



Oral Abstract #1

Title: A microfluidic bile-duct-on-a-chip platform for studying biliary epithelium in a dish

Abstract Topic: Translational: human in vitro and ex vivo

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Abstract Body: Background

Post-transplant cholangiopathies are much feared complications after liver transplantations as they are difficult to treat and can result in loss of graft function. These complications can arise when regeneration of damaged biliary epithelium is impaired. There are currently no accurate in vitro models for studying the effects of (prolonged) ischemia times and reperfusion injury on the integrity of the biliary epithelium. Three-dimensional intrahepatic cholangiocyte organoids (ICO) do allow for the in vitro expansion of cholangiocytes. However, the lumen of the ICO, representing the luminal side of bile ducts, can only be assessed after disrupting the three-dimensional organoid structure. Therefore, we aimed to establish a micro physiological bile-duct-on-chip (BDOC) to study biliary epithelium in vitro.

Methods

Four-channel BDOC (dimensions; L:1cm, WH:500µm) were prepared using polydimethylsiloxane (PDMS). All channels were filled with collagen type-I augmented human-derived liver extracellular matrix (ECM). Viscous finger patterning was used to create a lumen (Φ:300µm) inside the hydrogel. ICO were initiated from human donor liver biopsies (n=10) and seeded in the channels (105cells/channel). Cells were expanded for up to 21 days at 37 °C. Integrity of the biliary epithelium was monitored using confocal microscopy and histology. Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) was used to study protein turnover by the cells.

Results

Within the 21-day period the lumen of the BDOC was populated with columnar cholangiocytes expressing cholangiocyte markers cytokeratin-7 and 19. Zonula occludens-1 staining revealed that the cells were polarized like biliary epithelium. After 21 days, the cell layer formed an impermeable barrier against 70kDa FITC-Dextran. Sialylated carbohydrates were detected in the intact glycocalyx, and sialic acid residues could be enzymatically removed. Interestingly, the cells were able to restore the glycocalyx within 24-hours as shown by intact apical sialic acid staining. Moreover, SILAC

revealed that the cells had a different proteomic profile when compared to controls. In the BDOC, mostly collagens were produced as opposed to laminins, which were most abundantly produced in the controls.

Conclusion

The microfluidic BDOC platform allows for the study of healthy biliary epithelium in vitro, as the organoids self-organized in polarized cholangiocyte-like barriers. Moreover, SILAC results showed that the BDOC platform can be used to study complex cell-matrix interactions, which also play a key role in the development of intrahepatic post-transplant cholangiopathies.

Abstract Keywords

Post-transplant cholangiopathies, Bile duct on chip, microfluidic, cholangiocyte, organoid, Liver transplantation, translational

Oral Abstract #2

Title: High-resolution spatial transcriptomics of antibody mediated kidney transplant rejection uncovers topologically and functionally distinct inflammatory signatures.

Abstract Topic: Data/Science

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Abstract Body: Background

While spatial transcriptomic analysis of kidney transplant biopsies has the potential to provide novel insights into mechanisms of human allograft injury, inadequate tissue resolution and limited numbers of detectable genes prevent in-depth analysis for biomarker discovery and therapeutic development.

Methods

We used 10X Visium HD capable of subcellular resolution at 2 microns to perform spatial transcriptomics on kidney allografts and normal kidneys from fixed paraffin-embedded biopsies. Samples were sequenced and processed using 10X spaceranger for cell segmentation and gene quantification. Downstream cell type deconvolution and spatial analysis were carried out using R package Seurat and Giotto. Loupe Browser was used for tissue inspection and visualization.

Results

Initial analyses of a case of antibody-mediated rejection (AMR) confirmed sub-single cell resolution, identified > 35,000 individual cells, and > 18,000



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unique genes. Bioinformatics focused on identifying spatially distinct cell niches and their gene profiles to decipher the heterogeneity of tissue injury at a level of resolution not previously achieved. Compared with control, normal native (non-transplanted) kidney, the analysis of the allograft tissue with AMR uncovered two spatially and transcriptionally distinct B cell rich niches. One B cell niche, B-cell 1, was interspersed throughout the parenchyma and showed upregulation of B cell and antibody genes including IGHD, IGHA1, IGKC and showed overexpression of complement component C7. A second, spatially distinct B cell rich niche, B-cell 2, contained macrophages, with over-representation analyses indicating upregulation of distinct pathways including antigen processing and presentation, immune cell activation, phagocytosis, and TNF family genes signaling. We separately uncovered a spatially and transcriptionally distinct macrophage-rich niche, innate-immunity/macrophage, with pathway analyses showing enrichment for innate immune activation, including ERK1/2 cascade and chemotaxis. Relatively few CD4 and CD8 T cells and NK cells were detected within the biopsy; no distinct T cell or NK cell niche was identified. Studies of additional transplanted kidney biopsies are ongoing.

Conclusion

Our analyses newly identify the spatial and transcriptomic heterogeneity of a transplanted kidney with AMR at high resolution. The recognition that AMR involves distinct macrophage and B cell inflammatory niches associated specific transcriptomic profiles highlights the complexity of the AMR process and has implications for therapeutic interventions to optimize treatment capable of improving patient outcomes.

Abstract Keywords

kidney transplantation; kidney biopsy; spatial transcriptomics; antibody-mediated rejection

Oral Abstract #3

Title: UNOS-Based Risk Stratification of Extended Criteria Donors for HCC Recurrence Following Liver Transplantation: A Machine Learning-Enhanced Analysis

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Extended criteria donor (ECD) liver utilization remains controversial in hepatocellular carcinoma (HCC) recipients due to concerns about oncologic outcomes. Current allocation policies rely on inconsistent ECD definitions, limiting evidence-based decision-making. We evaluated the association between comprehensive donor quality metrics and HCC recurrence-free survival (RFS) using an evidence-based ECD classification system.

Methods

We conducted a retrospective cohort study of 21,000 adult HCC liver transplant recipients from the UNOS database (2010-2020). ECD status was defined using an 8-criteria composite model based on literature review: donor age >65 years, macrovesicular steatosis >30%, cold ischemia time (CIT) >12 hours, split liver graft, donor BMI >30 kg/m², hepatitis B/C positivity, or donation after circulatory death (DCD). Primary analysis compared RFS between ECD and non-ECD recipients, adjusting for recipient, tumor, and center-level factors. Secondary analyses examined individual ECD criteria effects, ECD criteria count stratification, and propensity score matching (1:1, caliper width 0.2 SD). A CatBoost model was applied to optimize RFS prediction using high-dimensional clinical variables.

Results

Of 21,000 recipients, 5,932 (28.2%) received ECD grafts and 14,547 (69.3%) received non-ECD grafts. ECD recipients were older (59.8 vs. 58.3 years), had higher MELD scores (median 16 vs. 15), and received grafts from older donors (51.7 vs. 38.2 years). Most common ECD criteria were donor age >65 years (40.0%), high BMI >30 kg/m² (30.0%), and steatosis >30% (20.0%). Median follow-up was 4.2 years. Five-year RFS was significantly lower in ECD recipients (67.8% vs. 72.4%, log-rank $p < 0.001$). In multivariable analysis, ECD utilization increased recurrence risk (adjusted HR 1.23, $p < 0.001$). However, individual criteria analysis revealed heterogeneous effects: CIT >12 hours (HR 1.42, $p < 0.001$), severe steatosis (HR 1.31, $p < 0.001$), and DCD (HR 1.28, $p = 0.004$) were significant, while donor age >65 years alone showed no independent association. ECD criteria count analysis demonstrated dose-response relationship: 1 criterion (HR 1.18), 2 criteria (HR 1.35), ≥ 3 criteria (HR 1.67), all $p < 0.001$. Propensity score matching ($n = 4,896$ pairs) confirmed primary findings (matched HR 1.21, $p < 0.001$). CatBoost model demonstrated an AUC of 0.78 for predicting five-year RFS, supporting its potential for personalized donor-recipient matching.

Conclusion

While ECD liver grafts modestly increase HCC recurrence risk through a dose-response relationship, this association is primarily driven by criteria such as CIT, hepatic steatosis, and DCD status, rather than age alone. These findings support development of nuanced, criteria-specific donor selection algorithms that could expand transplant access while maintaining acceptable oncologic outcomes.

Abstract Keywords

Liver transplantation Hepatocellular carcinoma (HCC) Extended criteria donor (ECD) Recurrence UNOS



Oral Abstract #4

Title: Immunosuppression Personalization with Immunobiogram

Abstract Topic: Clinical studies in humans

Author(s)

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Abstract Body: Background

Study's purpose: to evaluate whether in vitro response to immunosuppressive drugs (IMS) measured with Immunobiogram (IMBG) pre-transplant predicts first-year rejection or infection in kidney transplant (KT) patients.

Methods

IMBG examines isolated and stimulated PBMCs in 3D-cell cultures integrated with individual immunosuppression (IMS) concentration gradients along channels of a plate. PBMC evaluation of activation/proliferation determines the efficacy and pharmacodynamics of mycophenolate, tacrolimus, sirolimus, everolimus, and corticosteroids. Result interpretation translates into patient-specific dose-response curves and calculated negative or positive Z-Scores indicating individual IMS sensitivity related to a reference population. Patients received pretransplant IMBG testing and were followed for 12 months and evaluated for either BPAR or infection events. Patients with BPAR or Infections prior to 3 months post-transplant were excluded in order to evaluate outcomes related to baseline IMS from 3-12 months.

Results

Patients receiving Pre-TX Immunobiogram (n=107) were evaluated for increased or decreased sensitivity to baseline IMS. Patients without early rejection and IMS baseline treatment data at 3 months (n=91) were evaluated for BPAR events associated with IMS. BPAR events after 3 months (n=14) only occurred in patients with an average negative IMBG score <-0.187 (n=65), and all patients with an average negative Z-score above 0.187 were free of Rejection (n=21). Patients without early infection and IMS baseline treatment data at 3 months (n=84) were evaluated for infection events associated with IMS. Infection events (n=18) only occurred in patients with an average positive IMBG score >.206 (n=63) and all patients with an average positive Z-score below .206 were free of infection events.

Conclusion

IMBG offers a unique approach to determining optimal personalized immunosuppression

Abstract Keywords

Immunosuppression

Oral Abstract #5

Title: Glycosphingolipid catabolism via GM2A limits the spectrum of CD8+ T cell alloreactivity

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

T cell sensitivity to antigen is a critical parameter in controlling T cell-mediated alloimmunity. However, the molecular mechanisms governing the strength of the T cell allorecognition are incompletely understood. Here, we identified the glycosphingolipid-catabolizing protein, GM2A, as a novel regulator of TCR expression that limits the sensitivity of CD8+ T cells to alloantigen and controls the magnitude of the allogeneic response.

Methods

To assess the role of Gm2a in alloimmunity we utilized a fully allogeneic BALB/c to WT vs. Gm2a^{-/-} skin graft model, or minor OVA antigen mismatch model. T cell proliferation and alloreactive precursor frequencies were calculated following an in vivo mixed lymphocyte reaction. TCR sensitivity was assessed via flow cytometry by stimulating WT vs. Gm2a^{-/-} CD8+ T cells with OVA antigen, or low-affinity variants thereof. The immunosequencing of alloreactive TCR repertoire was evaluated using the TCR-β immuneSEQ assay from Adaptive Biotechnologies. To assess the therapeutic potential of GM2A, CTV-labeled PBMCs were treated with human recombinant GM2A prior to in vitro activation.

Results

Using data from the CTOT-09 clinical trial in which patients were weaned from tacrolimus immunosuppression, we discovered that CD8+ T cells from kidney transplant recipients that remained stable off tacrolimus exhibited increased expression of GM2A transcript as compared to patients who went on to reject their allografts. To determine if a causal relationship existed between Gm2a expression and alloimmunity, skin transplantation in WT vs Gm2a^{-/-} animals was compared. Gm2a deficiency significantly accelerated skin graft rejection (p=0.0108). Using a minor mismatch skin graft model to investigate the role of Gm2a in regulating CD8+ T cell immunity, we found that Gm2a^{-/-} CD8+ T cells exhibited increased accumulation and accelerated allograft rejection vs WT (p< 0.05), illuminating a CD8+ T cell-intrinsic role for Gm2a. Analysis of an in vivo mixed-lymphocyte reaction revealed increased alloreactive CD8+ T cell proliferation (p=0.0315) and a



50% augmentation in the frequency of alloreactive precursors among Gm2a^{-/-} vs CD8⁺ T cells ($p=0.0397$). TCR sequencing analysis demonstrated that Gm2a deficiency increased the number and diversity of alloreactive CD8⁺ T cell clones. Mechanistically, Gm2a^{-/-} CD8⁺ T cells exhibited sustained TCR expression upon antigen encounter compared to WT cells ($p < 0.05$), conferring increased responsiveness to low-affinity and low-dose antigens ($p < 0.01$). Finally, treating PBMCs with exogenous GM2A reduced CD8⁺ T cell proliferation ($p < 0.05$) and reduced TCR expression ($p=0.0003$).

Conclusion

We show for the first time that GM2A limits the CD8 T cell spectrum of alloreactivity by reducing surface TCR expression and attenuating CD8⁺ T cell sensitivity to allogeneic antigen. This work illuminates the potential of GM2A targeting to mitigate allograft rejection.

Abstract Keywords

GM2A, TCR, CD8 T cells, activation threshold

Oral Abstract #6

Title: Butyrophilin-2A2 (BTN2A2) Modulates Murine and Human Immunity via Interacting with Lymphocyte-Expressed CD45

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

B7 costimulatory family member Butyrophilin 2A2 (BTN2A2) is a transmembrane glycoprotein that was previously shown to play a role in maternal fetal tolerance. It is predominantly expressed by antigen presenting cells and has putative immunoregulatory properties for T cells, but molecular mechanisms are unclear.

Methods

We used immunoblots to analyze TCR-initiated signaling intermediaries, and used co-immunoprecipitation studies, confocal microscopy, structural modeling-guided mutational analyses, and microscale thermophoresis, to assess BTN2A2 interactions with CD45. We tested effects of recombinant BTN2A2 murine and human T cell responses as well as on B cell responses using in vitro systems including flow cytometry and fluorospot readouts. We tested the in vivo effects of recombinant BTN2A2 in the murine nephrotoxic

serum nephritis (NTS) model and assessed the in vivo effects of BTN2A2 deficiency (knockout mice) using the NTS model and heterotopic heart transplantation.

Results

Our findings show that BTN2A2 directly interacts with CD45RO on T cells, resulting in CD45 retention within the immune synapse during TCR activation. Recombinant BTN2A2 increased murine CD4⁺Foxp3⁺ regulatory T cells (Treg) and reduced T helper 17 (Th17) cells in vitro through mechanisms dependent on CD45 phosphatase activity. Using Fluorospot assays, we further show that BTN2A2 inhibits human Th1 and Th2: BTN2A2 significantly reduced tuberculin-derived protein derivative-induced interferon gamma, and house dust mite-induced IL-5 secretion in PBMCs obtained from antigen sensitized subjects. Separate assays using murine and human cells indicated that BTN2A2 induces Treg from naïve CD4⁺ T cells in the absence of TGFbeta and promotes Treg survival. Addition of recombinant BTN2A2 also prevented differentiation of human B cells in vitro, significantly reducing the numbers of R848-induced, IgG-secreting plasmablasts vs control ($p < 0.05$). In vivo, BTN2A2 treatment reduced clinical expression of murine nephrotoxic serum nephritis ($p < 0.05$), a Th17- and antibody dependent disease model, and increased spleen cell Treg/Th17 ratios. Conversely, analyses of BTN2A2-deficient animals showed exacerbated disease in response to nephrotoxic serum, associated with reduced Treg/Th17 ratios ($p < 0.05$), and allogeneic hearts transplanted heterotopically into BTN2A2-deficient recipients underwent accelerated rejection despite costimulatory blockade.

Conclusion

Together, our studies newly identify that BTN2A2 interacts directly with CD45RO on T cells, and functions as an immunomodulator of effector and regulatory T cells, as well as B cells. In addition to deciphering a physiological role for BTN2A2, the results have important therapeutic implications for T and B cell dependent autoimmune diseases and transplant rejection.

Abstract Keywords

Transplant immunology T cells Treg B Cells Heart Transplant

Oral Abstract #7

Title: TCF-1⁺ progenitor-like T cells sustain localized effector responses that mediate allograft rejection

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Graft rejection remains a significant barrier to the life-long acceptance of transplanted organs. Alloreactive T cells drive the many effector mechanisms of acute and chronic graft injury, including alloantibody production. T cells that infiltrate allografts are propagated and function locally without the need to home back to secondary lymphoid tissues, however the underlying mechanisms remain elusive.

Methods

Here, we present data from kidney and heart transplantation models in mice and clinical transplantation identifying a unique TCF-1+ progenitor-like subpopulation of T cells among graft-infiltrating cells that we hypothesize sustain localized effector responses.

Results

Analysis of scRNA-seq data of polyclonal T cells that infiltrate BALB/c, (B6xBALB/c)F1.OVA or human kidney allografts revealed the presence of Tcf7+ cells among Cd4+ and Cd8+ cells with transcriptional profiles similar to those of exhausted-progenitor T cells (Tpex) described in chronic infections and anti-tumor immunity. Tcf7+ cells that co-express Pdcd1 (PD-1) and the transcription factor Tox formed a unique cluster among kidney-infiltrating cells in mice while in human allografts Tcf7+ cells co-expressing Tox and Pdcd1 clustered among tissue-resident-memory and terminally-differentiated-effector-memory T cells. Using flow cytometry, we confirmed the presence of alloreactive TCF-1+ progenitor-like T cells in BALB/c kidneys transplanted into B6 mice, in which slow and chronic graft rejection is mediated by polyclonal CD8 cells reacting to the donor H-2Kd. Among the antigen-experienced CD44+PD-1lo and CD44+PD-1hi populations of CD4 and CD8 cells there were distinct subpopulations with TCF-1 and TOX expression. Notably, we observed very few CD44+PD-1+ cells in spleens of graft rejecting mice where the majority of T cells were of a naïve or central memory phenotype. Within kidney allografts, antigen-experienced TCF1+PD-1lo and TCF1+ PD-1hi subpopulations of CD4 and CD8 had variable expression of CD127, CXCR6 and CD28. Importantly, a fraction of these were positive for Ki-67, indicating they are actively cycling within allografts. We observed similar findings in BALB/c and bm12 heart allografts transplanted into B6 recipient mice. To test the function of Tcf7/

Tcf7-expressing cells in allograft rejection, we deleted Tcf7 in B6 recipients (Tcf7fl/fl;CreERT2) of BALB/c kidney allografts 6wks post-transplant and analyzed the mice 4-6wks later. Inducible Tcf7 deletion was highly efficient in T cells isolated from spleens and allografts. We observed no differences in the numbers of total cells, CD4, or CD8 cells in the spleens between the controls (Tcf7+/+CreERT2) and Tcf7 deleted mice. In contrast, we found significantly fewer numbers of total cells, including CD4 and CD8 cells, in kidney allografts from Tcf7 deleted mice. Moreover, BrdU analysis showed there were significantly fewer proliferating T cells within allografts from mice with induced Tcf7 deletion compared to controls, while no BrdU+ T cells were detected in the spleens of either group.

Conclusion

Taken together, these findings strongly support the notion that graft-infiltrating TCF-1+ progenitor-like T cells are the source of locally-derived alloreactive effectors that mediate rejection and require TCF-1 expression for their function.

Abstract Keywords

TCF1, progenitor-like, T cells

Oral Abstract #8

Title: Donor Thymic Epithelial Cell Engraftment Engineers a Hybrid Thymus That Promotes Long-Term Transplant Acceptance

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Despite many advances, rejection remains a major limitation of transplantation, requiring lifelong immunosuppression with significant morbidity. Novel approaches to induce donor-specific tolerance are urgently needed. Hematopoietic stem cell transplantation is to date the most effective experimental approach to promote transplant acceptance. However, its application is hindered by the toxicity of recipient conditioning, the risk of graft-versus-host disease, and unpredictable efficacy in MHC-mismatched combinations. The therapeutic efficacy of this approach revolves around a profound alteration of the process of thymic selection of developing T cells. To achieve a similar outcome, we explored the hypothesis that direct



engraftment of donor thymic epithelial cells (TEC) would create a “hybrid thymus” capable of training T cells on donor MHC molecules, generating a tolerogenic repertoire.

Methods

To test “engraftability” and function, we developed a mouse model using MHC-II knockout (KO) B6 mice as recipients of donor (congenic or allogeneic) TEC injection. Since MHC-II KO mice cannot generate CD4+ T cells endogenously, any CD4+ T cell development indicates successful donor TEC engraftment and function. We characterized the thymic reconstitution, T cell repertoire development, and regulatory T cell (Treg) generation. Functional assessment included adoptive transfer of TEC-trained CD4+ T cells into RAG-/- recipients followed by donor skin grafting. Finally, we tested the therapeutic efficacy of a Hybrid Thymus by injecting MHC-mismatched donor TEC into wild-type B6 recipients with simultaneous skin transplantation under a short term costimulation blockade protocol.

