novel gm porcine hearts and new medical treatments into NHP transplant. Hemoxygenase-1 and A20 decrease apoptotic and inflammatory reactions.

**Methods:** A biventricular working cardiovascular system was designed to perfuse both wild-type (wt) and quintuple gm (hemoxygenase-1/A20 plus hCD46, hCD55, hCD59) porcine hearts ex vivo; all hearts were (i) explanted with Bretschneider solution for cardioplegia, (ii) subjected to a cold ischemia for 150 minutes, (iii) reperfused with heparinized, diluted freshly drawn human blood. A 15 minutes reperfusion was performed before functional testing in a biventricular working heart mode. Cardiac function was investigated and necropsy performed after 180 minutes of perfusion.

**Results:** After 150 minutes of perfusion cardiac index (6.8 ±3.9 vs. 1.7 ±2.3 p = 0.04), stroke volume index (0.054 ±0.027 vs. 0.018 ±0.021; p = 0.01) and coronary perfusion index (3.7 ±1.5 vs.1.4 ±1.0; p =0.02) improved compared to wt. Cardiac weights were obtained both before and after perfusion. Gm hearts developed less myocardial edema (28 ±21 vs. 62 ±42 p; 0.03). Histology revealed less hemorrhage and structural damage in the gm hearts.

**Conclusion:** The tested genetic modifications significantly reduced acute xenoreactions. If combined with GFTA1-KO and h-thrombomodulin expression they are a promising basis to be used in future preclinical studies with non-human primates.

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**Mitochondrial Proteins Possess Antigenicity and Can Cause Rejection**

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**Purpose:** Recently, the generation of human embryonic stem cells by somatic nuclear transfer into an enucleated oocyte (NT-ESCs) has been achieved. It has been envisioned that autologous ESCs can thus be generated. However, we challenged this assumption and hypothesized that proteins coded from mismatched, allogeneic, oocyte donor-derived mitochondrial DNA (mtDNA) may induce alloimmunity and render NT-ESC derivatives amenable for rejection.

**Methods:** NT-ESCs were generated by transferring BALB/c (MHC H2d) fibroblast nuclei into enucleated (C57BL/6 × DBA/2)F1 oocytes. NT-ESC transplantation studies were then conducted across defined immunological barriers.

**Results:** mtDNA sequence analysis comparing C57BL/6 mitochondria of the NT-ESCs with BALB/c mitochondria revealed 2 non-synonymous SNPs within the mt-Co3 and mt-Cytb genes. Immune activation was substantial in isolated MHC-mismatched C57BL/6 recipients (MHC H2b), which was significantly stronger than those in isolated mitochondria-mismatched BALB/c or isolated minor histocompatibility antigen (MHA)-mismatched BDF1 (MHC H2b/d). Transplantation into T cell-deficient BALB/c nude mice showed no increase in NK cell-driven IFNγ Elispot frequency, excluding relevant innate rejection. Medawar experiments showed that tolerance against allogeneic mitochondria can reliably be induced and was further proof for the relevance of the adaptive immune system in rejecting mitochondria-mismatched grafts. In vitro Elispot assays with splenocytes form NT-ESC-immunized BalbC animals showed IFNγ responses against isolated NT-ESC mitochondria, but not cytosol, confirming the antigen within the mitochondrial fraction. Vector-based overexpression of C57BL/6 mt-Co3 or mt-Cytb in either BalbC ESCs or BalbC endothelial cells (ECs) caused an IFNγ response in Elispot assays with splenocytes from BalbC animals sensitized with NT-ESCs or NT-ECs, respectively. This confirmed the antigenicity of these two specific proteins. Sensitization against C57BL/6 mt-Co3 or mt-Cytb could also be induced using fully allogeneic C57BL/6 splenocytes for these two specific proteins. Sensitization against C57BL/6 mt-Co3 or mt-Cytb could also be induced using fully allogeneic C57BL/6 splenocytes for these two specific proteins.

**Conclusion:** This study revealed that an isolated mismatch of the mitochondrial proteins Co3 or Cytb in otherwise DNA-identical cells was sufficient to initiate marked T helper cell activation and to cause cell graft rejection.

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**Allogeneic and Xenogeneic Immunity Is Potently Suppressed by Expanded Regulatory T Cells (Tregs) from Human Thymus**

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**Purpose:** Tregs modulate immune responses directed to allo- and xenogenic antigens and are a promising therapeutic tool in organ transplantation. Challenges include Treg isolation and expansion to clinically-relevant numbers while maintaining stability. Clinical feasibility could be improved by efficient Treg cryopreservation for future infusions or expansions. We previously showed that abundant CD25+ thymocytes can be isolated from discarded pediatric thymus and expanded to highly suppressive and stable Tregs. Here we studied whether thymic Tregs (tTregs) can be cryopreserved and the potential of expanded tTregs to suppress allogeneic and xenogeneic immune responses.

**Methods:** Thymocytes were obtained by mechanical dissociation of thymuses collected during pediatric cardiac surgery. CD25+ Tregs were isolated by magnetic-bead cell separation and directly expanded or stored in liquid nitrogen for >3 months, thawed and expanded with anti-CD3, IL-2, rapamycin and artificial antigen-presenting cells. Treg characteristics were assessed by analyzing phenotype and suppressive capacity of proliferation of anti-CD3/28-stimulated T cells, T cells stimulated with irradiated allogeneic monocyte-derived dendritic cells, or peripheral blood mononuclear cells (PBMC) stimulated with irradiated neonatal porcine islets.

**Results:** The expansion capacity of cryopreserved tTregs was comparable to that of fresh tTregs (range: 11-36-fold expansion). Both expanded fresh and cryopreserved tTregs maintained high FOXP3 expression, contained few CD25+CD4+ Tfh cells recognized as an important cause of allograft dysfunction. We hypothesize that blockade of the CD28-CD80/86 co-stimulatory pathway hampers B cell differentiation into antibody producing plasma cells, and play a pivotal role in the generation of efficient antibody responses affected by cryopreservation. Expanded tTregs potentely suppressed allogeneic and xenogeneic-stimulated responder cells. These results indicate that discarded human thymus is an excellent source of abundant Tregs for cellular therapy in both allo- and xenotransplantation.

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**Targeting Human Follicular T-B Cell Interactions to Prevent Antibody Mediated Allo-Immune Responses**

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**Purpose:** Antibody-mediated rejection (ABMR) has been increasingly recognized as an important cause of allograft dysfunction. We hypothesize that FcαR-IgG complexes are crucial in ABMR. Germinal center CD4+CXCR5+PD1+ICOS+B6c/T follicular helper (Tfh)-cells activate B cells, and play a pivotal role in the generation of efficient antibody responses through costimulatory molecules and cytokines. Here, we investigated whether blockade of the CD28-CD80/86 co-stimulatory pathway and the IL-21 signaling pathway hampers B cell differentiation into antibody producing plasmablasts.

**Methods:** Fcs sorted T and B cells from human donor-derived spleens were co-cultured in a mixed lymphocyte reaction (MLR), mimicking lymphoid organ germinal center reactions in the absence and presence of an IL-21 receptor antagonist (anti-IL-21R) (5,10, 20 ng/ml), and belatacept targeting the CD28-CD80/86 (10 ng/ml). Proliferation and differentiation of T and B cell subsets were analyzed after 7 days using flow cytometry.

**Results:** CD4 T cell proliferation was not inhibited by anti-IL21R, while belatacept had a significant effect (3.7 fold reduction, p = 0.01). Furthermore, only in the presence of belatacept, a reduced number of alloactivated Tfh cells