TBI.