Results

Donor TEC successfully engrafted and restored CD4+ T cell thymic development in MHC-II KO recipients. TEC promoted recent thymic emigrants and the formation of a peripheral pool with Treg : T conventional cell ratios comparable to wild-type controls. TEC-trained T cells demonstrated donor-specific hypo-responsiveness in mixed lymphocyte reactions. Critically, adoptive transfer of TEC-trained CD4+ T cells supported the survival of donor-matched skin grafts compared to controls. In wild-type recipients, donor TEC injection combined with transient costimulation blockade promoted survival of donor MHC matched skin grafts compared to controls, with 50% of recipients going long term, demonstrating therapeutic synergy.

Conclusion

This study demonstrates that donor TEC engraftment creates a functional hybrid thymus capable of reshaping T cell selection toward transplant tolerance. TEC-trained T cells exhibit donor-specific hypo-responsiveness while maintaining immune competence. The combination of donor TEC with minimal immunomodulation represents a promising strategy to achieve lasting transplant acceptance without chronic immunosuppression, offering proof of principle of an innovative approach for transplant medicine.

Abstract Keywords

Transplant Tolerance; Thymus; Thymic Epithelial Cells; Thymic Selection; T cells; Skin Transplant

Oral Abstract #9

Title: Inhibition of CD154:CD11b interactions using a novel nanotherapeutic improves allograft survival

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

It is now appreciated that CD154 and CD40 may be differentially effective as therapeutic targets in transplantation owing to the ability of CD154 to bind to a second receptor, CD11b. We previously reported that the combination of anti-CD40 and a specific CD154:CD11b blocker enhanced allograft survival compared to anti-CD40 alone.

Methods

In the current study, we utilized a novel nanoparticle-based approach to more effectively deliver CD154:CD11b blockade during transplantation. Donor-reactive Thy1.1+ CD4+ and CD8+ T cells were transferred into WT recipients of allogeneic skin grafts, which were then treated with either CTLA-4Ig or the combination of CTLA-4Ig plus a hyaluronic acid nanoparticle-conjugated CD154:CD11b peptide inhibitor (iPepHANP). Donor-reactive CD4+ and CD8+ T cell responses were interrogated using flow cytometry. The graft survival of CTLA-4Ig-treated vs. CTLA-4Ig plus iPepHANP-treated recipients was determined.

Results

Results indicated that iPepHANP synergized with CTLA-4Ig in prolonging allograft survival and inhibiting donor-reactive CD4+ and CD8+ T cell responses. Specifically, frequencies of donor-reactive CD4+ and CD8+ T cells in the spleen were significantly reduced in iPepHANP+CTLA-4Ig-treated animals as compared to animals treated with CTLA-4Ig alone (p=0.0070 and 0.0036, respectively). Moreover, iPepHANP+CTLA-4Ig administration significantly reduced donor-reactive CD4+ T cell (p=0.0325) and activated CD8+ T cell infiltration into skin allografts compared to CTLA-4Ig alone (p=0.0284). Notably, mice treated for 100 days with the CD154:CD11b blocking nanoparticle demonstrated sustained transplantation tolerance following secondary graft challenge in the absence of any further immunosuppression.

Conclusion

Taken together, these data demonstrate that the combination of CTLA-4Ig and iPepHANP inhibits allospecific T cell responses more effectively than CTLA-4Ig alone and can promote allograft tolerance. Further development of CD154:CD11b-blocking nanoparticles as a therapeutic strategy in transplantation may be warranted.

Abstract Keywords

CD154:CD11b blockade, nanoparticles, allograft tolerance



Oral Abstract #10

Title: Macrophage Recognition of Donor MHC I Tunes IL-27 Production to Shape Tissue Repair Following Heart Transplantation

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Tissue damage in ischemic grafts early after transplantation requires a rapid and coordinated repair response by infiltrating recipient monocytes. Yet, the local stimuli that direct monocyte and macrophage (MΦ) differentiation into reparative subsets remains poorly understood. Likewise, allografts develop fibrosis and vasculopathy despite potent immunosuppressants targeting T and B cells suggesting undefined mechanism leading to these pathologies. Recent studies established that monocyte recognition of donor MHC I via Paired Immunoglobulin Receptors (PIRs) directs their differentiation into allostimulatory dendritic cells after organ transplantation (Tx). This raises important questions around how local graft MHC I regulates recipient myeloid-mediated tissue repair following Tx. As such, we hypothesize that dysregulated MΦ repair of ischemic allografts results when recipient MΦs recognize donor MHC I.

Methods

Ischemic hearts from C57BL/6 (B6) syngeneic (syn), D8-KOD0 (B6; H2-Dd+, H2-Db-, H2-Kb-), and MHC I-deficient KOD0 (B6; H2-Db-, H2-Kb-) were transplanted into T, B, NK, and ILC deficient Rag2-/-γc-/- B6 recipients and analyzed by trichrome staining at POD40. Rag2-/-γc-/-, Pira-/- and Pirb-/- B6 MΦ differentiation was assessed after heart Tx and exposure to allogeneic (BALB/c; H-2d; allo) or syn donor cells using flow cytometry and scRNAseq. BMDMΦ differentiation in the presence of recombinant MHC I was assessed in vitro by flow cytometry.

Results

Absence of graft MHC I led to extensive fibrosis associated with recipient myeloid cell differentiation into iNOS+ MΦ. Surprisingly, Allo H2-Dd MHC I in

donor hearts displayed decreased infiltration and reduced fibrosis compared to MHC I-deficient and syn grafts (Fig. 1A). Assessment of BMDMΦs stimulated by self or non-self MHC I revealed a novel axis where self-MHC I stimulated release of IL-27 whereas non-self MHC I promoted its intracellular accumulation (Fig. 1B). Mechanistically, we show that this involved PIR-independent, but SHP1 dependent signaling (Fig. 1B). Modulation of MΦ-derived IL-27 protein was observed in donor grafts at early and late timepoints after HTx in B6 Rag2-/-γc-/- recipients (Fig. 1C) and IL-27 directly stimulated fibroblast proliferation.

Conclusion

These data identify an unappreciated mechanism where MHC I allorecognition drives MΦ differentiation and function. More importantly, sustained MΦ IL-27 production due to persisting non-self MHC I may become dysregulated and contribute to graft fibrosis over time.

Abstract Keywords

Macrophage, Innate allorecognition, Tissue Repair

Oral Abstract #11

Title: Alloimmunity Generated Injury Signals Control Local Chemokine Levels in Areas of Cardiac Allograft Vasculopathy

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Cardiac allograft vasculopathy (CAV) is a major factor limiting long-term survival after heart transplant (HTx). CAV is a progressive,



immunosuppressant-resistant disease leading to chronic rejection (CR) after HTx. Almost 50% of recipients of a HTx develop CAV after ten years, and CAV remains a leading cause of death after HTx. Unfortunately treating patients with T cell centric immunosuppressants to a level which cause infections and malignancy has failed to overcome CAV. The precise cellular mechanisms underlying CAV remains poorly understood. Histological evidence, however, suggests that CR is characterized by persistent immune cell infiltration, progressive fibrosis, and vasculopathy, ultimately leading to graft failure. Our purpose was to develop and understand the immunological mechanisms shaping CR to then develop interventions that move beyond conventional immunosuppression.

Methods

Long ischemia and repeated alloimmune responses are directly implicated in CR. Both will provoke extensive graft injury that releases local injury signals or damaged associated molecular patterns, such as interleukin 33 (IL-33). IL-33 supports tissue repair by recipient regulatory T cells (Tregs) and macrophages but also fuel alloreactive CD4+ T cells. To define how injury signals and alloimmune cells influence each other in areas of CAV, we utilized IL-33 as a model injury signal in a CD4+ T cell driven mouse HTx CR model. C57BL/6 mice (B6; H-2b) received allogeneic (allo) HTx from MHCII-mismatched Bm12 (H2-I-Abm12) donors that were IL-33+/+ or IL-33-/. B6 IL-33+/+ or IL-33-/- syngeneic (syn) HTx in B6 recipients were also completed. On day 14 post-HTx scRNA-seq analysis was performed on CD45+ cells isolated from the allograft, while spatial transcriptomics on day 30 post-HTx was performed to identify immune cells and active pathways in the microenvironment of an occluded vessel (il33^{+/+} or il33^{-/-} Bm12 hearts transplanted into B6 recipients) and healthy vessels (il33^{+/+} or il33^{-/-} B6 hearts transplanted into B6 recipients)

Results

Spatial transcriptomic analysis confirmed expected upregulation of T cell activation-related pathways, Th1 signaling, interferon-gamma activation, and antigen presentation pathways along with fibrotic pathways like atherosclerosis signaling in occluded vessels. scRNA-seq analysis of heart allografts infiltrating leukocytes demonstrated persistent T effector and T memory population. Ingenuity Pathway Analysis and differential gene expression on spatial data revealed that the absence of IL-33 led to downregulation of repair pathways, including Th2 and macrophage-reparative genes such as Arginase 1, while augmenting TCR signaling and macrophage activation pathways in allo occluded vessels. Further gene expression analysis revealed that numerous chemokines were elevated in allo-occluded vessels in response to IL-33, with Ccl8 showing the highest upregulation. Single-cell RNA-seq analysis and co-localization studies using immunofluorescence in HTx models of CAV identified macrophages as the predominant source of Ccl8. Ccl8, also known as monocyte chemoattractant protein-2, binds multiple leukocyte receptors and recruits monocytes, T lymphocytes, natural killer cells, and mast cells. Interestingly, Ccl8 expression from macrophages required IL-33, suggesting that an axis where alloimmune cell mediated injury release of injury signals propagates chemokines that may further promote infiltration of the graft by additional immune cells.

Conclusion

Our findings demonstrate that IL-33 is a central injury-derived signal that dynamically shapes immune responses during CR after HTx. While IL-33

can support reparative pathways through Tregs and macrophages after HTx, persistent alloimmunity also amplifies local T cells responses and chemokine expression, most notably Ccl8, which can drive the recruitment of multiple inflammatory leukocytes. We identify macrophages as the predominant source of Ccl8 within occluded vessels, linking persistent injury and alloimmunity to pathogenic immune cell infiltration and impaired repair. These data establish IL-33-Ccl8 signaling as a critical axis in the progression of CAV and suggest that targeting injury-immune cross talk may provide a new therapeutic avenue beyond conventional T cell-centric immunosuppression to improve long-term graft survival.

Abstract Keywords

Alloimmunity, IL33-Ccl8 axis, macrophages, Cardiac allograft vasculopathy. T cells

Oral Abstract #12

Title: Anti-CD154 limits murine Epstein-Barr viral burden and induces a more polyfunctional CD8+ T cell response

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Current FDA-approved costimulation blockade for use in organ transplantation, Belatacept, significantly impairs the response to Epstein-Barr virus (EBV), increasing the risk of post-transplant lymphoproliferative disease (PTLD) and lymphoma. Anti-CD154 is a promising immunotherapeutic for solid organ transplantation with multiple ongoing clinical trials, but its impact on the immune response to EBV is unknown. The goal of this study was to determine the impact of anti-CD154 on protective immunity to a murine Epstein-Barr virus (EBV) homolog (MHV68) compared to currently available mainstay immunosuppression.

Methods

Mice were infected with a YFP-expressing strain of MHV68 and treated with anti-CD154, CTLA4-Ig (Belatacept homolog), or Tacrolimus. Virus-specific CD8+ T cells responses were measured using viral peptide:MHC tetramers for two different lytic viral antigens, p56 and p79. Differentiation and function were assessed using flow cytometry. Viral load was estimated by number of YFP-expressing infected germinal center B cells and confirmed using serum viral DNA qPCR.

Results

Results indicate that the frequency of virus-specific CD8+ T cells was not significantly reduced following anti-CD154 treatment. Unexpectedly, CD8+ T cells in anti-CD154-treated mice also exhibited significantly lower



frequencies of Tim-3+TCF1- terminally differentiated cells and an increase in polyfunctional producers of IL-2, IFN γ , and TNF α compared to untreated mice. Of note, anti-CD154-treated mice had significantly smaller spleens with a significant decrease in the number of bulk and MHV68-YFP+ germinal center B cells as compared to untreated mice on day 14. Upon comparison with current immunosuppression, CTLA4-Ig and Tacrolimus, anti-CD154-treated mice had the greatest inhibition of splenomegaly. Interestingly, CTLA4-Ig-treated mice exhibited the most significant impairment of CD4+ T follicular helper cells (TFH) compared to anti-CD154- and Tacrolimus-treated mice. Moreover, anti-CD154 did not significantly alter the frequency of TFH compared to untreated controls.

Conclusion

Overall, CD154 blockade does not significantly decrease the frequency or quality of EBV-specific CD8+ T cells in a mouse model. The ability of anti-CD154 to block the CD40 costimulation necessary for initiation of the germinal center response, limiting peak viral burden, may be a beneficial effect of anti-CD154 immunosuppression and may limit the potential for aberrant replication-associated complications like PTLD. Comparison of anti-CD154 to CTLA4-Ig during EBV infection suggests TFH impairment and potentially the antiviral antibody response may be the mechanistic source of impaired EBV response during CTLA4-Ig immunosuppression that is not replicated in the use of anti-CD154. Overall, this suggests anti-CD154 may be a promising alternate costimulation blockade strategy with regard to its ability to preserve protective immunity.

Abstract Keywords

CD154, CD40L, Epstein-Barr virus, CD8 T cells, Mouse, Protective Immunity

Oral Abstract #13

Title: An IL-2 Mutein (VIS171) Induces Regulatory T Cells and Prolongs Survival in a Rhesus Kidney Transplant Model

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Interleukin-2 mutein (mIL-2) therapies have demonstrated selective Treg expansion and prolonged half-life compared to wild-type IL-2, addressing the limitations of broad immunosuppression. This study evaluates the effects of the mIL-2 VIS171 on Treg expansion and immune modulation in a rhesus macaque kidney allotransplant model, exploring its potential to promote immune tolerance and improve allograft survival in kidney transplantation.

Methods

Eleven (11) rhesus macaques were included in this study. The treatment group (n=4) received VIS171 at 50 μ g/kg subcutaneously on days -5, 1, 15, and 29, along with daily rapamycin (trough levels: 8–12 ng/mL) from day 0 through day 180. Control animals (n=7) received daily rapamycin alone (Figure 1A). Treg populations (CD4+CD25+FoxP3+) and CD8+ cytotoxic T cells, along with other immune phenotypes, were assessed throughout the study using flow cytometry.

Results

Rejection-free graft survival was significantly prolonged in the group receiving VIS171 with Rapamycin compared to the Rapamycin alone group (Figure 1B). The median survival time in the VIS171 + Rapamycin group was 90 days versus 21.5 days in the Rapamycin alone group (p=0.045). We confirmed CD4+CD25+FoxP3+ regulatory T cells expanded 5 to 7 days after the administration of VIS171 (Figure 1C), with a maximum peak on the first day after kidney allotransplantation. Notably, the animals on rapamycin-only immunosuppression showed no significant increase in peripheral Tregs after the transplant, implying the crucial role of VIS171 in inducing Treg expansion post kidney transplantation. The data clearly demonstrate the biological efficacy and benefit of VIS171 in prolonging allograft survival.

Conclusion

The mIL-2 VIS171, combined with rapamycin, significantly improved graft survival and selectively expanded Tregs in a rhesus macaque kidney transplant model. The distinct peaks of Treg expansion, including the peak increase coinciding with antigen exposure, highlight the potential of VIS171 as an effective strategy for enhancing immune regulation and prolonging allograft survival.

Abstract Keywords

Tregs, Immune Tolerance, Kidney Transplantation

Oral Abstract #14

Title: Inhibition of Innate Immune Allorecognition Using Nanobiologics Effectively Overturns Ongoing Allograft Rejection

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro



INTERNATIONAL TRANSPLANTATION SCIENCE MEETING

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Abstract Body: Background

A key risk factor to long-term allograft outcomes is persistent and reoccurring early acute T cell mediated rejection (TCMR) episodes, which occur in a significant fraction of patients despite supplemented rejection treatment. Recent evidence strongly suggests that TCMR is driven by innate immune allorecognition memory responses (Allo-IMem). However, Allo-IMem is inadequately controlled by current immunosuppressive regimens, and thus alternative therapeutics are necessary. Consequently, we investigated whether therapeutically inhibiting Allo-IMem would repress ongoing TCMR, and thus provide novel therapeutic perspectives in transplantation.

Methods

WT B6 recipients of Balb/c islet allografts were left untreated for 7 days to allow acute TCMR to develop naturally. On days 7-12 during ongoing TCMR, innate immune cells were targeted using high-density lipoprotein nanobiologics, which are naturally and specifically incorporated by innate immune cells. Nanobiologics were either unloaded (control; U-NB), or loaded with the mTOR inhibitor rapamycin (mTORi-NB; 5mg/kg), and compared to the treatment of rapamycin in its free form (Rapa; 5mg/kg).

Results

The signals leading to Allo-IMem remain largely undefined. To this end, we uncovered that murine Allo-IMem responses signal through mTOR in innate immune cells in vivo and that these memory responses can be effectively inhibited with mTORi-NB treatment, as opposed to Rapa treatment. By experimentally segregating Allo-IMem and skin allograft TCMR temporally, we demonstrate that 1) established Allo-IMem significantly enhances subsequent acute TCMR, and that 2) the specific inhibition of Allo-IMem with mTORi-NB in complete absence of adaptive immune cells drastically reduces subsequent TCMR and significantly enhances allograft survival, compared to either control U-NB or Rapa treatments. Remarkably, a short

mTORi-NB treatment initiated during established and ongoing acute TCMR (days 7-12) of islet allografts protected mice from complete rejection, with 64% of mice surviving long-term and an additional 27% of recipients demonstrating significantly delayed rejection compared to U-NB treatment (Fig. 1A). Additionally, mTORi-NB treatment during ongoing TCMR was therapeutically advantageous over Rapa in promoting survival of both heart allografts, and of islet allografts in a stringent mouse model where recipients were reconstituted with donor-specific memory T cells prior to transplantation (Fig. 1B). ScRNAseq and flow cytometry analysis of innate immune cells infiltrating islet allografts on day 10 demonstrated that mTORi-NB treatment during ongoing TCMR inhibits cell cycle, glycolysis, and type I/II interferon signaling, while promoting TGF-beta signaling pathways, compared to control U-NB. In addition, mTORi-NB treatment during ongoing TCMR reduced pro-inflammatory tissue-resident macrophages, while promoting reparative macrophages within islet allografts. When assessed by ex vivo assays, innate immune cells infiltrating allografts from mTORi-NB treated mice showed a drastic reduction in their antigenic presentation capacity, compared to innate cells from U-NB treated mice. In turn, both effector T cell proliferation and IFN- γ effector T cell numbers within islet allografts were significantly decreased upon mTORi-NB treatment during ongoing TCMR, compared to U-NB.

Conclusion

Taken together, our data demonstrate that Allo-IMem responses signal through mTOR in innate immune cells and potentially drive TCMR. Consequently, inhibiting Allo-IMem responses using innate immune cell specific nanobiologics effectively overturns established adaptive immune responses and allograft rejection, providing a novel innate immune targeted therapeutic approach for acute TCMR treatment in transplant patients.

Abstract Keywords

Acute T cell mediated Rejection Innate immune allorecognition High density Lipoprotein Nanobiologics mTOR Rapamycin Reparative macrophages

Oral Abstract #15

Title: Tim-4 Effector B Cells Express a Th17-Like Proinflammatory Cytokine Module That Is Restrained by Nur77

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

Author(s)

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Abstract Body: Background

B cells expressing various proinflammatory cytokines potentiate immune responses in infection, autoimmunity and transplantation. However, specific



markers for such effector B cells (Beffs) are lacking and it is unknown whether Beffs expressing different cytokines share common phenotype or regulation. We showed that Tim-4⁺ B cells express IFN γ and promote islet allograft and tumor rejection. We now show that Tim-4⁺ B cells also express IL-17 and ask whether Tim-4⁺ B cells express other proinflammatory cytokines and examine how their proinflammatory function is regulated.

Methods

Transplants: 250 BALB/c islets under the kidney capsule of chemically diabetic B6 mice or heterotopic BM12 hearts (single H-2 mismatch; B6 recipients). Cytokine expression: intracellular flow cytometry (IFC) of splenocytes stimulated 5h with PMA, ionomycin, BrefeldinA. RNAseq/ATACseq and qPCR: +/- 24h stimulation with anti-IgM + IL-23.

