LOS013 VASCULARIZED HUMAN FINGER DECELLULARIZATION: A SUBUNIT APPROACH TO HAND TISSUE ENGINEERING

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Background: Hand allotransplantation breakthrough is still highly limited by the need for immunosuppression. From our previous results in the porcine ear and human face tissue engineering, we hypothesized an application to human finger and hand scaffold production.
Methods: Nine long-digit grafts were procured postmortem from four fresh human donors, and decellularized by sequential arterial perfusions of detergent/polar solvent solutions. Cellular clearance was assessed by DNA quantification and H&E staining. Extracellular matrix preservation was assessed by Masson's Trichrome (MT) for soft tissues and bone mineral density (BMD) for phalangeal bones. Acellular samples were cultured with fibroblastic cell lines, and examined with vital staining. To challenge *in vivo* the vasculature, a short reperfusion study was performed in a pig recipient. Finally, decellularization was applied to a whole human hand, and vascular tree assessed by angio-CT scan.
Results: Digital grafts were successfully decellularized, with a quick epidermolysis, nail loss and complete bleaching. Cell clearance was demonstrated on H&E sections, associated to 95.3% DNA reduction, compared to native tissues ($p < 0.0001$). MT staining showed microscopic structural preservation. Decellularized phalanx mean BMD was 510 mg hydroxyapatite/cm3, similar to control. Seeded cells were viable and homogeneously distributed on all scaffolds. *In vivo*, blood quickly reperfused the whole scaffold; after 3 h, the vascular tree was still patent, as demonstrated by fluoroscopy, fingertip pulse and 93% oxygen saturation. The extension to a whole-hand graft decellularization was achieved successfully, with an excellent preservation of the superficial and deep vascular tree.
Conclusion: We could produce finger and hand extracellular matrix scaffolds from human cadaveric source, with a preserved and perfusable vascular tree. These results could represent a true alternative to upper extremity allotransplantation.

LOS014 COMBINATION OF IL2/ANTI-IL2 COMPLEXES AND ANTI-INFLAMMATORY THERAPY FOR TOLERANCE INDUCTION

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Introduction: Interleukin-2 (IL2) complexed with a specific antibody against IL2 (IL2cplx) rapidly expands and activates Tregs *in vivo*. Treatment with IL2cplx has shown potency in inducing tolerance towards islet (but not skin) allografts. Here we investigated the potency of IL2cplx based therapy to prolong skin allograft survival and the mechanisms of tolerance.
Methods: Mice received fully or MHC mismatched skingrafts, tolerogenic therapy with IL2cplx and mTOR inhibition (0.5 mg IL-2/2.5 mg JES6-1; 1 mg/kg rapamycin; i.p.,3x/week 4 weeks) and short-term treatment with anti-IL6. Mechanisms of tolerance were investigated by analysis of donor-specific antibodies, flow-cytometric analysis and MLRs. Groups of mice were challenged with a second skingraft to test for infectious tolerance and for memory responses.
Results: We could show that combination of IL2cplx, rapamycin and anti-IL6 significantly prolongs survival of fully mismatched skingrafts (MST = 76 days, $p < 0.0001$) and leads to prevention of acute rejection, even after complete stop of treatment at d29. Importantly, the absence of minor antigens led to graft survival >100 days. Analysis of sera revealed complete absence of donor-specific antibodies ($p < 0.01$) and kinetics of rejection of a second graft suggested additional absence of Tmem response. Flow cytometric analysis of the graft indicates increased frequencies of intragraft Tregs and active regulatory mechanisms. Graft survival was demonstrated to be critically dependent on CD4 + CD25 + Tregs.
Discussion: We could show that Treg expansion via IL2cplx synergizes with rapamycin and anti-IL6, leading to significantly prolonged skin allograft survival and prevention of acute rejection even in the absence of ongoing treatment. Further experiments to uncover the underlying mechanisms are under way. We think these results will have significant impact on the development of new protocols for tolerance induction.

LOS015 DONOR SPECIFIC AND NON-DONOR SPECIFIC HLA ANTIBODIES AND LONG-TERM OUTCOME POST LUNG TRANSPLANTATION

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Donor specific antibodies (DSA) against HLA are associated with chronic lung allograft dysfunction (CLAD) and mortality post lung transplantation, but data concerning prevalence, time of onset, persistence and effects on long-term outcome remain scarce.

We assessed the association between HLA antibodies and CLAD-free and graft survival in a cohort of 362 patients undergoing lung transplantation between 2010 and 2015. We stratified our analysis according to DSA status, persistence of antibodies and timing of antibodies (pre-transplant, early or late post-transplant).

Within our cohort, 61 (17%) patients ever developed DSA (mostly against HLA-DQ), which was associated with worse CLAD-free survival ($p < 0.0001$) and a trend for worse graft survival ($p = 0.059$). Persistent ($p < 0.0001$ HR 3.386 CI 1.928–5.948) as well as transient ($p = 0.0045$; HR 2.998 CI 1.406–6.393) DSA were associated with shorter CLAD-free survival compared to patients without DSA. Persistent DSAs ($p = 0.0005$; HR 3.071 CI 1.632–5.778), but not transient DSA were negatively associated with graft survival compared to patients without DSA, likely due to the higher incidence of restrictive CLAD in the persistent DSA group. Non-DSA HLA antibodies and pre-transplant HLA antibodies had no effect on post-transplant outcome.

We demonstrated an important difference in prognosis between persistent and transient DSA. Moreover, the observed association between DSA

LOS016 HYBRID PSEUDOISLETS GENERATED FROM ISLET CELLS AND AMNIOTIC EPITHELIAL CELLS REVERSE DIABETES AFTER MARGINAL MASS TRANSPLANTATION IN A MURINE MODEL

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Aims: Inflammatory phenomena and delayed revascularization remain a significant obstacle to the success of clinical islet transplantation. Human amniotic epithelial cells (hAECs) exhibit anti-inflammatory, immunomodulatory and regenerative properties, and are widely available. In this study we have engineered functional hybrid pseudo-islets (HPI) composed of dissociated islet cells and hAECs and explored whether they can protect islet cells from ischemic and inflammatory damage and improve their function and engraftment
Methods: Rat pancreatic islets were dispersed into single cells for the generation of pseudo-islets (PI), consisting in 500 islet cells. HPIs were generated from 500 islet cells mixed with 500 hAECs on microwell platforms. PIs and HPIs were assessed for viability and function *in vitro*. *In vivo* function was assessed by transplantation into STZ-diabetic scid mice
Results: Significant enhancement of insulin/glucagon expression and of glucose-stimulated insulin secretion were observed from HPIs as compared to PIs. Transplantation of marginal mass HPIs, corresponding in size to 150 IEQs, under the kidney capsule of diabetic mice normalized blood glucose levels within 3–7 days. In contrast, transplantation of the same mass of PIs or native islets was unable to reverse diabetes. Removal of the islet graft-bearing kidney led to recurrence of hyperglycemia. HPIs were morphologically intact and expressed insulin and glucagon up to 4 months post-transplant. A two-fold increase in graft revascularization, assessed by CD34 staining, was seen in HPIs, compared to PIs and native islets. IPGTT at 1.5 and 3 months post-transplant were identical to non-diabetic controls
Conclusion: This data indicates that hAECs have a significant cytoprotective effect that enhances islet engraftment. hAEC-enriched pseudoislets may represent a novel approach to increase the success rate of islet transplantation and a first step towards the development of a bioartificial pancreas.

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