Results

Tim-4⁺ Beffs uniquely express ROR γ t, which along with IL-23, drive expression of a pathogenic Th17-like proinflammatory module comprising IL17a, IL17f, IL22, Csf2, IL6, and IL1b. Loss of IL-17 expression by Tim-4⁺ B cells results in their ectopic expression of IL-10 and Breg activity. RNAseq/ATACseq identified Nr4a1 (Nur77 transcription factor) as a potential candidate regulating Tim-4⁺ B cells (increased accessibility and expression at rest, downregulated upon activation). This was confirmed by IFC in splenic B cells from alloimmunized mice. Loss of B cell Nur77 (Nur77^{flox}Cd19^{Cre}+/-; Nur77 BKO) increased frequency of Tim-4⁺ Beffs (1.5x), while decreasing Tim1⁺ Breg frequency (30%). Furthermore, Tim-4⁺ Beffs from alloimmunized Nur77 BKO mice exhibited increased expression of ROR γ t (2.5x), IL17(1.7x), IL22(2x), GM-CSF(1.5x), IL6(2x), & IL1b(2x) compared to Cre-control Tim-4⁺ Beffs (IFC). After alloimmunization, Tim-4⁺ B cells from Nur77 BKO mice exhibited increased proliferation (35%) and decreased apoptosis (85%) vs those from Cre-controls. Finally, compared to control mice, Nur77 BKO mice exhibited shortened islet allograft GS (MST 14d vs 20d; $p < 0.05$) and accelerated rejection of BM12 hearts (MST 30d vs 65d; $p < 0.01$). Thus, Tim-4 is a broad marker for Beffs that promote allograft rejection via expression of a pro-inflammatory module driven by IL-23 and maintained by IL-17. Nur77 constrains Tim-4⁺ Beffs and maintains a normal balance between Beffs and Bregs, at least in part, by reducing Tim-4⁺ B cell viability and proliferation.

Conclusion

This enhances our understanding of B cell biology and provides insights highly relevant to immune tolerance.

Abstract Keywords

Tim-4; Effector B Cells; IL-17; Nur77

Oral Abstract #16

Title: Deletion of Blimp1 to Prevent Plasma Cell Differentiation Paradoxically Promotes Tolerance Through B Cell-Intrinsic Induction of Bregs

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

IL-10⁺ B cells (Bregs) express TIM-1 and play an important role regulating immune responses in autoimmunity and transplantation. Plasma Cells (PCs) are a major source of B cell IL-10. Further, PC-deficient mice (Prdm1 ("Blimp1") B cell KO) exhibit more severe EAE than CD19^{Cre}/+ (Cre) controls. This led many to conclude that "PCregs" actually underlie Breg activity. However, PCs are enriched in the meninges and may be particularly important in neuroinflammation. Thus, to determine whether PCregs also play an essential role as Bregs in transplantation, we bred and examined Blimp1^{f/f}/fl.CD19^{Cre}/+ (Blimp1 BKO) mice.

Methods

Islet transplants: STZ-induced diabetic mice (B6) received 250 BALB/c islets under the kidney capsule \pm anti-TIM-1 (RMT1-10) mAb. Cytokine expression by intracellular flow cytometry: Splenocytes stimulated 5h with LPS, PMA, ionomycin, monensin. Mixed BM chimeras: Irradiated CD45.1/2 hosts expressing an irrelevant tg-BCR were reconstituted 1:1 with bone marrow (BM) from CD45.1+ Cre and CD45.2+ Blimp1 BKO mice.

Results

Rather than accelerated rejection, Blimp1 BKO mice exhibited prolonged islet allograft survival vs. Cre controls (Fig 1). Blimp1 BKO mice had enlarged spleens with increased number (3X) and frequency (2.5X) of TIM-1⁺ IL-10⁺ Bregs. To determine whether this was B cell extrinsic (e.g. loss of PCs altering unknown feedback control or microbiota), or an intrinsic effect of low-level Blimp1 expression unrelated to PC differentiation, we generated mixed BM chimeras (see Methods). Compared to B cells derived from control BM, those from Blimp1 BKO BM exhibited an ~3-fold increase in TIM-1⁺ IL-10⁺ Bregs, indicating that Blimp1 has an intrinsic effect on B cells prior to PC differentiation which constrains Breg generation. scRNAseq analysis of TIM-1⁺ and TIM-1⁻ B cells isolated from these BM chimeras revealed that the cluster expressing TIM-1 transcripts (Havcr1) was enriched for immunoregulatory genes (e.g. Il10, Ebi3, Il12a, Fgl2, Ctla4, Lag3, Cd39, and Cd73). Cells within this cluster were markedly increased in cells derived from Blimp1 BKO vs. control BM ($p < 0.0001$), as were genes associated with activation (receptor tyrosine kinases, actin remodeling, CDKs, Myb, Cd38, Tlr9), Ag presentation (Cd80), and metabolic/ER remodeling programs characteristic of pre-PCs.



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Conclusion

This “hybrid” regulatory-activated state suggests that BLIMP1 normally constrains the emergence of highly suppressive, metabolically primed TIM-1+ Bregs, whose expansion obviates the requirement for regulatory PCs in enhancing allograft survival.

Abstract Keywords

Allograft Transplant tolerance regulatory B cells

Oral Abstract #17

Title: Fibrinogen-like protein 1 prevents antibody production in a LAG3 dependent manner

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Coinhibitory receptor Lymphocyte Activation Gene 3 (LAG3) is expressed on a wide range of immune cells. We have established that LAG3 deficient kidney transplant recipients, acutely reject their kidney allografts via antibody mediated rejection.

Methods

Based on this, we hypothesized that stimulation of LAG3 would diminish antibody production.

Results

To test whether LAG3 agonism could diminish antibody responses we performed plasma cell ELISPOT assays and crosslinked LAG3 with primary and secondary antibodies or with LAG3 ligand Fibrinogen-like protein 1 (FGL1), resulting in decreased IgG production by plasma cells. Furthermore, use of conditional knockout mice indicated this was plasma cell intrinsic. We next tested our hypothesis in an immunization model. Mice immunized with NP-KLH and treated with FGL1 had decreased anti-NP antibodies compared

to untreated immunized mice. Finally, we tested the ability of LAG3 to regulate antibody responses in a model of antibody mediated rejection in kidney transplantation. Untreated mice acutely reject their kidney grafts (MST = 7d), while LAG3 agonism via FGL1 treatment significantly prolonged graft survival with 66% of recipients surviving beyond day 80.

Conclusion

Together these findings demonstrate that LAG3 is a mediator of humoral immune responses, with therapeutic potential in transplantation.

Abstract Keywords

ABMR, LAG3

Oral Abstract #18

Title: Marchf1 Ablation Mitigates ABMR by Suppressing Memory B Cells and Expanding B10 Bregs

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Antibody-mediated rejection (ABMR) remains a major cause of graft failure, and current T cell-targeted immunosuppression is insufficient for ABMR control while causing significant side effects. B cells contribute to ABMR through antigen presentation, donor-specific antibody (DSA) production, and memory B cell sensitization, with MHC II presentation being central to these processes. The E3 ubiquitin ligase Marchf1 regulates MHCII surface expression and B cell function, but its specific role in ABMR is unclear. We investigated the impact of Marchf1 ablation on B-cell-mediated alloimmunity in a murine ABMR model.

Methods

A fully allogeneic heart transplant model was used to determine if Marchf1 knockout (Marchf1^{-/-}) prolongs allograft survival. To assess effects on anti-human HLA antibody responses, B6 HLA-A2⁺ transgenic hearts were transplanted into wild-type (WT) B6 or Marchf1^{-/-} recipients. Serum anti-HLA-A2 IgG was measured weekly by ELISA. On day 42 post-transplant, allografts were subjected to histopathological evaluation, and splenocytes were assessed by flow cytometry for B cell subsets to investigate underlying mechanisms.



Results

Marchf1^{-/-} recipients demonstrated significantly prolonged allograft survival compared with WT controls ($p=0.0148$, Fig 1A). WT recipients developed high-titer HLA-A2-specific IgG by day 21, which remained significantly lower in Marchf1^{-/-} mice. (Fig. 1B). Histological analysis of HLA-A2 grafts from WT recipients revealed severe edema, hemorrhage, and fibrosis consistent with ABMR, whereas grafts from Marchf1^{-/-} recipients exhibited improved pathology (Fig. 1C). Mechanistically, Marchf1 ablation reduced the frequency and number of splenic IgG1⁺ memory B cells (Fig. 1D). This reduction correlated with both the proportion and absolute number of IL-10⁺ B10 Bregs, which also exhibited enhanced IL-10 expression (Fig. 1E).

Conclusion

Our findings suggest that Marchf1 plays a critical role in regulating B cell responses during ABMR by limiting pathogenic memory B cells and promoting regulatory B cell expansion. Targeting Marchf1 may represent a novel therapeutic strategy to mitigate ABMR and improve long-term allograft survival.

Abstract Keywords

Antibody-mediated rejection; Tolerance; Marchf1; Heart Transplantation

Oral Abstract #19

Title: Pegcetacoplan for posttransplant patients with complement 3 glomerulopathy or primary (idiopathic) immune-complex membranoproliferative glomerulonephritis

Abstract Topic: Clinical studies in humans

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Abstract Body: Background

Complement 3 glomerulopathy (C3G) and primary (idiopathic) immune-complex membranoproliferative glomerulonephritis (IC-MPGN) are rare, chronic kidney diseases that often recur after transplantation despite conventional immunosuppression. NOBLE (Phase 2, NCT04572854) and VALIANT (Phase 3, NCT05067127) showed efficacy and favorable tolerability

of pegcetacoplan (C3/C3b inhibitor) for adults and adolescents with native or posttransplant recurrent C3G or primary (idiopathic) IC-MPGN. Here, we describe pegcetacoplan for kidney transplant recipients in these studies.

Methods

Eleven posttransplant patients with ≥ 1 g/g proteinuria at baseline received pegcetacoplan (NOBLE, n=6; VALIANT, n=5). Study designs and biopsy schedules differed between studies, but all patients received pegcetacoplan 1080 mg subcutaneously twice weekly for at least 24–26 weeks. Efficacy endpoints included change from baseline in proteinuria, estimated glomerular filtration rate (eGFR), and C3c staining on kidney biopsy. Treatment-emergent adverse events were also noted.

Results

The safety profile of pegcetacoplan was favorable for posttransplant patients, with no graft loss or rejection reported during 6 months of treatment. Pegcetacoplan-treated patients demonstrated decreased proteinuria and stable eGFR in both studies (Supplemental Table). C3c staining reduction was observed at Weeks 12 and 52 in NOBLE, and at Week 26 in VALIANT, suggesting that pegcetacoplan leads to early and sustained histopathological improvements.

Conclusion

Six months of pegcetacoplan treatment was safe and well tolerated for posttransplant patients with C3G and primary IC-MPGN. Patients achieved decreased proteinuria, stable eGFR, and improved C3 staining.

Abstract Keywords

GFR, Glomerulonephritis, Kidney, Proteinuria

Oral Abstract #20

Title: CAR-Tregs synergize with anti-thymocyte globulin and rapamycin to delay skin allograft rejection

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Solid-organ transplantation is the only curative therapy for end-stage organ failure. Nevertheless, recipients must undergo intensive immunosuppressive treatment, which increases their susceptibility to secondary infections and malignancies. As an alternative, regulatory T cell (Treg) therapy is being tested as a new way to induce allograft tolerance. We previously showed that Tregs engineered to express an HLA-A2-specific chimeric antigen receptor (A2-CAR) delayed HLA-A2+ skin graft rejection. However, their persistence and suppressive effect was transient. We hypothesized that adjunct immunosuppression may synergize with A2-CAR Tregs. To reflect the clinical context in which A2-CAR Tregs are being tested, we aimed to investigate the ability of a combination therapy including anti-thymocyte globulin (ATG, rabbit anti-mouse), rapamycin, and A2-CAR-Tregs to delay rejection in a model of F1 (BALB/c x B6.HLA-A2) skin to C57BL/6J mice (B6 mice) recipient transplant model.

Methods

Wild type B6 mice were transplanted with circular skin patch from F1 skin (using BALB/c mice which additionally expressed the 2W-OVA transgene, BALB/c 2W-OVA x B6.HLA-A2) or syngeneic B6 mice. ATG recipients received two intraperitoneal injections (500ug/dose) on day -1 pre and day 3 post-transplant. Rapamycin (1mg/kg) was administered for 10 consecutive days from day 14. Mice were infused with 1M A2-CAR-Tregs into the tail vein on day 20 post-transplant, a time point at which we determined the T cell depleting effects of ATG were minimal. Skin grafts were monitored at least three times per week, and weekly blood samples were collected to determine CAR-Treg engraftment and phenotype.

Results

The median graft survival for untreated or A2-CAR-Treg alone was 14 days. Treatment with ATG, ATG and A2-CAR-Tregs, or ATG and rapamycin all moderately extended median graft survival to 20-26 days. However, the combination of ATG, rapamycin and A2-CAR-Tregs resulted in significant prolongation, extending the median graft survival to 43 days. Circulating A2-CAR Tregs were detected in the peripheral blood of ATG and rapamycin treated mice, and they maintained FOXP3 and CAR expression. Mechanistically, when A2-CAR-Tregs were used in combination with ATG and rapamycin, there was a significant reduction in the frequency of OVA-specific CD8 T cells in the draining lymph nodes. Furthermore, A2-CAR-Tregs significantly reduced donor-specific antibody production by inhibiting T follicular helper cells and germinal center B cells.

Conclusion

These data show that A2-CAR-Tregs synergize with ATG and rapamycin and can be used clinically as a combination therapy for solid-organ transplantation. The findings have important implications for the application of A2-CAR Treg therapy in humans.

Abstract Keywords

CAR-Treg, cell therapy, transplantation, tolerance, immune therapy

Oral Abstract #21

Title: MHC II-specific chimeric antigen receptor regulatory T cells for transplantation tolerance

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Cell therapy with regulatory T cells (Tregs) expressing a donor antigen-specific chimeric antigen receptor (CAR) is a promising alternative approach for promoting allograft tolerance. CAR-Tregs targeting ubiquitously expressed mismatched donor MHC I alleles (HLA-A2) have entered clinical trials for transplantation. However, donor MHC II alleles, with expression primarily on antigen-presenting cells (APCs) and upregulation under inflammatory conditions, are key drivers of alloimmunity and may be superior targets for CAR-Tregs to promote allograft tolerance. Using in vitro and in vivo immunocompetent models of fully mismatched transplantation, we tested the hypothesis that MHC II-specific CAR-Tregs could inhibit APC function, reduce alloimmunity, promote donor-specific tolerance and extend allograft survival.

Methods

To study the therapeutic potential of donor MHC II-specific CAR-Tregs in



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immunocompetent models of organ transplant, we developed CAR-Tregs specific for the BALB/c mouse MHC II allele I-Ed (I-Ed-CAR-Tregs). We tested their ability to suppress BALB/c APCs in vitro and prolong allograft survival in combination with α CD154 co-stimulation blockade in fully allogeneic BALB/c.2W-Ova to C57BL/6 skin and heart transplantation.

Results

I-Ed-CARs demonstrated high target specificity and minimal cross-reactivity toward other mouse MHC molecules, and selectively mediated Treg activation marker upregulation (CD25, CTLA-4, PD-1, LAP) and proliferation in response to BALB/c, but not C57BL/6, APCs. In vitro suppression assays, I-Ed-CAR-Tregs inhibited co-stimulatory molecule (CD80, CD86) expression on BALB/c CD11c+ dendritic cells (DCs), depleted I-Ed target molecules from DCs via trans-endocytosis, and potently suppressed BALB/c DC-stimulated activation and proliferation of alloreactive C57BL/6 CD4+ and CD8+ T cells. In vivo, I-Ed-CAR-Tregs synergized with low-dose α CD154 to significantly prolong BALB/c.2W-Ova allograft survival across skin and heart transplantation models. Mechanistically, I-Ed-CAR-Tregs trafficked selectively to allografts and draining lymph nodes, where they suppressed germinal centre responses among B cells with shared (I-Ed) and distinct (I-Ad, H-2Kd/H-2Ld) BALB/c MHC allele specificities, reducing levels of circulating donor-specific IgM and IgG. I-Ed-CAR-Tregs also controlled total and donor (2W/Ova)-specific CD4+ and CD8+ T cell responses and mediated infectious tolerance by promoting the accumulation of Tregs with distinct donor (2W)-specificity.

Conclusion

Donor MHC II-specific CAR-Tregs potently suppress APCs and MHC II-driven alloreactivity, and are effective tolerogenic therapies in multiple preclinical models of immunocompetent organ transplantation. Our data support the clinical development of CAR-Treg therapies targeting donor MHC II alleles as a new distinct approach to reduce immunosuppressive burden and promote donor-specific tolerance in transplantation.

Abstract Keywords

Solid organ transplantation, chimeric antigen receptor, regulatory T cell, cell therapy, MHC Class II, antigen-presenting cell, immune tolerance

Oral Abstract #22

Title: Allogeneic Treg Programmed to be Reparative is a Therapeutic Cell Therapy with Clinical Potential for the Resolution of Tissue Damage and Inflammation following Tissue Injury

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe respiratory complications that can occur following hematopoietic stem cell transplantation or lung transplantation (Tx). It is characterized by immune cell infiltration, excessive pro-inflammatory cytokine release, and alveolar-capillary membrane damage, leading to high morbidity and mortality. There is a critical need for effective therapies that can both rapidly suppress inflammation and quickly promote lung tissue repair to limit disease progression. FoxP3+ regulatory T cells (Tregs) play a vital role in maintaining lung homeostasis by suppressing other immune cells. Previous studies in our lab demonstrate that upon tissue damage, alarmin IL-33 is released from the injured stroma to stimulate to cells expressing the IL-33 receptor, ST2. Regulatory T cells (Treg) expressing ST2 respond to IL-33 by proliferating and secreting repair-promoting molecules like IL-13, which act locally on macrophages to promote a reparative phenotype. However, during severe lung injury leading to ARDS, the endogenous Treg response is often insufficient to control inflammation and support repair. While adoptive Treg therapy (ACT) holds promise, the use of autologous Treg products is limited in the setting of acute lung injury (ALI) because manufacturing requires several weeks, missing the critical early window in which intervention could alter disease progression. For acute injuries like ALI, an allogeneic Treg product would be more feasible, enabling rapid administration compared to the delayed availability of autologous cells. Thus, we tested the hypothesis that IL-33 can program allogeneic Treg with reparative functions, providing a potential new immunotherapy for acute lung injury (ALI).

Methods

Tregs were generated and expanded from C57BL/6 (B6; Syn), BALB/c (Allo), or B6xBALB/c (F1) mice. Conventional suppressive Treg (SupTreg) were expanded for 14 days with IL-2 and CD3/CD28 stimulation where reparative Treg (RepTreg) were expanded with the addition of IL-33. B6 mice were treated with 3 U/kg Bleomycin or 1000PFUs of H1N1 to induce ALI, with Syn, F1, or Allo SupTregs or RepTregs adoptively transferred 24 hours post-ALI. Mortality, morbidity, and lung pathology was assessed. Local lung immune populations were analyzed using flow cytometry.

Results

IL-33 programs Treg that are suppressive, retain FoxP3 expression, and produce IL-13. After infusion Syn and Allo Tregs were both present in the lung interstitium at day 7 post-delivery, but only Syn persisted beyond day 20. Importantly, both Syn and Allo RepTregs significantly reduced morbidity when delivered intravenously 24 hours post ALI, whereas SupTreg significantly increased morbidity in both ALI models. Protection was associated with an augmented presence of alveolar and CD301b* monocyte-derived macrophages.

Conclusion

Using allogeneic RepTreg immunotherapy represents a novel approach to reprogram Treg effector functions with IL-33 to not only suppresses



inflammation but actively promote tissue repair by locally modulating reparative macrophages. These data demonstrate RepTreg efficacy after cryopreservation, making this a clinically feasible, off-the-shelf intervention for acute lung injury and transplantation, with broad potential across tissue injury and immune-mediated diseases.

Abstract Keywords

Regulatory T Cells Tissue Repair Immunotherapy Inflammation Lung

Oral Abstract #23

Title: Initial analysis of innate and adaptive immune responses in heart transplant recipients of the ALLIN HEART (tocilizumab) trial

Abstract Topic: Clinical studies in humans

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Abstract Body: Background

Outcomes after heart transplant (HT) are suboptimal. We hypothesize that early post-HT inflammation and other downstream effects of interleukin-6 (IL-6) contribute to graft injury and survival. IL-6 is a multifunctional mediator of post-HT inflammation. One downstream component of the IL-6 signaling pathway is phosphorylation of STAT3 proteins, which then translocate to the cell nucleus and regulate gene expression for inflammatory cytokines. This influences T cell differentiation, among other effects. The ALLIN HEART trial is a randomized, blinded, placebo-controlled multicenter study testing the hypothesis that a 5-month course of tocilizumab (anti-IL-6 receptor, IL-6R) plus standard 3-drug immunosuppression will improve HT outcomes. The trial enrolled 151 subjects to be followed for 1 year. Unblinding of treatment assignments and study outcomes is anticipated to occur by October 2025. Herein, we examined innate and adaptive immune responses in peripheral blood of study subjects by assessing serum levels of inflammatory markers, analyzing RNAseq on peripheral blood, quantifying phosphorylated STAT3 (pSTAT3) levels after IL-6 stimulation, and assessing regulatory T cell (Treg) function via suppression assays.

Methods

Peripheral blood samples from subjects were collected at 1 week, and 1, 3, 6, and 12 months post-HT. As IL-6 signaling induces liver production of C-reactive protein (CRP), and serum CRP levels correlated with treatment assignments in rheumatoid arthritis trials, we analyzed serum CRP levels in each subject. We also analyzed serum levels of 10 cytokines/chemokines by Luminex and quantified T cell subsets by flow cytometry. We performed bulk RNA sequencing using Next Generation Sequencing on serially collected peripheral blood. Computational analysis was performed to determine the differential expression of the genes across the aforementioned timepoints. In peripheral blood mononuclear cells (PBMCs) from study patients at the one-month post-HT timepoint, we detected IL-6 induced pSTAT3 levels using phosphoflow cytometry. We tested Treg suppressive capacity using in vitro autologous suppression assays with PBMCs and Tregs from the same patient.

Results

CRP was examined 1-month post-HT in 96 study subjects, and segregated them into 2 groups – high (n=48, mean 5011.8 ng/mL) vs. low CRP (n=48, mean 27.5 ng/mL). The high CRP group demonstrated significantly higher levels of serum TNF- α and MCP-1 than the low CRP group over the initial 6 post-HT months (Figure 1A). Differences between cytokine levels between groups were seen as early as 7 days post-HT and then diminished beyond 6-months (after cessation of study drug). In a subset of 38 patients, flow cytometry analyses showed significantly higher ratios of Tregs (CD4+CD25+CD127lo) / IL-17 producing T cells (Th17) (CD4+CXCR3-CCR6+CXCR5+PD1+) in the low CRP group, during the initial 6 post-HT months (Figure 1A). RNAseq gene enrichment analyses performed on serial samples from 48 subjects showed differences between groups in gene sets associated with IL-6 production and regulation, and Th17 cell differentiation, among other gene ontology (GO) terms between week 4 and 6-months post-HT. In a small number of patients studied (n=4), IL-6 induced pSTAT signaling in CD4+ and CD8+ cells correlated inversely with Treg suppressive capacity (Figure 1B).

Conclusion

Together, our findings of differential serum levels of inflammatory cytokines, Treg/Th17 ratios, temporal IL-6-related gene expression patterns that correlated with serum CRP levels, along with decreased pSTAT3 signaling associated with enhanced Treg function, suggest that IL-6R blockade has significant effects on innate and adaptive immune responses known to be IL-6 dependent. Further analyses and correlations with study outcomes will be required to make definitive conclusions.

Abstract Keywords

Phosphoflow cytometry RNAseq Regulatory T cell Heart transplant Interleukin-6

Oral Abstract #24

Title: Targeting Immune Metabolism Via PKM2 Inhibition: A Promising Approach For Alloimmune Modulation

Abstract Topic: Basic Immunobiology: preclinical models in vivo and in vitro



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Abstract Body: Background

Organ transplantation is a life-saving therapy, but rejection continues to compromise long-term graft survival. Current immunosuppressants mitigate early rejection but are associated with toxicity and do not prevent chronic immune injury. Emerging evidence highlights that immune activation relies on metabolic reprogramming, particularly the switch from mitochondrial respiration to aerobic glycolysis (Warburg effect), which fuels the bioenergetic and biosynthetic demands of pro-inflammatory cells. Pyruvate kinase M2 (PKM2), a glycolytic enzyme upregulated in activated immune cells, plays a dual role: as a tetramer promoting glycolysis, and as a dimer that translocates to the nucleus to regulate pro-inflammatory gene transcription. While PKM2 has been implicated in various immune-mediated diseases, its role in transplantation remains largely unexplored. We hypothesized that PKM2 is critical for the effector functions of alloimmune T cells and that its inhibition could mitigate rejection and improve transplant outcomes.

Methods

To assess PKM2 expression dynamics following transplantation, female C57BL/6 (B6) mice received heart grafts from syngeneic B6 or fully mismatched BALB/c (B/c) donors. Grafts were analyzed by flow cytometry on post-operative days (POD) 3 and 7, with naïve B6 and B/c hearts used as baseline controls. To examine the impact of PKM2 inhibition on T cell alloimmunity, CD3⁺ cells were isolated from B/c mice and treated for 24h with PKM2 inhibitors (PKM2i, Shikonin or TEPP-46) or vehicle. These T cells were then co-cultured with LPS-stimulated allogeneic BMDCs from B6 mice and used as responders in a mixed lymphocyte reaction (MLR) assay. For in vivo studies, fully mismatched heart grafts (B/c→B6) were performed and recipients treated intraperitoneally with PKM2i or vehicle twice weekly from POD1–28. Skin grafts from male B6 donors were also transplanted onto female B6 recipients, with the same treatment schedule, to evaluate mechanisms via flow cytometry and histology on POD14.

Results

Flow cytometry analyses revealed that PKM2 expression was markedly upregulated in graft-infiltrating immune cells during fully mismatched heart transplantation, with the highest levels observed on T cells by POD7, while syngeneic grafts maintained baseline expression (Fig. 1A). Pharmacological inhibition of PKM2 significantly prolonged heart graft survival in comparison to vehicle-treated controls (Fig. 1B). Treatment of CD3⁺ T cells with PKM2 inhibitors significantly reduced IFN- γ production in mixed lymphocyte reactions in vitro (Fig. 1C,D). Mechanistic studies in vivo using skin transplant models demonstrated that PKM2 inhibition reduced the presence of graft-infiltrating T cells (Fig. 1E,F) and diminished expression of activation markers (CD25 and Ki67) (Fig. 1G,H) and effector cytokines (IFN- γ) (Fig. 1I). Histological analyses confirmed decreased immune infiltration and

attenuated tissue injury in PKM2i-treated grafts. These data support PKM2 as a key metabolic regulator of alloimmune responses

Conclusion

PKM2 orchestrates both metabolic and transcriptional programs that drive T cell-mediated rejection. Its pharmacologic inhibition attenuates effector T cell activation and prolongs allograft survival. These findings identify PKM2 as a promising target for metabolic immune modulation in transplantation.

Abstract Keywords

Immunometabolism; PKM2; Alloimmunity; graft survival.

Poster # 1

Title: Donor Macrophage-Derived CCR5 Ligands Interfere with Post-Transplant Tolerization

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

Author(s)

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Abstract Body: Background

Using donor splenocytes fixed with cross-linker ethylcarbodiimide (ECDI-SPs), our lab has induced tolerance in a variety of murine and non-human primate models when one dose of ECDI-SPs is administered before transplant and one dose after. We aimed to induce tolerance post-transplant in a murine model by depleting donor macrophages prior to transplant. Once achieved, we then investigated the role of donor-derived chemokines in graft infiltration and failure of tolerization.

Methods

We transplanted BALB/c pancreatic islets under the kidney capsule of C57BL/6J mice made diabetic with streptozotocin. Prior to islet harvest, donors were treated with either anti-CD115 antibody to deplete macrophages or an isotype control. Recipients received two doses of ECDI-SPs following transplant. Graft rejection was defined as hyperglycemia for two consecutive days. Grafts and spleens were harvested and evaluated with flow cytometry. Functional assays were performed with splenic T cells from recipients. In vitro assays were performed by culturing harvested pancreatic islets with IFNG. We then evaluated islets and supernatant with qPCR and multiplex immunoassay.

Results

Pancreatic islet grafts depleted of donor macrophages were successfully tolerized with two doses of post-transplant ECDI-SPs. In vitro culture showed that islets depleted of macrophages produced less cytokines at the mRNA



and protein levels. In particular, CCL3, CCL4, and CCL5 (RANTES) were all significantly decreased with macrophage depletion. These cytokines are ligands for CCR5, a chemokine receptor associated with islet allograft rejection. Depletion of donor macrophages resulted in decreased innate infiltration on POD 1. To investigate the possibility of donor-derived chemokines contributing to this change, control islets were transplanted into recipients treated with maraviroc, a pharmaceutical CCR5 inhibitor. We found that treatment of the recipient resulted in a decrease in POD 1 graft infiltration.

Conclusion

Depletion of donor macrophages strongly improves the ability of EC1D-SPs to produce delayed tolerization. We showed that donor macrophages are linked with production of CCL3, CCL4, and CCL5, all ligands of receptor CCR5. Targeting CCR5 with an inhibitor resulted in a decrease of innate graft infiltration similar to that seen with donor macrophage depletion. These results suggest that the beneficial effect seen from donor macrophage depletion may be caused in part by the reduction of donor-derived chemokines acting on recipient CCR5.

Abstract Keywords

Islet, macrophage, cytokines, tolerance

Poster # 2

Title: Complement-dependent regulation of the Memory B cell repertoire

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Memory B cell (Bmem) survival is essential for guarding against reinfection, yet the processes that ensure their longevity remain unclear. Understanding the mechanisms of B cell longevity are relevant to transplantation as Bmem are key participants in humoral rejection. In previous work we showed that germinal center (GC) B cells transcriptionally repress surface expression of the complement regulatory protein decay-accelerating factor (DAF, CD55), among other complement regulators, resulting in GC B cell C3a- and C5a-receptor signaling required for effective GC responses. As DAF is re-expressed on newly formed Bmem exiting the GC, herein we investigated the effects of deleting DAF on murine Bmem.

Methods

See below, included in results section.

Results

We adoptively co-transferred congenically marked, wild-type (WT) and DAF^{-/-} B cell receptor transgenic B1-8hi B cells (specific for nitrophenol, NP) into naïve congenic hosts. Following immunization with NP/ovalbumin, our kinetic flow cytometry analysis initially showed equivalent formation of WT and DAF^{-/-} Bmem but then uncovered a specific and progressive reduction in DAF^{-/-} Bmem numbers over six weeks ($p < 0.01$), without a change in total (WT + DAF^{-/-}) Bmem pool size. To isolate any potential effects of DAF deficiency to Bmem survival, four weeks after immunizing WT and DAF^{-/-} B1-8hi mice with NP/ovalbumin, we co-transferred WT and DAF^{-/-} Bmem into unimmunized congenic hosts in the absence of antigen. Over 7-14 d, flow analyses showed that the WT Bmem proliferated to sustain stable Bmem pool sizes, reflecting homeostatic proliferation (HP). In contrast, we observed reduced HP and increased cell death in DAF^{-/-} Bmem coupled with upregulation of Btg2 (RNAseq and flow), a transcriptional regulator of proliferation and immune cell death. Separately, following transfer into C3^{-/-} hosts, WT Bmem HP was augmented ($p < 0.05$), further linking systemic complement activation to inhibition of Bmem HP. These findings raised the intriguing concept that complement activation that occurs during immune responses could function as a bystander mechanism to reduce in Bmem HP, thereby regulating the existing Bmem repertoire composition to accommodate the newly developing Bmem induced by the vaccination. To test this, we adoptively transferred equal numbers of WT B1-8hi Bmem (and control naïve B1-8hi B cells) into 2 groups of naïve congenic hosts. Building upon studies that RNA-based vaccines (among other adjuvants) activate complement, we immunized one group of animals with an RNA vaccine to SARS CoV2 spike protein (WH-1, no cross reactivity to NP) and injected the control group with saline. We analyzed the B1-8hi Bmem in the recipients by flow cytometry 7-10 days later. Consistent with the previous experiments, in the control saline-treated recipients, the NP-specific Bmem underwent HP and expanded. In contrast, administration of the WH-1 vaccine prevented B1-8hi Bmem proliferation/expansion ($p < 0.05$ vs control). We observed no effects on transferred naïve B cells in either group demonstrating specificity for Bmem.

Conclusion

Together, the results confirm an under-appreciated role for HP in maintaining Bmem longevity. Moreover, the data newly indicate that complement activation initiated by vaccination (and likely during other immune responses), prevents Bmem HP to "make room" for newly forming Bmem. These fundamental observations have important implications for designing therapies aimed at altering Bmem repertoires in response to immunological stimuli, including those induced by vaccines, infections, autoimmune diseases and transplantation.

Abstract Keywords

B cells, memory, humoral immunity

Poster # 3

Title: Blocking cGAS-STING to Promote Heart Transplantation Survival in Mice

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro



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Abstract Title

Blocking cGAS-STING to Promote Heart Transplantation Survival in Mice

Abstract Body: Background

Inflammation caused by ischemia-reperfusion injury (IRI) remains a major challenge in solid organ transplantation. IRI triggers cell death induced DNA release and activation of the cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway, amplifying inflammation and accelerating rejection. TREX1, a cytosolic exonuclease, degrades cytosolic DNA and limits cGAS-STING activation. Previous work by our group showed that TY1, a novel non-coding RNA that can be administered via nanoparticles, upregulates TREX1 in macrophages and reduces post-ischemic injury in murine myocardial infarction models. We hypothesize that TY1 reduces IRI-driven inflammation via a TREX1-dependent mechanism that limits cGAS-STING activation and thereby promotes graft survival.

Methods

We transplanted BALB/c hearts heterotopically into B6 recipients treated for 3 days prior to transplant with 0.19 µg/g of TY1 or saline (n=6-10/group). After surgery, recipients were treated daily with either TY1 or saline for 7 days then twice weekly. 150 mcg of CTLA4-Ig was administered intraperitoneally on post-op day 2. Graft survival was monitored by palpation, with rejection defined as cessation of heartbeat. A subset of animals (n=3 per group) was sacrificed on day 14 for phenotypic analysis of heart and splenic lymphocytes. TREX1 expression was quantified by RT-qPCR. Statistical significance was defined as p-value < 0.05.

Results

TY1+CTLA4-Ig significantly prolonged median survival (35 vs. 18 days with CTLA4-Ig alone, p< 0.05). On day 14, TY1+CTLA4-Ig animals show significantly reduced graft infiltrating IFN-γ and TNF-α secreting CD8+ T cells. Analysis of intra-graft macrophages showed higher frequencies of LY6Clo (regulatory) macrophages and significantly lower frequencies of LY6Chi (proinflammatory) macrophages (p< 0.05). TY1 significantly increased TREX1 expression.

Conclusion

Our findings support the conclusion that TY1 administration reduces early posttransplant inflammation likely via limiting cGAS-STING activation. As TY1 under review by the FDA for use in humans, our findings support the need for testing this approach to improve human transplant outcomes.

Abstract Keywords

TREX1, cGAS-STING pathway, Non-coding RNA

Poster # 4

Title: Deciphering the Secret Handshake Between T cells and APC Required for Tolerance Induction via CD154 Blockade

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Costimulation blockade (CoB)-based immunotherapy is a very promising approach for transplantation, especially with the ongoing trials of improved blockers of the CD40/CD154 pathway. However, factors determining CoB efficacy remain poorly understood, limiting the capacity for clinical optimization. In this regard, different antigen presenting cells and associated anti-inflammatory cytokines have been implicated in the tolerogenic process of CD154 blockade: B cells and IL-10, as well as cDC1 and TGFβ. However, we don't know the T cell-intrinsic factors that determine CoB success. We then aimed at elucidating the role that direct signaling of IL-10 in T cells has on the outcome of CoB therapy.

Methods

C57BL/6 (B6) mice with T cell-restricted expression of a dominant negative IL-10 receptor (10R-DN) received Balb/c skin transplants and were compared to wt B6 mice. Recipients received peri-transplant donor specific infusion (day 0) and three anti-CD154 mAb doses (days 0, 7, 14). The phenotype and behavior of wt and 10R-DN T cells under modulation by CoB were analyzed ex vivo via flow cytometry and in vitro functional assays.

Results

Unmanipulated 10R-DN recipients rejected their transplant with dynamics identical to those of wt animals. However, graft survival in 10R-DN could not be promoted by CoB (median survival 29 vs 88 days in 10R-DN vs wt recipients respectively) revealing a novel and important effect of IL-10 signaling directly on CD4 T cells. This “uncontrolled” rejection correlated with increased production of TNF-α, IFN-γ and IL-17 by T cells in draining lymphoid tissues. MLR experiments outlined that lack of IL-10 signaling



did not diminish the ability of anti-CD154 to modulate alloreactive T cell proliferation. However, the absence of this pathway impaired the regulation of TH1 differentiation. Critically, the lack of IL-10 signaling eliminated completely the conversion of T cells into Tregs. Interestingly, when tested in a standard Treg induction assay (activation in the presence of TGF β), 10R-DN T cells showed preserved responsiveness to exogenous TGF β . This result indicates that IL-10 signaling in T cells is a prerequisite for the successful actuation of Treg induction ascribed to CD154 blockade.

Conclusion

Overall, these findings reveal IL-10 signaling directly in T cells is a prerequisite for the therapeutic efficacy of CoB, adding an important block to our understanding of this approach. Rather than functioning through passive blockade of costimulation, successful CoB requires active tolerogenic signaling involving coordinated production and sequential signaling of IL-10 and TGF β in T cells. Given known polymorphism in the IL-10 signaling pathway, our study identifies critical patient selection criteria and suggests that complementary interventions targeting IL-10 responsiveness may be necessary to optimize CoB protocols in clinical transplantation.

Abstract Keywords

Tolerance; Costimulation Blockade; T Lymphocytes; IL-10 signaling; Regulatory T cells; Skin Transplant

Poster # 5

Title: Targeting Alloreactive CD4+ Peptide MHC-II Using a Novel pMHC-5M-CAR Mimotope Library

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Allogeneic transplantation is limited by robust T cell-mediated responses that drive allograft rejection and graft-versus-host-disease (GVHD). Current transplant regimens require life-long immunosuppression to inhibit T cell mediated pathology. This approach is incompletely effective and results in increased susceptibility to allogeneic peptide-MHC complexes, underlines these complications. A major gap in therapy is the inability to selectively target alloreactive T cells, forcing prophylactic treatment for organ rejection and GVHD to rely on broad lymphocyte suppression. The critical need is to identify specific peptide-MHC determinants of alloreactivity and define approaches for their selective targeting. The central hypothesis is that clinically relevant alloreactive T cells can be selectively

identified and eliminated to reduce transplant pathology without broad immunosuppression. We hypothesize that selective targeting of T cells capable of direct alloreactivity, recognition of non-self MHC, is dependent on the specificity of peptide antigens presented by the allogeneic MHC. To test this, we utilize a I-Ab peptide mimotope display library generating a 9-mer peptides with defined I-Ab anchor residues in MHC anchor positions 1, 4, 6, and 9 with random use of all 20 naturally occurring amino acids in TCR contact positions 2, 3, 5, 7, and 8 in combination with a 5-module Chimeric Antigen Receptor System using I-Ab as the receptor (pMHC-5M-CAR) developed in the Kuhns laboratory. To test this system and define alloreactive responses against I-Ab, the peptide mimotope library and pMHC-5M-CAR were co-transfected into TCR deficient 58ab- thymoma cells stably expressing NFAT-GFP reporter (58.NFAT, gift of Ken Murphy, Washington University). Using this system, we demonstrate specific recognition of I-Ab by allogeneic BALB/C H-2d and CBA (H-2k) splenic CD4+ T cells. Identification of GFP+ 58.NFAT.pMHC-5M-CAR cells enables FACS isolation and high-throughput sequencing of the library of mimotope peptides driving responses against allogeneic I-Ab by CD4+ T cells from different mouse allotypes. We have subsequently introduced the peptide mimotope library and pMHC-5M-CAR into primary BALB/c CD8+ cells to enable the targeted killing of I-Ab-reactive T cells. We are currently testing this system for the ability to inhibit in T cells alloreactivity as well as inhibit GVHD in parent-to-F1 model and rejection of MHC-mismatched heterotopic heart transplants.

Methods

1. We propose using the described peptide display system to enable antigen-specific targeting of alloreactive CD4+ T cells. Given the demonstrated importance of peptide specificity in T cell alloreactivity, we hypothesize that presentation of a broad and diverse repertoire of allo-pMHC ligands will be essential for targeting of alloreactive T cells by 5M-CAR-CTLs. To test the importance of presented peptide diversity in targeting alloreactive T cells using the 5M-CAR-CTL system, we will generate I-Ab-expression construct libraries with scaled peptide mimotope diversity (libraries encoding 2.6x10⁸, 2.6x10⁶, 2.6x10⁴, and 2.6x10² peptides). Singular expression of OVA:I-Ab will be used as a 0 diversity control. OVA:I-Ab will also be included in the mimotope library pools as an internal positive control for 5M-CAR-CTL function (measuring frequency of OVA:I-Ab tetramer reactivity after 5M-CAR-CTL co-culture to test antigen-specific elimination). I-Ab peptide mimotope 5M-CAR-CTLs will be generated in BALB/c (H-2d) CD8+ T cells via transfection with 3 polycistronic retrovirus constructs as previously described²³. Transfected cells will be co-cultured at graded effector:target (E:T) ratios ranging from 0.1:1 to 10:1 with 10⁶ splenic CD4+ T cells from immunologically naive BALB/c mice. After 16 h co-culture, CD4+ splenic T cells will be recovered by paramagnetic bead enrichment and tested for alloreactivity in 1-way MLR using CFSE-dilution and ELISPOT assays (Table 1). Elimination of OVA-specific CD4+ T cells will be measured using OVA:I-Ab tetramers to demonstrate efficacy of experimental conditions⁶³. Experiments will be performed in triplicate replicates for confident quantitative assessment of reductions in CD4+ T cell alloreactivity. We will use adult (6-12 wk) mice for all experiments to eliminate potential confounding variables that could arise from age-related changes to the T cell repertoire. We plan to use equal numbers of male and female mice, as sex-related differences in direct T cell alloreactivity have not been identified, though responder and stimulator cells will be sex-matched in experiments to avoid potential confounders of Y-chromosome-derived minor histocompatibility antigens.
2. We hypothesize that 5M-CAR-CTLs can be effectively used for targeted elimination of alloreactive CD4+ T cells in vivo. To test this hypothesis, we will generate I-Ab peptide mimotope 5M-CAR-CTLs



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in BALB/c CD8+ T cells as described in Aim I.A (using the same mimotope library constructs). Transfected cells (6x10⁶)23 will be adoptively transferred into immunologically naive BALB/c mice, and 7 d later CD4+ splenic T cells (isolated by bead enrichment) from treated and untreated mice will be recovered for in vitro measurement of alloreactivity in 1-way MLR using CFSE-dilution and ELISPOT assays (Table 1). Elimination of OVA-specific CD4+ T cells will be measured using OVA:I-Ab tetramer, as an internal positive control. Experiments will be performed in 3 mice in each group in triplicate replicate experiments using sex-matched, adult male and female mice, with the rationale as described in Aim I.A.

Results

This specific CAR system, known as the biomimetic five-module chimeric antigen receptor (5MCAR), has been successfully used to target and eliminate pathogenic CD4+ T cells responsible for type 1 diabetes in a mouse model. The engineered 5MCAR cytotoxic T lymphocytes rapidly found and eradicated autoreactive T cells, preventing diabetes onset and reducing pancreatic damage in treated animals, with long-term engraftment suggesting durable protection against disease development. Preliminary data from matched and mismatched haplotype mixed lymphocyte reaction experiments demonstrate consistent upregulation of alloreactivity, as measured by GFP expression from the NFAT reporter 58ab- cell line, in mismatched samples compared with matched and negative controls. This pattern indicates that the NFAT-driven reporter system sensitively reflects T cell activation and allogeneic response in vitro, supporting the validity of the assay for assessing alloreactive T cell function in this model.

Conclusion

The 5MCAR system originally developed for targeting pathogenic CD4+ T cells in type 1 diabetes now shows promising applicability for measuring alloreactivity in the context of transplant immunology using matched and mismatched haplotype CD4+ mixed lymphocyte reaction experiments described in the abstract. The consistent upregulation of NFAT-driven GFP expression in mismatched versus matched and negative controls indicates effective detection of alloreactive T cell responses. Ongoing experiments to optimize CD8+ T cell transduction with the mimotope library aim to enhance the precision and efficacy of this system, potentially providing a powerful tool for immune monitoring and therapeutic intervention in transplantation settings.

Abstract Keywords

Alloreactive T cells Peptide-MHC specificity Chimeric antigen receptor (CAR) Peptide mimotope library NFAT-GFP reporter assay Direct alloreactivity CD4+ T cells CD8+ T cells Transplant immunology Haplotype mismatched Mixed lymphocyte reaction (MLR) Immune targeting Transplant tolerance Graft rejection inhibition

Poster # 6

Title: Recipient TLR9 Is Required for Endogenous Donor-Reactive Memory CD8 T Cell Proliferation and Effector Function to Mediate High Risk Allograft Rejection

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

Author(s)

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Abstract Body: Background

Donor-reactive memory T cells are a major barrier to successful solid organ transplants. We previously reported that increasing duration of cold ischemic storage (CIS) from 0.5 to 8 hrs prior to transplant of heart allografts in unsensitized mice increases early innate and memory T cell infiltration into the graft with increased p40 homodimer (HD)/IL-15 induced donor-reactive heterologous memory CD8 T cell proliferation to mediate CTLA-4Ig-resistant rejection of the high-risk allografts. Administration of exogenous p40 HD induces IL-15 production driving donor-reactive heterologous memory CD8 T cell proliferation within low ischemic allografts but did not increase their expression of effector functions to mediate acute graft injury. On this basis, we hypothesized that the increased ischemic environment in high-risk allografts provided mechanistic components provoking the memory CD8 T cells to both proliferate and express effector functions mediating acute graft injury and that a pathogen recognition receptor induced by ischemia/reperfusion injury is likely to drive this increased intra-graft alloimmune response.

Methods

To test this, we transplanted complete MHC mismatched A/J heart allografts subjected to 8 hrs CIS into wild type or TLR9^{-/-} B6 mice.

Results

High ischemic allografts in B6.TLR9 deficient recipients had marked decreases in macrophage and Ly6C+ monocyte infiltration and memory CD4 and CD8 T cell proliferation on day 2 post-transplant with extended survival in CTLA-4Ig conditioned recipients (MST 42 days vs. 27 days in CTLA-4Ig conditioned wild type recipients). Depletion of recipient CD11b+ cells using diphtheria toxin treatment of CD11b-DTR/TLR9-sufficient recipients abrogated the early donor-reactive heterologous memory CD4 and CD8 T cell proliferation within the high risk allografts and CTLA-4Ig resistant rejection (MST 49 days vs. 24 days in CTLA-4Ig conditioned recipients treated without DT). Memory CD8 T cell expression of effector function related genes (IFN- γ , FasL and Granzyme B mRNA) was increased in the high vs. low ischemic allografts in wild type but not TLR9-deficient recipients. TLR9 agonist oligonucleotide promoted the heterologous donor-reactive memory CD8 T cell proliferation within low ischemic allografts that was accompanied by increased macrophage and Ly6C+ monocyte infiltration vs. non-treated recipients. Interestingly, deficiency of recipient TLR9 had no impact on the de novo priming of donor-reactive T cells or their ability to mediate graft rejection on day 7-9 post-transplant in the absence of CTLA-4Ig conditioning.

Conclusion

These results indicate that myeloid cells infiltrating high ischemic allografts are activated through TLR9 signaling to provide factors increasing early post-transplant inflammation that promote donor-reactive heterologous memory CD8 T cells proliferation and expression of effector functions mediating acute graft injury and CTLA-4Ig resistant rejection.



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Abstract Keywords

Inflammation, Ischemia/reperfusion injury, CTLA-4lg, Co-stimulation blockade, Memory T cells

Poster # 7

Protection of Transplanted Liver Function by Regulation of Na⁺/K⁺-ATPase Activity

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

Author(s)

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Abstract Body: Background

Liver transplantation is the final treatment option for patients with end-stage chronic or severe acute liver diseases. Ischemia–reperfusion injury (IRI) remains a major obstacle to graft survival. Na⁺/K⁺-ATPase dysfunction, an early and critical consequence of ischemia caused by ATP depletion, may contribute significantly to IRI. Therefore, preserving Na⁺/K⁺-ATPase activity could be a key strategy for protecting liver grafts. We have developed a novel technique, the improved Synchronization Modulation Electric Field (i-SMEF, patent pending), which preserves Na⁺/K⁺-ATPase function and enhances ATP generation and may consequently protect the liver graft from ischemia injury.

Methods

We evaluated i-SMEF in both ex vivo and in vivo models. Mouse livers were preserved in cold Histidine–Tryptophan–Ketoglutarate (HTK) solution for 10 hours with or without continuous i-SMEF stimulation (150 Hz). Biopsy collected to assess ATP content, mitochondrial function Pig pre-ischemic livers underwent 6 hours of machine perfusion with or without continuous i-SMEF stimulation, with perfusate and tissue collected to assess graft viability. For in vivo studies, orthotopic liver transplantation (OLT) was performed in male C57BL/6 mice (10–12 weeks, 30–35 g, n=5/group). Blood samples were collected at baseline and at 1, 3, and 7 days post-transplant for liver chemistry analysis, and liver tissue was examined on day 7 for histology. In addition, i-SMEF was tested in a warm ischemia–reperfusion model using female Yorkshire pigs (35–45 kg, n=3/group). Liver injury and function were assessed by plasma chemistry and histological evaluation.

Results

In the mouse ex vivo model (n=5 mice/group), i-SMEF significantly preserved ATP levels, maintaining 74% and 52% of basal ATP in liver tissue after 1 and 2 hours of preservation, respectively, compared to 42% and 21% in controls. Even after 6 hours, i-SMEF–treated livers retained ~35% of ATP, whereas control livers showed nearly complete depletion. Mitochondrial function was

also better preserved, with 80–130% higher ATP production and maximal respiration relative to controls. In the ex vivo porcine liver preservation study (n=4 pigs/group), i-SMEF demonstrated a synergistic effect when combined with NOMP, as the NOMP+i-SMEF group showed ~50% less injury than controls based on AST levels and H&E staining, compared to ~30% with i-SMEF alone and ~18% with NOMP alone. In the porcine liver ischemia reperfusion model, i-SMEF–treated livers exhibited 33–49% lower liver enzyme levels after 6 hours of reperfusion compared with controls. Histological analysis confirmed reduced sinusoidal dilation, hepatocyte necrosis, and cell death. Finally, in the mouse OLT model, recipients of i-SMEF–treated standard criteria donor (SCD) livers showed 43–51% lower ALT and AST levels, while recipients of i-SMEF–treated donation after circulatory death (DCD) livers exhibited even greater reductions (58–67%), along with reduced inflammation.

Conclusion

i-SMEF application during donor liver preservation maintains Na⁺/K⁺-ATPase activity, reduces ischemic injury, and improves graft function in both mouse and porcine models. These findings suggest i-SMEF as a promising therapeutic strategy to mitigate ischemia–reperfusion injury in liver transplantation.

Abstract Keywords

Liver, ischemia reperfusion injury, machine perfusion, graft function, Synchronization Modulation Electric Field, Na⁺/K⁺-ATPase

Poster # 8

Title: Graft-Derived IL-33 and Peripheral CD4 T Cells Cooperatively Drive Thymopoiesis After ATG-Induced Lymphoablation

Poster Topic: Basic Immunobiology: preclinical models in vivo and in vitro

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Abstract Body: Background

Anti-thymocyte globulin (ATG) is the most widely used lymphoablative induction therapy, applied to more than 75% of kidney recipients in the U.S. ATG eliminates donor-reactive T cells, but outcomes are determined not simply by depletion efficacy, but also by T cell recovery rate. Homeostatic proliferation of memory T cells promotes rejection, while de novo thymopoiesis restores protective naïve T cell repertoires. We previously showed that graft inflammation is an important driver of T cell reconstitution, but underlying mechanisms and relevant danger associated molecular patterns (DAMPs) and cytokines remain largely unknown. The goal of the current study is to test the effects of graft-derived alarmin IL-33 on recipient T cell reconstitution after lymphoablation in transplant recipients.

Methods

We used a murine heterotopic heart transplantation model and murine ATG treatment for the following donor/recipient strain combinations: BALB/c (H-2d) to B6.WT, BALB/c to B6.ST2^{-/-}, BALB/c to B6.IL-33^{-/-}, B6.WT to BALB/c, B6.IL-33^{-/-} to BALB/c and B6.IL-33^{-/-} to B6. CD4CreST2fl/fl and CD4Cre littermates were used to determine whether T cell expression of ST2 is required T cell recovery. To test whether ST2 on B cells is required for T cell recovery, we generated mixed bone marrow chimeras with B-cell-specific ST2 deletion. To test the possibility of direct ST2 signaling driving CD8 T cell expansion, we co-injected CD45.1+ B6.WT and CD45.2+ B6.ST2^{-/-} CD8 T cells into CD45.1/2 recipients followed by heart transplantation and ATG. Flow cytometry was used to quantify thymocyte subsets (DN, DP, SP CD4+, SP CD8+) and splenic RTEs (Recent Thymic Emigrants) (CD24hi Qa2lo).

Results

Recipient ST2 deficiency impaired CD8 T cell reconstitution and prolonged heart allograft survival. The defect in CD8 T cell recovery was also observed in ST2 deficient isograft recipients demonstrating that IL-33/ST2 signaling supports homeostatic T cell expansion rather than alloantigen driven responses. ST2 expression on either B or T cells was not required for rapid T cell recovery. Donor IL-33 expression proved essential: BALB/c recipients of B6.IL-33^{-/-} grafts demonstrated significant decrease in T cell reconstitution and extended heart allograft survival. The absence of donor IL-33 also impaired T cell recovery in isograft recipients. This was associated with reduced numbers of RTEs in the recipient spleen, 70–80% reduction in thymic cellularity, and blocked DN to DP progression of thymocyte development. We have previously reported that depletion-resistant CD4 T cells re-entered the thymus after ATG and enhanced thymopoiesis. The migration of peripheral CD4 T cells into the thymus was diminished in the absence of donor IL-33.

Conclusion

Taken together, our results identify a novel graft-to-thymus communication axis. We propose that graft-derived IL-33 conditions the thymic stroma, enabling CD4 T cell recruitment and promoting thymic regeneration. These findings provide insight into a new mechanism of T cell reconstitution after ATG treatment in allograft recipients, and identify IL-33/ST2 as a therapy target pathway to improve the efficacy of lymphoablation in transplant recipients.

Abstract Keywords

ATG, IL-33/ST2, Thymopoiesis, RTEs(Recent thymic emigrants)

Poster # 9

Title: Older circulatory death donor liver grafts show increased utilization with machine perfusion

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Machine perfusion (MP) has become essential in liver transplantation, especially for donation after circulatory death (DCD) donors. Both in situ normothermic regional perfusion (NRP) and ex situ MP improve graft outcomes; however, their clinical implementation remains inconsistent. This study evaluates procurement techniques and preservation strategies to identify predictors that maximize liver graft utilization among DCD donors.

Methods

We analyzed Organ Procurement and Transplantation Network (OPTN) database for all DCD heart transplanted donors from October 2020 to December 2024. Donors were categorized by procurement method: super-rapid recovery (SRR) versus thoraco-abdominal NRP, and by preservation method: static cold storage (SCS) versus MP. Grafts were all recovered for utilization and were classified based on utilization status (Transplanted vs Not Transplanted). Donor characteristics and utilization rates were compared using logistic regression analysis.

Results

A total of 1,327 liver grafts were recovered from DCD donors, with an overall utilization rate of 87% (1,154 grafts transplanted; 173 not transplanted). Median donor age was higher for grafts treated with NRP-MP (33 vs. 31 years; $P = 0.039$). Transplanted graft donors were younger on average compared to non-transplanted donors (31.1 ± 9.4 vs. 32.67 ± 9.2 years; $P = 0.04$). Lower macrosteatosis, absence of fibrosis, and absence of heavy alcohol consumption correlated with higher utilization ($P \leq 0.022$). SRR-SCS showed the highest non-utilization rate (28.5%), whereas adjunct perfusion methods significantly reduced non-utilization to 3.4% (SRR-MP), 8.0% (NRP-SCS), and 4.2% (NRP-MP; $P < 0.001$). Biopsies were performed in 32% of recovered grafts, significantly more frequent among non-utilized grafts (47.1%) than utilized grafts (29.9%; $P < 0.001$). Multivariable analysis showed biopsied grafts even with minimal pathology had lower odds of utilization compared to unbiopsied grafts (OR range 0.04–0.50; all $P \leq 0.011$). Each additional year in donor age raises the odds of utilization with adjunct machine perfusion by 5-9% compared to SRR-SCS ($P < 0.001$).



Conclusion

MP, particularly with NRP, significantly improves utilization of DCD livers, especially from older donors traditionally considered marginal. Integration of macroscopic evaluation and MP viability assessment to reduce biopsy reliance may further optimize the liver graft utilization rate from DCD donors.

Abstract Keywords

Liver transplantation, machine perfusion, donation after circulatory death

Poster # 10

Title: Successful Use of Belatacept as Short-term Renal Rescue Immediately Post Lung Transplantation

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Belatacept is a fusion protein that binds to CD80 and CD86 preventing costimulation with CD28 thus preventing T cell activation. It is approved for use in kidney transplant only, and limited published data is available in lung transplantation. In this article, we present two cases of belatacept use in lung transplant as a method for renal rescue in place of tacrolimus.

Methods

Single-center experience on the use of belatacept as a CNI sparing therapy to allow for renal recovery.

Results

Patient #1 is a 65-year-old Hispanic male who received a bilateral lung transplant secondary to ILD. He received induction with basiliximab and steroids. Maintenance immunosuppression included mycophenolic acid 1gm BID starting post-operative day (POD) 0, a prednisone taper, and tacrolimus starting POD 3 with a trough goal of 12-15 ng/mL. His post-operative course was complicated by hemorrhagic shock resulting in acute kidney injury immediately after surgery. The patient required continuous renal replacement therapy on POD 5 due to worsening azotemia and hyperkalemia. The post-operative course was also complicated by epileptiform discharges and generalized periodic discharges (GPDs). Unfortunately, renal function continued to worsen, and neurological status did not improve. Therefore, the decision was made to discontinue tacrolimus and switch the patient to a triple immunosuppression regimen with mycophenolic acid, prednisone, and belatacept starting on POD 22. Creatinine at the time of belatacept initiation was 2.68 mg/dL and GFR was 26. Belatacept 10mg/kg was given on day 0,

4, 14, and 28 then monthly thereafter. On POD 31 creatinine had improved to 1.18 mg/dL and GFR >60. The patient was transitioned back to tacrolimus on POD 98 and belatacept was discontinued. His renal function remained stable, and subsequent biopsies remained negative for acute cellular rejection (ACR) and antibody mediated rejection (AMR) at 6 months post-transplant. DSAs remained negative. Patient #2 is also a 65-year-old Hispanic male who received a bilateral lung transplant secondary to combined pulmonary fibrosis and emphysema. He received induction with basiliximab and steroids. Maintenance immunosuppression included mycophenolic acid 1gm BID starting POD 0, prednisone taper, and tacrolimus starting POD 3 with a trough goal of 12-15 ng/ml. The patient's post-operative course was complicated by sepsis and acute kidney injury related to perforated sigmoid diverticulitis with purulent peritonitis requiring sigmoid colectomy and diverting colostomy on POD 9. Creatinine had increased from 0.80 mg/dL to 2.11 mg/dL. The decision was made to hold tacrolimus and initiate belatacept 10mg/kg on POD 10 due to worsening acute kidney injury. He received three doses total at day 0, 4, and 14. After his last dose of belatacept, the patient's creatinine was 0.99 mg/dL with GFR >60. Tacrolimus was reinitiated on POD 34. His renal function remained stable until approximately 3 months post-transplant, at which time he developed stable chronic kidney disease related to calcineurin inhibitor therapy. Subsequent biopsies have been negative for ACR or AMR. DSAs remained negative.

Conclusion

Belatacept based immunosuppression in combination with mycophenolic acid and prednisone appears to provide an effective renal rescue option in the cases presented above without compromising risk of rejection at 3 and 6 months. More robust data is imperative to determine long term safety and efficacy of belatacept use in lung transplantation.

Abstract Keywords

immunosuppression belatacept IL2RA

Poster # 11

Title: Symptomatic gut dysbiosis is associated with chronic allograft dysfunction in pediatric kidney transplant recipients

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Post-transplant diarrhea (PTD), a frequent but neglected complication of kidney transplantation (KTX), may lead to serious adverse outcomes. As PTD is commonly caused directly by immunosuppressive drugs or infections it is sometimes difficult to track. Previously, we identified enteric patient-reported outcome measures (PROMs) to be associated with allograft dysfunction in pediatric KTX. While investigations in adult transplant recipients highlight the potential role of gut dysbiosis in the development of PTD, pediatric studies are missing. In this study we investigated the association between the gut microbiome, PROMs, and allograft dysfunction in pediatric KTX recipients.

Methods

This was a prospective observational pilot study during routine follow-up in pediatric KTX recipients at the Medical University of Vienna. PROM and symptom questionnaires, fecal and blood samples were collected cross-sectional at regular outpatient clinic visits. Fecal samples were analyzed with metagenomic shotgun sequencing. Taxonomies and metabolic pathways were identified using Metaphlan and Humann. Group comparisons were calculated with Mann Whitney U tests and permutational multivariate analysis of variance (alpha and beta diversity) and ANCOM-BC II (on individual species and metabolic pathway level of metagenomic sequencing data).

Results

We included a total of 16 pediatric KTX recipients. Patients were at median 15 years old (IQR 12-17 years), 4.7 years post-transplant (IQR 1.3-13.8 years), 10 were male, 11 received deceased donations, and 13 were on their first kidney allograft. We identified PTD in 25% of patients (4/16). In patients with PTD gut microbiome alpha diversity was significantly lower ($p=0.02$) than in those without. Beta diversity did not differ between these groups. In fecal samples of PTD patients *Clostridium innocuum* ($p=0.0016$) and *Bacteroides xylanisolvens* ($p=0.038$), among others, were significantly increased while *Faecalibacillus intestinalis* ($p=0.038$) and the metabolic pathway nucleotide synthesis ($p=0.004$) were significantly reduced. Higher abundance of *C. innocuum* was further significantly associated with worse allograft function ($p=0.045$) and the amount of nucleotide synthesis present within the gut microbiome with patients' tacrolimus trough levels ($p=0.066$).

Conclusion

Previous studies have demonstrated tacrolimus inactivation by Clostridiales in vitro. Thereby, the observed increase of *Clostridium innocuum* in fecal samples of pediatric KTX recipients with PTD, alongside significantly reduced nucleotide synthesis (i.e., DNA damage repair) may potentially link symptomatic PTD to worse allograft outcomes. Here we demonstrated, for the first time, the potential benefits of PROM assessment in pediatric KTX recipients linking symptomatic gut dysbiosis to allograft dysfunction.

Abstract Keywords

pediatric, kidney transplantation, gut microbiome, dysbiosis, allograft dysfunction, patient-reported outcomes, patient-reported outcome measures, PROs, PROMs, diarrhea, post-transplant diarrhea

Poster # 12

Title: The endogenous self-peptide repertoire with high binding-strength to allogeneic MHC I in the serum of pediatric kidney transplant recipients correlates with minimized rejection-free immunosuppression

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Allograft rejection and adverse effects of immunosuppression limit long-term kidney graft and patient survival. With 10-year graft survival rate of 61-77%, pediatric kidney transplant (KTX) recipients require multiple kidney transplantations throughout their lifetime. Minimization of immunosuppression and tolerance could potentially solve this issue. In experimental mouse models, tolerance is dependent on endogenous self-peptides presented by the allogeneic MHC I. Here we investigated such tolerance-associated signatures within the serum self-peptide repertoire of pediatric KTX recipients.

Methods

This was a prospective observational pilot study in pediatric KTX recipients at the Medical University of Vienna. Serum samples were acquired cross-



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sectional during routine follow-up. The serum proteome was measured via mass spectrometry on an Orbitrap Exploris 480 with data-independent acquisition. Two groups of patients were defined and matched for the number of HLA-mismatches. One group constituted of patients with unusually low maintenance immunosuppression without rejection or donor-specific antibody development during follow-up and matched to a second group of patients with substantially higher immunosuppression and alloimmune events. MHC binding-strength for each protein was calculated using netMHCpan and high-resolution HLA typing. Differential abundance and associations were calculated using linear mixed-effects models for microarray data and proteomics and spearman correlations.

Results

We included a total of 3 pediatric KTX recipients with minimized immunosuppression and no alloimmune events during follow-up alongside 4 matched control patients in this study. The included patients were at median 15 years old (IQR 12-16 years), 6.7 years post-transplant (IQR 5.9-7.2 years), 57% received deceased donations, the median HLA-mismatch was 3 (IQR 2-3), and 90% were on triple immunosuppression. We identified 395 significantly increased (FDR $p < 0.05$) vs 509 decreased plasma proteins with high binding-strength to their mismatched allogeneic donor HLA I molecules in patients with minimized immunosuppression without alloimmune events (e.g., ALDOB, RPS18, RACK1). Enrichment analysis of these serum proteins indicates an increase in amide metabolism. A total of 31 proteins correlated negatively ($p < 0.01$) with tacrolimus trough levels (e.g., HOOK3).

Conclusion

Here we identified a serum protein signature based on the endogenous self-peptide repertoire with binding-capacity to mismatched donor HLA I in pediatric kidney transplant recipients without alloimmune events despite minimized immunosuppression. Proteins of this signature were found in tolerized murine kidneys (ALDO, RPS18), identified as key regulators of T-cell homeostasis (RACK1), Treg differentiation (amide metabolism), and in graft-vs-host-disease (HOOK3). This serum signature comprises first evidence of a potential biomarker signature to guide future interventional trials aiming to achieve operational tolerance in pediatric KTX.

Abstract Keywords

pediatric, kidney transplantation, kidney, tolerance, mhc, immunosuppression

Poster # 13

Title: Sirolimus Utilization in Pediatric Heart Transplantation: Experience from China

Poster Topic: Clinical studies in humans

Author(s)

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Abstract Body: Background

Sirolimus is widely used in adult heart transplantation for its pivotal roles in preventing allograft rejection, delaying the progression of cardiac allograft vasculopathy, and mitigating calcineurin inhibitor (CNI)-associated nephrotoxicity. However, its application in pediatric heart transplantation remains understudied. Existing literature is limited by small sample sizes, low-grade evidence, and inconsistent conclusions, while no experience from China has ever been reported.

Methods

A retrospective study was conducted involving 77 pediatric heart transplant recipients who underwent transplantation at Fuwai Hospital, Chinese Academy of Medical Sciences, from January 2015 to May 2024. Among these, 25 patients (32.5%) had documented sirolimus administration during follow-up. Transplant registry records, electronic medical charts, and longitudinal follow-up data were systematically reviewed to summarize the single-center experience of sirolimus utilization in pediatric heart transplantation.

Results

In the 25 patients treated with sirolimus, the median age at transplantation was 13 years (interquartile range [IQR], 10–15 years), and the median age at sirolimus initiation was 15 years (IQR, 11–17 years). Indications for sirolimus therapy included renal insufficiency and acute rejection. At the latest follow-up, 13 patients remained on continuous sirolimus treatment, all for managing renal insufficiency, with therapy initiated at a median of 25 months (IQR, 15–49 months) post-transplantation. The median preoperative serum creatinine was 67.9 $\mu\text{mol/L}$ (IQR, 61.3–90.1 $\mu\text{mol/L}$), which increased to 122.2 $\mu\text{mol/L}$ (IQR, 94.1–143.3 $\mu\text{mol/L}$) prior to sirolimus initiation; one year after treatment, the median serum creatinine decreased to 89.8 $\mu\text{mol/L}$ (IQR, 70.7–108.4 $\mu\text{mol/L}$). Five patients treated for acute rejection discontinued sirolimus after rejection resolution, with no recurrence observed during follow-up. One patient who initiated sirolimus on postoperative day 6 discontinued treatment due to impaired wound healing. Six patients developed infections leading to sirolimus withdrawal, including two fatal cases: both patients initiated sirolimus on postoperative day 7, with causes of death identified as acute pancreatitis and thrombotic microangiopathy, respectively.

Conclusion

Sirolimus demonstrates efficacy in pediatric heart transplantation for treating acute rejection and improving renal function. However, careful selection of treatment timing and dosage optimization are critical, and close monitoring for adverse events, is essential to ensure safety.

Abstract Keywords

Heart transplantation, Pediatric Transplantation, Immunosuppressive Therapy, Sirolimus



Poster # 14

Title: Pregnancy Outcomes Post-Renal Transplantation

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Renal transplants are becoming increasingly more common, and as care for transplant recipients continues to advance, so has the frequency of these patients experiencing pregnancy. Our study describes pregnancy outcomes in renal transplant recipients.

Methods

This was a single-center retrospective cohort of renal transplant recipients who underwent pregnancy at a tertiary referral hospital in Louisiana between January 2012 and June 2025. Baseline demographics, transplant information, kidney function, medical comorbidities, and pregnancy outcomes were collected through chart abstraction. Our primary maternal outcomes included pregnancy-induced hypertension (PIH), kidney function at the time of delivery, and the development of a CDC-defined severe maternal morbidity (SMM). Fetal outcomes included spontaneous miscarriage < 20 weeks (SAB), spontaneous preterm birth (sPTB), small for gestational age (SGA), and NICU admission. Outcomes in patients with and without prednisone prescription were compared, as well as patients undergoing pregnancy before and greater than 24 months post-transplant. Data were analyzed via student t-test for continuous variables and chi-squared for categorical variables where appropriate.

Results

29 pregnancies were noted from 19 identified patients. Intrinsic renal disease was the most common indication for kidney transplant (84%, n=16). 17 patients (89%) entered pregnancy >1yr post transplant, 15 > 2yr post transplant. 75% (n=14) of these patients entered pregnancy with pre-existing hypertension. Preeclampsia was a common complication (29%, n=17). 52% of patients underwent an unplanned cesarean section (n=15). Kidney function was not significantly altered by pregnancy (average decrease in creatinine 0.3, SD 0.41). Overall SMM was 10% (n=3), comprising only the need for blood transfusion. There were 3 (10%) first trimester SABs, 2 second trimester losses (7%), and 2 patients underwent termination (7%). Of the live births, there were 18 (62%) preterm and 4 (14%) term deliveries. No pregnancies >20 weeks resulted in stillbirth or neonatal death. 9 of the 22 liveborn infants (41%) were admitted to the NICU. Outcomes were compared between patients prescribed prednisone versus those who were not as part of their transplant regimen. Our data demonstrates an increased rate of

sPTB in patients not on prednisone (p=0.007) compared to those taking prednisone. There was no significant difference in the development of PIH, gestational diabetes, or the development of an SMM. Similarly, there were no differences in the development of SGA infants nor for SAB rates. Baseline demographics were similar between the two groups except for race, noting that patients self-identifying as Black were less likely to be prescribed prednisone (p=0.027). Outcomes were also compared between patients who were pregnant less than two years after transplantation (n=4) to those who were more than two years past their transplant (n=15); no statistically significant differences in pregnancy outcomes were noted.

Conclusion

Renal transplant patients undergoing pregnancy have high rates of preeclampsia, preterm birth, and unplanned cesarean section, but overall low SMM. Kidney function was not clinically impacted by pregnancy in this cohort. Babies born to transplant recipients have high NICU admission rates. The addition of prednisone may be associated with a reduced risk of preterm birth and is not associated with increased risk of SGA. Delaying pregnancy for more than 24 months does not appear to improve outcomes.

Abstract Keywords

Renal transplantation, pregnancy outcomes, prednisone, preeclampsia, preterm birth, unplanned cesarean section

Poster # 15

Title: Mucosa-Associated Microbial and Fut2 Genetic Signatures Correlate with Clinical Outcomes in Patients with Primary Sclerosing Cholangitis

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Primary Sclerosing Cholangitis (PSC) is a progressive hepatobiliary disorder influenced by complex interactions between host genetics, mucosa-associated microbiota, and systemic inflammation. This study aimed to identify microbial taxa and FUT2 non-secretor genotype associations with clinical biomarkers of inflammation, liver injury, electrolyte disturbances, prognostic indices, and mortality in PSC patients.



Methods

Pre-transplant colonic mucosal biopsies from PSC patients were analyzed by 16S rRNA gene sequencing, and FUT2 genotyping (rs601338) was performed to determine non-secretor status. Correlative analyses examined relationships between microbial abundances, FUT2 genotype, inflammatory markers (e.g., CRP), liver enzymes, electrolytes, prognostic scores (MELD, Mayo Risk Score, Amsterdam-Oxford Model), posttransplant bacteremia incidence, and mortality.

Results

The FUT2 non-secretor genotype was strongly associated with intestinal bacteremia ($r = 0.432$, $p = 0.001$) and moderately correlated with increased postoperative mortality ($r = 0.257$, $p = 0.016$). Non-secretor status also correlated with elevated markers of liver injury and cholestasis, including total bilirubin ($r = 0.216$, $p = 0.045$), gamma-glutamyl transferase (GGT; $r = 1.001$, $p = 0.013$), and aspartate aminotransferase (AST; $r = 0.214$, $p = 0.040$), as well as reduced hemoglobin ($r = -0.247$, $p = 0.021$) and increased platelet counts ($r = 0.317$, $p = 0.003$), indicating systemic inflammation and hepatic dysfunction. Mucosal microbial profiles were intricately linked to clinical biomarkers. Actinomycetales and Rhodobacterales abundances correlated positively with elevated C-reactive protein (CRP), AST, total bilirubin, MELD scores, and mortality, suggesting these taxa are associated with inflammation, hepatocellular injury, and poor prognosis. In contrast, Flavobacteriales showed inverse correlations with CRP ($r = -0.341$, $p = 0.005$), AST, and mortality ($r = -0.250$, $p = 0.019$), indicating potential protective or anti-inflammatory roles. CRP levels positively correlated with Actinomycetales and negatively with Lactobacillales and Flavobacteriales, linking microbial shifts to systemic inflammation. Electrolyte levels were also influenced by microbial composition: serum calcium and potassium positively correlated with Escherichia/Shigella and Flavobacteriales, and negatively with Actinomycetales and Rhodobacterales. Sodium levels were positively associated with Lactobacillales and negatively with Rhodobacterales, Caulobacteriales, and Pseudomonadales, reflecting the interplay between gut microbes and fluid-electrolyte regulation. Prognostic indices, including MELD, Mayo Risk Score, and the Amsterdam-Oxford Model, were positively associated with taxa enriched in advanced disease and cholestasis (e.g., Actinomycetales, Rhodobacterales) and negatively with Enterobacteriales (Escherichia/Shigella). Bacteremia was significantly associated with recurrent PSC diagnosis ($r = 0.275$, $p = 0.010$), elevated CRP ($r = 0.298$, $p = 0.004$), and increased mortality ($r = 0.278$, $p = 0.009$), underscoring its clinical relevance as a marker of disease severity.

Conclusion

Our findings demonstrate that mucosa-associated microbiota and FUT2 non-secretor genotype are intricately linked to inflammation, liver injury, electrolyte imbalance, prognostic indices, and mortality in PSC. These results highlight the interplay of host genetics and mucosal microbiota in disease progression and identify potential biomarkers and therapeutic targets for personalized PSC management.

Abstract Keywords

Primary Sclerosing Cholangitis, mucosal microbiome, FUT2, bacteremia, liver injury, systemic inflammation, prognostic scores, mortality

Poster # 16

Title: Donation After Circulatory Death: Insights from Donor and Non-Donor Comparisons in a Local Cohort

Poster Topic: Clinical studies in humans

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Abstract Body: Background

Donation after circulatory death (DCD) significantly expanded the availability of donor organ grafts. Despite this, DCD donation presents unique logistical and clinical challenges as patients must undergo withdrawal of life-sustaining measures (WLSM) and declaration of death prior to graft procurement. Determining which patients are likely to become organ donors remains challenging. Furthermore, there is a paucity of literature exploring quality control measures employed during this process. The aim of this study was to explore predictors of successful DCD donation and quality control measures employed during the DCD process.

Methods

We prospectively enrolled 35 patients undergoing WLSM at Vancouver General Hospital between May 2023 and December 2024. We subsequently conducted retrospective chart review and data collection. Patients were categorized as DCD donors or non-donors. Data collected included demographic variables, primary diagnosis, cause of death, comorbidities, lab values preceding WLSM, and documented rationale for procurement outcomes. Laboratory values (e.g., creatinine, liver enzymes, arterial blood gases) and other continuous variables were compared between donor and non-donor groups using unpaired t-tests or Mann-Whitney U tests as appropriate. Categorical variables, including cause of death and comorbidities, were compared between donor and non-donor groups using Chi-square or Fisher's exact tests as appropriate.

Results

The average age of patients was 58.81 ± 14.67 years ($n = 32$), with 7 females (21.88%) and 25 males (78.13%). Of the 32 patients, 15 (46.88%) died of hypoxic-ischemic brain injury, 5 (15.63%) from intracranial hemorrhage, 6 (18.75%) from sepsis, and 6 (18.75%) from traumatic brain injury. Only 8 (25%) patients proceeded to successful organ procurement, with 9 attempted procurements, while 24 (75%) did not. Preliminary analysis suggests that there were no statistically significant differences in baseline lab values preceding WLSM between donors and non-donors ($p > 0.05$). However, of the 24 patients who did not proceed to organ donation, documentation of the medical rationale and exclusion criteria for procurement decisions was incomplete. Specifically, 1 (4.17%) patient



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had exceeded the warm ischemic time limit, 2 (8.33%) had medical contraindications, and the substitute decision maker declined for 8 patients (33.33). For the remaining 13 patients (54.17%), there was no clear documentation for why they did not proceed to procurement. Furthermore, documentation of the donated organs was found for only 3 (37.5%) of the 8 successful procurements.

Conclusion

Our findings highlight variability and gaps in the documentation of eligibility assessment and procurement outcomes in patients considered for DCD organ donation. Standardizing eligibility assessment templates and mandatory rationale fields in electronic medical records may improve clarity, facilitate QI monitoring, and benefit the identification of suitable DCD donors. Future work will focus on implementing and evaluating a standardized documentation framework to support consistent decision-making and maximize organ donation opportunities.

Abstract Keywords

Donation after circulatory death, organ donation, eligibility assessment, quality improvement, documentation, demographics

(1) Poster # 17

Title: Achieving and Maintaining Target Immunosuppression Levels by Combined Pharmacogenetic and Torque Teno Viral Load Testing

Poster Topic: Data/Science

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Abstract Body: Background

Tacrolimus is an immunosuppressant commonly prescribed to kidney transplant patients. We evaluated the feasibility of a novel approach for personalizing tacrolimus treatment by combining pharmacogenetic typing obtained pre-transplant with post-transplant monitoring of immune response levels using a common non-pathogenic virus as a biomarker. Tacrolimus is metabolized by the CYP3A5 enzyme, activity of which directly influences pharmacokinetics of the drug. Three CYP3A5 alleles (*3, *6 and *7) are associated with full or partial loss of enzymatic activity, depending on zygosity. The Dutch Pharmacogenetic Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC)

agree on recommendations for initial tacrolimus dosing based on CYP3A5 genotyping for these alleles. Pre-transplant genotyping for CYP3A5 may help to plan treatment more efficiently and achieve target concentrations of tacrolimus in the patient's blood more quickly. Additional biomarkers enabling monitoring variations in individual response to treatment will be beneficial in long-term patient management. One such biomarker is the human Alpha Torquetenovirus (TTV), a non-pathogenic virus that is highly prevalent in the human population. Multiple published clinical studies demonstrated significant associations between TTV titers and individual immunosuppression levels.

Methods

Multiplex TaqMan technology was utilized in combination with QuantStudio 5 instruments and proprietary Thermo Fisher reagents.

Results

We evaluated the feasibility of developing a pre-transplant qPCR assay for genotyping *3, *6, and *7 alleles in CYP3A5 in a single 6-plex reaction. We tested our new qPCR assay using well-characterized DNA and primary human samples including whole blood and buccal swabs demonstrating feasibility of single 6-plex assay to provide CYP3A5 genotyping results. Future use of a single multiplex assay for CYP3A5 genotyping may provide benefits for laboratory practices by simplifying test workflows. We evaluated the feasibility of developing a new multiplex post-transplant qPCR assay to uniformly measure common TTV species, using both TTV template DNA as well as donor urine and blood samples. Our post-transplant TTV assay demonstrated a significantly tighter distribution of Cq values in comparison to previously published assays when tested with 27 different TTV sequences. Testing the assay against a high titer (up to 5×10^6 copies per reaction) of closely related beta and gamma Anelloviruses and large amounts of human genomic DNA (up to 700 ng) demonstrated a lack of detectable cross-reactivity with large amounts of human DNA and closely related viruses. Linearity for all TTV species was demonstrated in the range from 10 to 1×10^8 copies per reaction, with a lower limit of detection of 1 copy per reaction. We evaluated our ability to quantify TTV in blood samples from 80 donors demonstrating 81% TTV prevalence in the studied population, which is in alignment with previously published data. We compared TTV titers in whole blood and the plasma of 16 healthy donors and demonstrated significantly higher TTV titers detectable in whole blood in comparison to plasma. We also demonstrated feasibility to detect and quantify TTV in the urine of transplant patients and healthy individuals, with significantly higher TTV titers detected in the urine of kidney transplant patients.

Conclusion

We demonstrated feasibility to use a multiplex qPCR assay to genotype 3 SNPs in the CYP3A5 gene in a single reaction. We also demonstrated feasibility to accurately and consistently quantify a variety of TTV species by multiplex qPCR. Combining pre-transplant CYP3A5 genotyping with TTV monitoring post-transplant may further enhance immunosuppression management in kidney transplant patients.

Abstract Keywords

Pharmacogenetics, PGx, TTV, CYP3A5, qPCR, Immunosuppression, Tacrolimus



Poster # 18

Title: Analysis of Risk Factors of Myocardial Infarction & Cardiac Arrest after Hepatectomy

Poster Topic: Data/Science

Author(s)

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Abstract Body: Background

Myocardial infarction and cardiac arrest significantly contribute to mortality after non-cardiac surgeries. This study aimed to identify hepatectomy-specific risk factors for cardiac complications and assess whether adding new information during hospitalization changes these risk factors.

Methods

A retrospective analysis of the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) database identified patients who underwent hepatectomy between 2015 and 2019. Risk factors were grouped into the preoperative, intraoperative, and postoperative phases. Elements were selected by bootstrap resampling to determine their association with cardiac events. Significance was determined using logistic regression, with the characteristics found to be significant for bootstrap sampling.

Results

Within one month of hepatectomy, 325 of 11093 (2.9%) patients had cardiac complications. Surgery-specific perioperative risk factors independently associated with increased cardiac complications included hepatic failure (3.61 [2.64-4.93], $p < 0.001$), biliary leakage (1.77 [1.30-2.42], $p < 0.001$), open hepatectomy (1.71 [1.13-2.60], $p = 0.029$), operative time > 300 min (1.32 [1.03-1.70], $p = 0.029$), and reintubation (2.15 [1.38-3.33], $p < 0.001$). Other significant perioperative risk factors included male sex (1.58 [1.23-2.03], $p < 0.001$), age > 50 years (2.89 [1.86-4.43], $p < 0.001$), and diabetes (1.97 [1.54-2.53], $p < 0.001$). Adding new variables improved the statistical model performance. The C-index improved from 0.727 in the preoperative risk assessment to 0.811 in the final analysis of all perioperative risk factors.

Conclusion

Pre-, intra-, and postoperative risk factors are linked to cardiac complications. Incorporating new information during hospitalization enhances risk assessment and statistical prediction of cardiac complications. This study could equip surgeons with information to better stratify patients at high risk for major postoperative cardiac events.

Abstract Keywords

Hepatectomy, cardiac event, transplant surgery

Poster # 19

Title: Pancreas Graft Failure Risk Modeling: How Endpoint Choice Shapes Machine Learning Predictions

Poster Topic: Data/Science

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Abstract Body: Background

Heterogeneous definitions of pancreas graft failure complicate risk prediction after simultaneous pancreas-kidney (SPK) transplantation. We evaluated how endpoint choice influences model performance and the hierarchy of predictors.

Methods

We conducted a single-center retrospective cohort study of 190 adult simultaneous pancreas-kidney (SPK) transplant recipients between 2015 and 2023. Three specified endpoints were modeled: (A) a modified OPTN/UNOS composite outcome defined as sustained insulin requirement ≥ 0.5 U/kg/day for ≥ 90 days, with deaths with a functioning graft censored; (B) any post-transplant insulin use at any dose, with death considered a failure event; (C) a sensitivity analysis excluding graft explants attributed to surgical complications and defining failure as sustained insulin requirement ≥ 0.5 U/kg/day for ≥ 90 days. Categorical variables were one-hot encoded and continuous variables were standardized. Four preprocessing strategies were compared, varying in the use of simple imputation and missingness indicators. For each endpoint, the final model was selected based on the highest precision-recall area under the curve (PR-AUC), using the Brier score to break ties. We used L2-regularized logistic regression as the classifier. Model performance was evaluated using AUROC, PR-AUC, Brier score, and F1 score at the tuned operating threshold. Adjusted effects were reported as odds ratios per one standard deviation increase. Model interpretability was assessed using permutation feature importance.

Results

Endpoint A: modified OPTN/UNOS composite (sustained insulin requirement ≥ 0.5 U/kg/day for ≥ 90 days; deaths with a functioning graft censored). AUROC 0.777, PR-AUC 0.394, Brier score 0.070, F1 0.211 at threshold 0.517. Strongest adjusted associations: pancreas slow function, defined as insulin requirement within 30 days post-transplant (OR 3.07); higher pre-transplant insulin per kg (OR 2.98); dialysis history (OR 2.57);



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longer pancreas cold ischemia time (OR 2.31); prior pancreas transplant (OR 2.25); and higher recipient BMI (OR 2.09). Endpoint B: any insulin use or death counted as failure. AUROC 0.662, PR-AUC 0.526, Brier score 0.216, F1 0.058 at threshold 0.989. Notable adjusted effects: donor pancreas type (DBD vs DCD; OR 2.68); pancreas slow function (OR 1.98); donor sex category (OR 1.87); and higher pre-transplant insulin units (OR 1.59). Sensitivity C: early explants attributed to surgical complications excluded and deaths with a functioning graft censored; failure requires sustained insulin ≥ 0.5 U/kg/day for ≥ 90 days. AUROC 0.613, PR-AUC 0.099, Brier score 0.066, F1 0.189 at threshold 0.068. Despite lower event prevalence and attenuated discrimination, effect directions were consistent, led by higher pre-transplant insulin per kg (OR 4.69), prior pancreas transplant (OR 2.45), baseline BMI (OR 2.39), dialysis history (OR 2.07), pancreas slow function (OR 1.81), and longer pancreas cold ischemia time (OR 1.42).

Conclusion

Across endpoints, pre-transplant insulin burden and early pancreas slow function were the most consistent predictors, with additional contributions from pancreas cold ischemia time, dialysis exposure, recipient BMI, prior pancreas transplant, and donor factors. Endpoint definition materially altered apparent performance: the any-insulin or death endpoint showed higher PR-AUC but poorer calibration, whereas the stricter metabolic endpoint calibrated better. The single-center sample and modest event counts limit power, precision, and generalizability; external validation is warranted.

Abstract Keywords

Simultaneous pancreas–kidney transplantation; Machine Learning; pancreas graft failure; insulin dependence; missing data; predictive modeling.

Poster # 20

Title: Sentinel Skin Flap in Liver Transplantation: Patient Acceptability of a Novel Immune Surveillance Biomarker

Poster Topic: Data/Science

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Abstract Body: Background

Allograft rejection is a major barrier to long-term transplant success. Diagnosis currently depends on non-specific clinical features and invasive biopsy, which carry risk, cause anxiety, and delay intervention. A sentinel skin flap (SSF), created by co-transplanting a vascularised 3×10 cm donor skin flap to the forearm during liver transplantation, provides an externally visible and immunologically active site for early detection of alloimmune activity. As clinical translation depends on patient perspectives, we evaluated the acceptability of SSF among liver transplant recipients.

Methods

A prospective cross-sectional survey was conducted among adult liver transplant recipients attending outpatient clinics at a National liver transplant centre. After a standardised briefing with clinical images and schematics, participants completed a previously validated questionnaire capturing demographics, transplant history, biopsy experience, and attitudes to SSF. Domains included cosmetic concerns, cultural and religious attitudes, stigma, and perceived benefits (reassurance, empowerment, self-monitoring, adherence). Likert, binary, and free-text responses were analysed descriptively; chi-squared and non-parametric tests explored associations.

Results

Fifty patients participated (29 male, 21 female; mean age 53.4 years). Seven (14%) were post-transplant; the remainder were waitlisted. Acceptability was high: 92% would consent to SSF, 96% anticipated reassurance, 92% empowerment, and 84% reduced distress. Concerns were modest—cosmetic (34%), social (16%), surgical (12%)—with no religious objections. Women more frequently reported cosmetic concerns ($p = 0.020$). Waitlisted patients expressed greater surgical concerns ($p = 0.007$) but also higher reassurance ($p = 0.026$) and anatomical site acceptance ($p = 0.042$).

Conclusion

Liver transplant recipients strongly supported the SSF concept, with only modest concerns and subgroup differences suggesting areas for targeted counselling. This study establishes patient endorsement of SSF monitoring in liver transplant recipients and provides feasibility evidence to justify clinical trials and the integration of patient-centred immune surveillance into practice.

Abstract Keywords

Sentinel skin flap; Patient acceptability; Solid organ transplantation; Liver transplantation; Immunological monitoring

Poster # 22

Title: Assessment of DSA by serial titration associates with acute rejection severity in kidney transplant recipients

Poster Topic: Miscellaneous



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Abstract Body: Background

Multiplexed flow cytometry-based single antigen bead (SAB) assays are the standard for detection of anti-HLA alloantibodies. The presence of donor-specific antibodies (DSA) is diagnostic in antibody-mediated rejection (AMR) and higher levels of DSA are associated with risk of AMR. The output of the SAB assay, Mean Fluorescence Intensity (MFI), is correlated with antibody concentration and higher MFI with AMR risk. However, limitations of MFI including non-linearity, saturation, reagent variability, and potential for assay inhibition limit assessment of DSA levels and risk assessment. We propose DSA quantification by titration is a more informative measure of antibody quantity and is associated with biopsy-proven rejection.

Methods

We performed SAB titration in 19 serum samples from adult and pediatric kidney transplant recipients with known DSA at time of for-cause kidney biopsy. SAB testing was performed using LABScreen Single Antigen Class I and II reagents on a FLEXMAP 3D analyzer with each sample tested by serial dilution (neat, 1:4, 1:10, 1:64). DSA >1000 MFI at any dilution were included for analysis.

Results

There were 35 DSA detected from 19 subjects. Biopsy results included five AMR, two T cell mediated rejection (TCMR), eight mixed TCMR/AMR, and four no rejection. Serum dilution demonstrated non-proportional decreases in MFI, highlighting the nonlinear relationship between MFI and DSA concentration (Fig 1). MFI for undiluted serum underrepresented level of DSA in patients with AMR, as these samples showed persistent DSA at 1:10 and 1:64 dilutions, while DSA were not present at these dilutions in non-AMR samples. In those with any AMR, the initial 4-fold DSA dilution resulted in an average 15% increase in MFI, followed by 45% and 44% decrease with subsequent dilutions. This is in contrast to those without AMR, where there was a consistent decreased across serial dilutions with an initial 6% decrease in MFI from neat to 1:4 followed by 72 and 80% for 10 and 64-fold dilutions. Serial titration demonstrated greater sensitivity and a stronger relationship with more severe rejection phenotypes and presence of microvascular inflammation at higher dilutions (Table 1). Using a cutoff of 3000 MFI, a higher proportion of those with AMR had DSA at 1:10 compared to those with TCMR or no rejection (53% vs 21%, p=0.02).

Conclusion

Our results highlight the limitation of using MFI for evaluation of DSA and indicate serum dilution and titration by SAB may provide a more accurate measure of DSA concentration. DSA present at 10-fold dilution correlates with presence and severity of rejection. These results have important clinical implications, particularly when making decisions about the urgency of biopsy

or treatment initiation. Future work aims to evaluate reduction in antibody concentration with clinical response to rejection treatment.

Abstract Keywords

Donor specific antibody, histocompatibility

Poster # 23

Title: Social Support and Donor Inquiry Among Kidney Transplant Candidates

Poster Topic: Miscellaneous

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Abstract Body: Background

The degree of social support may influence living donor kidney transplant (LDKT) access, yet measures of social support are infrequently assessed in LDKT research. We aimed to examine the association of three measures of social support on receiving a living kidney donor inquiry. Social support measures included Bernal's Brief Scale Support Scale (6 items on emotional and tangible support, range 0-18), living situation (single yes/no question about the presence of another adult in the household), and social engagement (number of close family and friends that 'you feel at ease with and can talk to about what is on your mind', < 4 vs. 4 or more).

Methods

We conducted a cross-sectional cohort study of adult de novo pre-transplant candidates who completed baseline surveys in two prior studies that tested a behavioral intervention for LDKT access at a single transplant center between April 2009 and December 2024. The minimum follow-up was one year. Multivariate Cox models included baseline variables significantly associated with at least one inquiry sent to the transplant center at p < 0.05. Variables included social support measures, sociodemographics, health, household income, health literacy, and technology use and access.

Results

Of 411 candidates, 131 (32%) received a donor inquiry. Candidates were 57 ± 13 years old, 41% female, 31% African-American, 46% Medicaid-insured, and 48% < \$30,000 annual household income. On multivariate analysis, social engagement of 4 or more close friends and family was independently associated with living donor inquiry [aHR = 1.548; 95% CI 1.045-2.294; p = 0.029]. Several other variables approached significance (p < 0.10) including race, not on dialysis, and estimated post-transplant survival score [Table 1].



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Conclusion

Extent of social engagement was strongly associated with live kidney donor inquiry. Findings highlight the importance of including social support measures in LDKT research.

Abstract Keywords

kidney transplant living donor social support patient education

Poster # 24

Title: Obesity and Outcomes in Living Kidney Donors: A Systematic Review

Poster Topic: Miscellaneous

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Abstract Body: Background

Living kidney donors (LKD) present hope to a continually increasing waitlist of recipients in the face of stagnant supply of deceased-donor allografts. Increasing prevalence of obesity (BMI [body mass index] ≥ 30 kg/m²) and its health effects may affect candidacy of potential LKDs.

Methods

Systematic review of select articles from PubMed, Embase and ResearchGate was conducted (Figure 1) to explore impact of obesity on outcomes in LKDs. Adverse outcomes were categorized as direct surgery-related events, cardiovascular complications and renal/urologic findings.

Results

Multiple studies demonstrated a strong correlation between obesity and surgery-related factors including increased intraoperative bleeding, prolonged operative time and hospital stay, higher readmission rate, and wound complications such as incisional hernia but not surgical site infection. Obese donors were at significant risk of developing hypertension but not major adverse cardiovascular events. The risk of developing diabetes in

obese donors predisposed them to chronic kidney disease (CKD). Significant evidence suggestive of increased incidence of microalbuminuria/proteinuria in obese donors, especially with reduction in glomerular filtration rate or creatinine clearance, is lacking.

Conclusion

Obesity in LKDs is associated with adverse surgery-related factors, development of hypertension, and CKD possibly due to their additional predisposition to diabetes. Prospective studies comparing outcomes of donors ranging from overweight to obesity classes (I to III) may establish non-arbitrariness in selection of LKD candidates based on BMI.

Abstract Keywords

Living kidney donation, kidney transplantation, obesity, living donor outcomes

Poster # 25

Title: Accelerating Kidney Transplants: An Innovative Referral Screening Tool

Poster Topic: Miscellaneous

Author(s)

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Abstract Body: Background

Timely assessment and evaluation of patients with end-stage renal failure are crucial steps in the transplantation process. Currently, the median time from patient referral to the pre-kidney evaluation program to the Patient Selection Committee (PSC) decision is seven months. Several key issues contribute to this delay: 1. Lead Time to Initial Evaluation: The lead time to the initial physician evaluation is four months. 2. High No-Show and Late Cancellation Rates: These rates result in underutilized resources and extend the time to physician visits due to the need to reschedule patients. 3. Clinical Contraindications: A significant number of patients are evaluated despite having clinical contraindications present upon referral. 4. Referral Processing Time: The time spent processing referrals is increasing due to a higher number of referrals, including re-referrals from previously deferred (ineligible) candidates, with no increase in staffing resources. The CARES Act of March 2020 penalizes nephrologists and dialysis centers for not referring their patients for kidney transplant evaluations. This has led to an increase in redundant referrals of patients who are ineligible for transplant due to contraindications. There is no objective or standardized method to identify and weigh relative contraindications early in the process, and there is limited MD involvement with referrals that have multiple relative contraindications. Additionally, there is no clear visibility into the screening process to determine the coordinator's progress.



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Methods

We assembled a multidisciplinary team to identify barriers to timely patient progression from referral to decision. Initially, we created a current state process map to outline the roles and tasks of each team member, highlighting areas where delays were observed. A Rapid Improvement Event (RIE) was then conducted, led by a medical center process engineer and attended by all core members of the Kidney Pre-Transplant program. During this event, relevant historical data was presented, including: - Referral volume growth to over 2,000 per year - Increase in scheduling lead time for new patients to five months - No-show/late cancellation rate at almost 40% The group identified over 100 barriers delaying patient progress, categorizing them and narrowing the focus to the fifteen highest impact ideas (Figure 1). As a result of the RIE, five project groups were formed to address: 1. Referral review and screening process 2. Patient no-shows and cancellations 3. Patient scheduling and completion of testing 4. Process checklist 5. PSC preparation Workgroup #1 focused on developing a new and innovative way to screen patients more quickly and accurately. The existing screening tool was subjective and required MD review for absolute contraindications to determine if a patient should be seen as a consult only. The new referral screening tool is a systematic approach, starting from the highest significance down. This tool is documented in a progress note and screened in the following order (Figure 2):

- Information to verify patient meets criteria for kidney transplant
- Have they been declined previously? Why? What has changed?
- Absolute contraindications – MD review followed by a compassionate decline via phone call and letter to the patient and referring provider
- Relative contraindications – scored tool to determine medical eligibility

The new scored screening tool was developed utilizing months of data analysis and practice screens to ensure the scores were as accurate as possible, thereby expediting the process of getting patients to the correct appointment.

Results

We successfully reduced the time from referral to the Patient Selection Committee by an average of 119 days, achieving a 40% reduction (Figure 4). Key changes that contributed to this improvement included: 1. Efficient Referral Processing and Screening Tool: Implementing a more streamlined referral processing and screening tool. 2. No-Show/Late Cancellation Policy: Introducing a policy to address no-shows and late cancellations. 3. Testing Completion Requirement: Mandating the completion of testing during evaluation within six months. The development of a process checklist within Epic was crucial for maintaining throughput and ensuring follow-up for all team members. This checklist enabled the referral processing team to work more efficiently and handle more referrals without needing additional staff. In 2021, we introduced a new role for a part-time screening coordinator, which increased the number of referrals screened out before clinic appointments. Since the implementation of the new screening tool in October 2023, the number of referrals screened out has risen from 38 in 2021 to 404 in 2024 (Figure 3). Additionally, the median progression time from referral to evaluation decreased from 145 days in 2020 to 74 days in 2024 (Figure 5).

Conclusion

Despite significant advances in medicine and technology, as well as increased awareness of organ donation and transplantation, there remains a substantial gap between the supply of and demand for organs. According to the United Network for Organ Sharing (UNOS), there are currently 103,707 people in the United States awaiting an organ transplant. In 2023, 46,630 transplant surgeries were completed, leaving 57,077 individuals still waiting

for lifesaving surgery (UNOS, 2024). The volume of patient referrals to kidney transplant programs has increased, and with 50% of kidney transplant centers participating in the mandatory IOTA model, there is a pressing need to improve the time from referral to decision for patients. It is crucial to complete evaluations as quickly and safely as possible to determine whether patients qualify for a kidney transplant, thereby addressing the urgent need for timely and effective transplantation processes.

Abstract Keywords

kidney transplant, referral optimization, access to transplant, patient throughput

Poster # 26

Title: Extended Post-Prophylaxis Monitoring Reduces CMV-Related Hospitalizations in Kidney Transplant Recipients with CMV Mismatch (D+/R-)

Poster Topic: Miscellaneous

Author(s)

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Abstract Body: Background

Cytomegalovirus (CMV) is a major source of morbidity and mortality in kidney transplant recipients (KTRs). Without prophylaxis, 40–100% of KTRs develop CMV infection, with the highest risk in CMV-seronegative recipients of CMV-seropositive donors (D+/R-), known as CMV mismatch (CMV MM). Despite prophylaxis, CMV disease occurs in up to 20% of CMV MM patients after cessation of therapy, with most infections arising within the first 100 days post-transplant or shortly thereafter. Aims- This study aimed to (1) assess the rate of CMV disease post-prophylaxis in CMV MM KTRs, (2) determine hospitalization rates due to CMV infection, and (3) evaluate the effect of biweekly CMV PCR monitoring for 3 months post-prophylaxis on clinical outcomes.

Methods

Two cohorts were analyzed. The first cohort included KTRs transplanted between July 1, 2021 and June 30, 2023. The second cohort included patients transplanted between Sept 1, 2023 and Aug 31, 2024. All CMV MM recipients received valganciclovir 900 mg PO daily for 6 months (dose-adjusted for renal function), followed by a post-prophylaxis protocol consisting of biweekly CMV PCR for 3 months, patient education on symptom reporting, and serology recheck at 1 year.

Results

In the first cohort, 1,007 KTRs were recorded, with 187 (18.5%) identified as CMV MM. Among these, 83 patients (44%) developed CMV disease and 33 (17%) required hospitalization for CMV-related complications. In the second



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cohort of 492 transplants, 89 (18%) were CMV MM. Post-prophylaxis, 23 of 89 patients (25%) developed CMV viremia, while 5 (5%) required hospitalization due to CMV disease. All hospitalized patients had peak viremia >100,000 IU/mL, and one patient died of CMV-related gastrointestinal bleeding. Early detection through monitoring facilitated timely interventions, reducing complications (Image). Statistical comparison between cohorts confirmed a significant reduction in CMV disease incidence (44% vs. 25%; $Z = 3.06$, $p \approx 0.002$).

Conclusion

Biweekly CMV PCR monitoring post-prophylaxis in CMV MM patients is effective in reducing CMV-related hospitalizations and may contribute to mortality prevention. These findings support the continuation and potential expansion of extended surveillance protocols in high-risk kidney transplant populations.

Abstract Keywords

1. Prophylaxis 2. Solid organ transplant recipients 3. Cytomegalovirus infection

Poster # 27

Title: Protocol 4 Month MRI Detects Silent Biliary Pathology in DCD Liver Transplant Recipients

Poster Topic: Miscellaneous

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Abstract Body: Background

Biliary complications are a major cause of morbidity and mortality after donation-after-circulatory-death (DCD) liver transplantation. DCD liver transplants have an associated increased risk for ischemic cholangiopathy. Machine perfusion has helped to decrease this incidence but is also associated with the development of anastomotic strictures. Most transplant centers rely on abnormal liver tests or symptoms such as new onset jaundice or pruritus to prompt further investigation with cross-sectional imaging. In January 2023, our center began performing routine 4-month post-transplant Magnetic Resonance Imaging with Cholangiopancreatography (MRI/MRCP) on all DCD recipients to try and detect cholangiopathy earlier. To our knowledge, no other institution performs universal screening MRI in this cohort for this purpose.

Methods

We performed a HIPAA-compliant retrospective review of all patients who underwent DCD liver transplantation at our institution from January 2023 to December 2024. We excluded any patient that did not complete a screening MRI/MRCP at 4 months. Imaging findings were categorized as normal,

anastomotic stricture or cholangiopathy (minor, confluence dominant, multifocal progressive or diffuse necrosis). All cases were reviewed and classified at a multidisciplinary conference including transplant hepatology, advanced endoscopy, abdominal radiology, and transplant surgery.

Results

297 DCD recipients underwent MRI/MRCP between 2-6 months post-transplant. 24 (8%) of 297 were asymptomatic with normal liver enzymes but had intervenable biliary pathology detected on the 4-month imaging. Intervenable pathology was defined as biliary dilation with filling defects/debris noted within the ducts. This was reviewed at our multidisciplinary conference with consensus achieved prior to proceeding with any intervention. 17 individuals had anastomotic strictures and 7 had ischemic cholangiopathy – 2 minor, 4 confluence dominant, and 1 multifocal progressive. Only 1 patient – from the cholangiopathy group – required re-transplantation.

Conclusion

Routine 4-month MRI identified clinically silent but treatable biliary disease in 8% of DCD liver transplant recipients. These findings would not have been detected by lab monitoring alone, suggesting that a routine protocol for imaging in DCD liver transplant recipients may allow for earlier intervention and potentially prevent development of infection, hospitalization, progression of cholangiopathy and even graft loss.

Abstract Keywords

Transplant Hepatology, MRI/MRCP, Ischemic Cholangiopathy, donation-after-circulatory-death (DCD), Anastomotic stricture

Poster # 28

Title: Orthodontic Derived Microplastics Modulate Macrophage Differentiation and Homeostasis

Poster Topic: Miscellaneous

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Abstract Body: Background

Synthetic polymers are widely used in an abundance of commercial products, including medical and dental materials and devices. The potential adverse effects of released micro- and nano-plastics on both the environment and



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living organisms are starting to be studied. There is a lack of research comparing the release of microplastics from traditional thermoformed versus more recent direct 3D printed products. Microplastics have been detected in a variety of human tissues such as, lung, liver, spleen and kidneys. Each of these sites contain local resident macrophages (MΦ) tasked with maintaining homeostasis by clearing cell debris and foreign bodies. Like these organs, the oral cavity has an extensive population of MΦ that complete immune surveillance and support oral tissue health. Microplastics from orthodontic procedures may have profound local and systemic effects. We hypothesize that dissemination of microplastics into tissues influences local MΦ differentiation as they attempt to clear these non-self and foreign materials.

Methods

Polymers with the following compositions were obtained, 3D printing material: polyurethane methacrylate-based photopolymer, urethane dimethacrylate-based photopolymer, polyamide, thermoforming material: PETG copolyester, thermoplastic polyurethane, and multi-layer polyurethane with PETG core. We tested material stability by submerging equal-sized disks in artificial saliva, incubating them, and vortexing daily for 30 seconds for a period of one week. Flow cytometry was used to quantify particles and transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to visualize. We observed MΦ uptake of microplastics with timelapse microscopy. Bone marrow-derived MΦ differentiation was assessed by flow cytometry 3 days following co-culture with microplastics.

Results

All products released detectable plastic particles, with 3D printed materials releasing the highest concentration. Furthermore, 3D printed materials shed more micro-sized plastics, whereas thermoforming materials released more nano-sized plastics. MΦs predominantly phagocytosed plastic that were typically $\geq 5 \mu\text{m}$. Following co-culture with microplastics, the 3D printed materials comprised of urethane drove MΦ differentiation into M1-like iNOS+CD86+ subsets.

Conclusion

Our findings reveal that commonly used orthodontic materials release micro- and nano-plastics that are readily taken up by MΦ, with 3D printed polymers releasing higher levels of immunostimulatory particles. The resulting shift toward pro-inflammatory MΦ phenotypes raises concern that material choice could influence not only local oral health but also systemic immune responses. These results underscore the importance of evaluating microplastic release in dental and medical devices to guide safer material selection and minimize unintended biological consequences for patients.

Abstract Keywords

Macrophage, Microplastics

Poster # 29

Title: Late-Onset JC Polyomavirus-Associated Nephritis in a Renal Transplant Recipient

Poster Topic: Miscellaneous

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Abstract Body: Background

JC Polyomavirus-Associated Nephritis (JC-PVAN) is a rare complication in kidney transplant patients caused by the reactivation of the latent JC virus. JC virus is a non-enveloped, double-stranded DNA virus in the Polyomaviridae family. It is highly prevalent, with a seropositivity of about 80% in the adult population. While primary infection is typically asymptomatic, the virus poses a risk in immunosuppressed individuals due to its potential for reactivation. Reactivating the JC virus in immunosuppressed individuals can lead to graft damage as well as the demyelinating disease, progressive multifocal leukoencephalopathy (PML), and JC viral encephalitis. We present an atypical case of JC virus manifesting several years post-transplant in a severely immunosuppressed patient.

Methods

Case Report

Results

Case description: A 61-year-old African American woman with a history of renal transplantation 7 years prior presented with an elevated serum creatinine level of 1.6 mg/dL following several years of stable graft function. Following transplantation, the patient received Campath for induction therapy. Her maintained immunosuppressive regimen included Tacrolimus (4-7 ng/dl), CellCept, and Prednisone. Given repeatedly negative plasma BK virus PCR results (three separate assays), testing for JC virus was pursued and returned positive. The patient underwent immunosuppressive reduction (get specifics) to enable host defense against JC virus reactivation. This intervention proved effective, with JC viral load declining from 81,600 copies/mL to 74 copies/mL over a six-year period. Additionally, the patient did not develop cognitive impairments caused by PML or JC virus encephalitis. Discussion: In immunosuppressed patients, JC virus nephropathy should be considered as a possible cause of elevated creatinine even years after transplantation. Differentiation from more common forms of polyomaviruses, such as BKV-PVAN, is essential for proper treatment.

Conclusion

This case illustrates that JC nephritis can be a late cause of allograft dysfunction. Diagnosis can lead to a significant reduction in viral load, which may prevent serious conditions such as JC virus encephalitis and PML.

Abstract Keywords

JC Virus, Kidney, Transplant



Poster # 30

Title: Empiric Adjustments of Tacrolimus when Discontinuing Voriconazole and Posaconazole

Poster Topic: Miscellaneous

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Abstract Body: Background

Tacrolimus trough stability is important for longevity of transplanted grafts. Drug interactions such as those with triazole antifungals can cause significant instability in tacrolimus trough levels. At this center, patients receive antifungal prophylaxis with a triazole for 4-6 months following lung transplantation. Prior to this study, a single tacrolimus dose adjustment was recommended with repeat tacrolimus trough 3-5 days after triazole discontinuation. This approach was followed by 2-6 weeks of tacrolimus level instability requiring multiple tacrolimus dose adjustments. This project aimed to characterize tacrolimus trough stability using coefficient of variation (CV) after implementation of an empiric tacrolimus dose adjustment calendar used at the time of triazole discontinuation.

Methods

Calendars for tacrolimus dose titrations and associated data collection were prepared for outpatient pediatric lung transplant recipients transplanted between November 2019 and November 2024 who transitioned off voriconazole or posaconazole. Calendars were developed based on previous tacrolimus dose needs prior to triazole, current tacrolimus dosing and previous trough levels. Team anticipated patient to need 3-4 times their current tacrolimus dose to account for change in metabolism due to drug interaction. Tacrolimus trough CV before and after transition as well as p value were calculated to assess tacrolimus trough variability.

Results

Titration calendars were prepared for 10 pediatric lung transplant recipients with 17 patients excluded due to death prior to triazole therapy completion (n=7), transitioned as inpatient (n=3), and adherence concerns (n=7). Titration calendars contained an average of 2.9 steps (range 2-3) over 6.4 days (range 5-8) with tacrolimus trough levels drawn approximately twice weekly (range 1-2) during the titration. Tacrolimus trough values were collected for 2 months prior to triazole discontinuation and for at least 2 months after discontinuation of triazole. Patients had an average of 8.5 tacrolimus troughs assessed prior to triazole discontinuation (range 2-17) and an average of 5.4 tacrolimus troughs assessed after discontinuation of triazole (range 2-7). Paired t-test average difference in CV of 4.7548% indicates CV after discontinuation of triazole (29.4062%) is not significantly higher than CV on triazole (24.6515%) (p=0.3758).

Conclusion

Tacrolimus trough CV was not significantly higher after discontinuation of triazole with use of an empiric tacrolimus dose titration calendar. This approach has been adapted as this center's standard of care in effort to minimize tacrolimus trough variability as triazole is discontinued.

Abstract Keywords

tacrolimus, posaconazole, voriconazole, triazole, lung transplant, pediatric

Poster # 31

Title: Sublethal doses of genotoxic immune-conditioning drugs, antivirals, and chemotherapeutics promote BK-polyomavirus-induced hemorrhagic cystitis

Poster Topic: Translational: human in vitro and ex vivo

Author(s)

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Abstract Body: Background

Hemorrhagic cystitis (HC) is a severe complication associated with hematopoietic stem cell transplantation, thought to arise from a combination of damage to the bladder and uncontrolled replication of BK polyomavirus (BKPyV) from immune suppression. Many of the drugs commonly used for immune conditioning prior to transplantation kill replicative immune cells through genotoxic activity. HC has also been reported in association with chemotherapy, but the role of virus infection in these cases has not been evaluated. Not all patients undergoing these regimens develop HC and the full risk criterion for HC development are unknown. To date, no studies have evaluated the effects of these genotoxic regimens on BKPyV replication, and how this may impact HC development, either in transplant recipients or individuals undergoing chemotherapy. In this study, we hypothesize that low-level genotoxic activity initiates a modest stress response that primes the host cellular environment to be more conducive to the replication of this host-dependent DNA virus.

Methods

We used immortalized human bladder epithelial cells (HBLAKs) as our primary in vitro model and complemented those experiments with organotypic HBLAK cultures and BKPyV-positive urine specimens collected from transplant recipients at the start of immune conditioning and throughout and after transplantation. Cells were infected with BKPyV and then either treated concurrently with drugs or pretreated for six hours before infection and washed to remove the compound prior to viral exposure. Drug exposures were chosen to model clinically relevant, low-cytotoxicity conditions (IC10 and IC20). The agents tested spanned immune-conditioning drugs (cyclophosphamide, fludarabine, busulfan), common chemotherapeutics (etoposide, cisplatin, 5-fluorouracil), and antivirals (cidofovir, ganciclovir, acyclovir). We included heat shock and paclitaxel as controls to distinguish a general stress response



from effects specific to genotoxic mechanisms. Viral load was quantified by qPCR, infected cell frequency was assessed by immunofluorescence, and organotypic infection assays validated findings in a tissue-like context. To probe viral genome integrity and replication intermediates we performed both short-read and long-read DNA sequencing, and to characterize host responses we used RNA-seq and ATAC-seq to map transcriptional changes and chromatin accessibility, respectively.

Results

Results are clear and consistent across models. Mild genotoxic exposure following infection increased BKPyV replication ~4–5-fold relative to untreated cells; the effect grew with longer treatment. Strikingly, a 6-hour pre-treatment before infection, with no continued drug exposure, enhanced viral load by ~11–15-fold at 3 days post-infection. Heat shock and paclitaxel did not reproduce these effects, indicating this is not a generic stress response but linked to genotoxic mechanisms. Immunofluorescence showed increased numbers of susceptible/infected cells after genotoxic exposure, suggesting the primary effect is on host susceptibility to infection rather than a simple acceleration of viral genome replication. Organotypic HBLAK cultures infected with wildtype/archetype BKPyV recapitulated the findings. Deep sequencing (short- and long-read) of *in vitro* models and BKPyV-positive urine from patients beginning immune conditioning did not reveal increased mutagenesis, rearrangements, or notable changes in viral replication intermediates attributable to drug exposure. In other words, drug treatment did not appear to directly alter viral genome integrity or replication mechanics. Transcriptomics revealed substantial overlap across treatment and infection conditions, with pronounced changes in histone-related genes and chromatin remodeling pathways. ATAC-seq showed widespread, common changes in chromatin accessibility across treatments; these altered regions were significantly enriched for AP-1 family transcription factor binding motifs. AP-1 factors are known to bind the BKPyV noncoding control region (NCCR) and can function as pioneer factors to open chromatin, providing a plausible mechanistic link between genotoxic exposure, altered chromatin state, and enhanced BKPyV infection.

Conclusion

What this really means is: low-level DNA damage from immune conditioning or certain chemotherapies can reprogram urothelial chromatin accessibility in ways that increase BKPyV susceptibility and replication. The effect is driven by host chromatin and transcriptional changes, notably AP-1 motif enrichment and histone-related transcriptional shifts, rather than by induction of viral genome mutation or gross changes in replication intermediates. Clinically, these findings offer a mechanistic explanation for why some patients develop BKPyV-related HC after conditioning or chemotherapy and point to possible interventions: (1) risk stratification based on host chromatin/transcriptional markers, (2) timing or choice of conditioning agents to minimize permissive chromatin remodeling, and (3) targeted therapeutics aimed at blocking the host pathways (e.g., AP-1 signaling or chromatin modifiers) that increase infection susceptibility. Ongoing work will validate these markers in larger patient cohorts, assess whether early antiviral or chromatin-modulating strategies can prevent HC, and evaluate how emerging BK therapeutics interact with this host-centric mechanism.

Abstract Keywords

BK polyomavirus, Hemorrhagic cystitis, Transplantation, Immune conditioning, Genotoxic stress, Chromatin accessibility, ATAC-seq, AP-1 transcription factors, HBLAK, Organotypic culture

Poster # 32

Title: Co-stimulation Blockade Impairs Hybrid Immunity to SARS-CoV2 Immunization

Poster Topic: Translational: human in vitro and ex vivo

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Abstract Body: Background

Hybrid immunity describes the phenomenon of enhanced SARS-CoV2 vaccine protection among SARS-CoV2 infection experienced individuals compared to infection-naïve individuals. Hybrid immunity has been shown to confer superior protection from breakthrough SARS-CoV2 infection in healthy and immunocompromised hosts. The quality of hybrid immunity by immunosuppressive class has yet to be described. Here we seek to address this knowledge gap by comparing the properties of vaccine-elicited antigen-specific B cells in kidney transplant recipients (KTRs) by class of maintenance immunosuppression and prior SARS-CoV2 infection status.

Methods

We analyzed a cohort of 15 kidney transplant recipients who received the primary two-dose SARS-CoV-2 mRNA vaccine series treated with belatacept or standard of care immunosuppression. Participants were divided based on prior history of SARS-CoV-2 infection. Peripheral blood was collected before immunization, 21 days after the first dose, and 21 days after the booster dose. We used high-parameter spectral flow cytometry, including a SARS-CoV-2 spike protein receptor-binding domain (RBD) tetramer, along with B cell markers CD27, CD38, IgD, IgM, IgG, CD11c, FcRL5, CXCR5, CD21, CD24, CD71, CD62L, CD80, and CD86. Data was analyzed using the OMIQ platform. Following compensation and quality control, unsupervised clustering was performed on total B cells and RBD-specific B cells using UMAP and FlowSOM.

Results

Nine kidney transplant participants (60%) received belatacept-based immunosuppression and 6 participants (40%) received standard of care immunosuppression. 33.3% of belatacept treated subjects and 50% of



standard of care immunosuppression treated subjects were SARS-CoV-2 infection experienced prior to vaccination. Median time after transplant was 116 days (IQR 94-343). 11 participants (73.3%) received the Pfizer BNT162b2 vaccine, and 4 participants (26.6%) received the Moderna mRNA-1273 vaccine. Unsupervised clustering of total B cells revealed that belatacept-based immunosuppression was associated with an expansion of isotype-switched CD86+ B cell subsets over standard of care immunosuppression. Frequency of RBD-specific B cells was reduced in infection-experienced KTRs on belatacept versus infection-experienced KTRs on standard of care immunosuppression. Unsupervised clustering of RBD-specific B cells revealed that an IgG and CD11c expressing B cell population lacking complement receptor 2, CD21, was poorly identified among infection-experienced KTRs on belatacept after immunization, while these could be readily identified after immunization of infection-experienced KTRs on standard of care immunosuppression.

Conclusion

Collectively, our data suggest that hybrid immunity to SARS-CoV2 is modified by costimulation blockade therapy with belatacept in KTRs. Personalized vaccine strategies for KTRs on co-stimulation blockade with belatacept are required and will likely be distinct from KTRs on standard of care immunosuppression.

Abstract Keywords

hybrid immunity, vaccines, SARS-CoV2, kidney transplantation, immunosuppression, costimulation blockade, belatacept

Poster # 33

Title: Indocyanine green (ICG) on normothermic machine perfusion for liver allograft viability and functional assessment

Poster Topic: Translational: human in vitro and ex vivo

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Abstract Body: Background

Normothermic machine perfusion (NMP) has facilitated utilization of historically discarded liver allografts. Current liver viability and assessment tools offer limited insight into post-transplant outcomes. Indocyanine green (ICG), which is taken up by hepatocytes and excreted by cholangiocytes, is widely used to assess tissue perfusion (Figure 1). We hypothesize ICG administered to livers on NMP will allow us to distinguish uptake and excretion characteristics, which will correlate with liver allograft outcomes, enabling insight into hepatocellular and cholangiocyte function. To

investigate this, we have implemented an ICG administration protocol for all livers maintained on NMP at our transplant center. ICG uptake and excretion characteristics will be correlated with clinical outcomes and histologic characteristics

Methods

In a pilot study to assess the feasibility and utility of ICG as a viability marker for livers on NMP (IRB #810421), ICG (0.25mg) was administered and liver parenchymal uptake was measured serially over one hour (SpyPhi, Stryker) in 18 liver allografts on NMP (Figure 2). Color histogram analysis was used to quantify liver parenchyma and bile fluorescence over time. Perfusate, bile, and tissue samples were collected for spectrophotometric quantification. ICG uptake and clearance will be correlated with currently available viability markers, including bile pH and glucose, perfusate pH and lactate, and routine histologic analysis, as well as clinical outcomes of interest.

Results

To date, eighteen liver allografts have undergone ICG evaluation; fourteen livers underwent transplant, and four livers were discarded due to histologic moderate to severe necrosis. ICG uptake demonstrated a difference between the two groups, with transplanted livers increasing at an average of 55.8% from 0 and 5 minutes compared to 31.9% in discarded livers. While bile production was variable across livers, transplanted livers demonstrated faster clearance of ICG (mean 32 min, range 9-45 min, no bile production in three livers) as compared to declined allografts (mean 45 minutes in two livers, no bile production in two livers) (Figure 3).

Conclusion

In this pilot study, we have demonstrated different ICG uptake and clearance patterns between declined and transplanted livers and a potential association between uptake time and liver utilization and viability assessment by clinical criteria. ICG is a feasible point of care tool to assess hepatocyte and cholangiocyte function for livers maintained on NMP. Additional work is warranted to determine the relationship between ICG uptake and clearance characteristics and discrete clinical outcomes, namely ischemic cholangiopathy, early allograft dysfunction, and primary non-function.

Abstract Keywords

normothermic machine perfusion, extended criteria, DCD, viability assay, biomarker

Poster # 34

Title: Differential Human and Pig Plasma Protein Profiles Following Pig Hepatocyte Exposure in Patients during Bioartificial Liver Treatments

Poster Topic: Xenotransplantation



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Abstract Body: Background

Recent advances in pig-to-human xenotransplant (XTx) using genetically engineered pigs highlights its therapeutic potential. Bioartificial liver (BAL) therapy using wild-type pig hepatocytes offers a unique clinical model to study the xenogeneic immune activation. Characterizing human and pig protein abundance during BAL treatments can provide critical insights to optimize future XTx strategies. Objective: To profile dynamic changes in the human plasma proteome following BAL treatment using nanoparticle-based enrichment technology.

Methods

Six patients who underwent 1-7 BAL treatments, each consisting of 6 hours of plasma perfusion through a cartridge containing pig hepatocytes, were included. For patients receiving multiple treatments, the BAL was repeated within 24 hours. Plasma samples were collected from the patients before each BAL treatment ("Pre"), and up to 2 weeks thereafter. Additional samples were collected from the BAL circuit as plasma returned to the patients post-BAL treatment ("Post"). As Controls, normal human plasma, and wild-type pig plasma were used. Proteomic analysis of 80 samples was performed using Seer Proteograph™ Assay (nanoparticle-based enrichment) and quantitative mass spectrometry

Results

Mass spectrometry analysis (DIA-NN v.1.8.1) using human and pig UniProt sequences identified 159,601 unique peptides (1% FDR), including 65,093 human-specific and 25,539 pig-specific peptides. Using these species-specific peptides, 8,744 human and 4,225 pig protein groups were reconstructed. The graphic shows the human plasma proteins (light blue) and pig proteins (deep blue) identified in each sample, highlighting trends in xeno-related protein dynamics overtime. Human protein abundance showed inter-patient variability prior to BAL exposure, indicating potential heterogeneity in immune responsiveness. Immediately post-BAL ("Post"), an average of 3157 ± 415 pig proteins were detected in plasma, confirming xenogeneic protein transfer. Within 24 hours, pig protein levels significantly decline to 581 ± 175 , suggesting rapid clearance or immune-mediated degradation.

Conclusion

This study provides the first comprehensive characterization of the human plasma proteome following porcine hepatocyte exposure in BAL therapy. Pig proteins significantly increase post-BAL, confirming xenogeneic protein transfer during BAL treatment, followed by rapid clearance, reflecting clearance or immune processing.

Abstract Keywords

Xenotransplantation, Xenoimmune response, Bioartificial Liver, Liver Xenotransplantation, Proteome, Mass spectrometry.