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Natural Killer Cells Recognize Pulmonary Epithelial Stress Molecules during Primary Graft Dysfunction

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seen in BTG (platelet activation) levels, F1+2 (thrombosis) levels, or neutrophil counts. h*pvWF lung baboon recipients trended toward longer survival (p=0.07) with attenuated thrombocytopenia compared to reference lungs (p<0.01)(Fig.2).

Conclusion: When perfused with human blood, h*pvWF lungs had reduced sequestration of platelets compared to pvWF lungs, with similar reductions in lung xenotransplant recipients. "Humanization" of porcine vWF attenuates the nonphysiologic platelet adhesion and aggregation associated with xenograft rejection. Further study will focus on Fc-receptors, adenosine and coagulation pathway dysregulation, and activated neutrophils through additional drugs and genetic modifications.

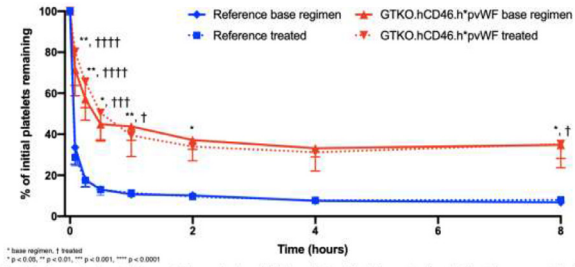

Fig. 1. Ex vivo perfusion of pig lungs with humanized von Willebrand factor (blue) demonstrating significant improvement in platelet sequestration compared to lungs with porcine vWF (red), regardless of treatment (dashed).

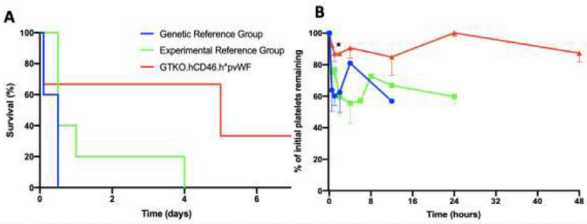

Fig. 2. In vivo lung xenotransplantation demonstrating (A) improved survival of baboon recipients of pig lungs with h*pvWF (red), compared to both genetic controls (blue) and experimental controls (green). The genetic reference groups represent GTKO.hCD46 lungs that had a slightly varied experimental technique, while the experimental reference group includes additional genetic modifications unrelated to platelet activation with identical experimental technique. (B) The h*pvWF lungs also showed improvement in platelet sequestration in the first 48 hours after transplantation compared to both reference groups.

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Natural Killer Cells Recognize Pulmonary Epithelial Stress Molecules during Primary Graft Dysfunction

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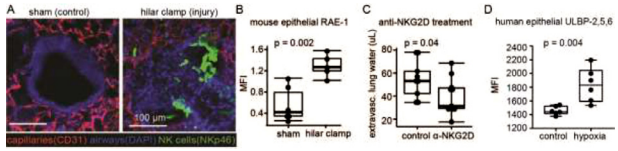
Purpose: Primary graft dysfunction (PGD) occurs in 1/3 of all lung transplants. It is defined by epithelial dysfunction and innate immune cell infiltration. Natural killer (NK) cells are innate lymphocytes that are activated by NKG2D receptor binding of specific stress molecules. We hypothesized that NK cells mediate PGD by recognizing stress molecules induced on lung epithelial cells and causing injury via direct cytotoxicity.

Methods: In an established experimental PGD model, left hilar clamp (HC) was compared to sham (S) surgery in C57BL/6 mice. Stress molecules (RAE-1, MULT1) and NK cell receptors were measured by flow cytometry median fluorescent intensity (MFI) on dissociated lung cells. Mice were given blocking anti-NKG2D or isotype control antibodies preceding HC. Human stress molecules (MICA, MICB, ULBP1, 3, and 2/5/6) were measured by flow cytometry on bronchial epithelial cells incubated for 4 hours in hypoxia (1% O_2) versus normoxia conditions. The Mann-Whitney U test was applied for pairwise comparisons.

Results: NK cells were increased as a percent of lymphocytes ($p = 0.008$) and by count ($p = 0.04$) in HC ($n = 5$) versus S ($n = 5$) lungs and infiltrated airways (Figure 1A). The NKG2D receptor was increased on NK cells following HC ($p = 0.005$). The stress molecules MULT1 ($p = 0.0002$) and RAE-1 (Figure 1B, $p = 0.002$) were increased on epithelial cells in HC compared to S lungs. NKG2D blockade ($n = 9$) resulted in less pulmonary edema following HC compared to isotype control antibody (Figure 1C, $n = 9$, $p = 0.04$). Hypoxic human bronchial epithelial cells ($n = 6$) had increased MICB ($p = 0.008$), ULBP-1 ($p = 0.05$), and ULBP-2,5,6 (Figure 1 D, $p = 0.004$) compared to normoxic cells ($n = 6$).

Conclusion: PGD induces NK cell stress ligands leading to NK cell recruitment that contributes to PGD. NKG2D blockade, under investigation in clinical trials of other diseases, resulted in decreased lung injury. The induction of human NKG2D stress molecules during hypoxia suggests that this NK cell receptor-ligand interaction may mediate PGD.



(337) *Reprinted from the April 2020 meeting issue by approval*

Deconvolution of Donor and Recipient Transcripts from Frozen Lung Transplant Biopsies

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Purpose: Despite years of study using conventional cellular molecular approaches applied to both humans and animal models, the biology of lung transplantation remains incompletely understood. We aimed to develop a new strategy to study molecular mechanisms using human lung transplant samples and modern sequencing technology. The interaction between donor and recipient cells is unique to transplantation. As such, we developed a sequencing and bioinformatic strategy to help deconvolute recipient from donor specific gene expression in a frozen lung tissue biopsy.

Methods: We performed whole exome sequencing on frozen biopsies of pure donor and recipient lungs, followed by RNA-seq on a frozen lung biopsy taken at 2h after reperfusion. For each patient, donor/recipient specific single nucleotide variations (SNVs) were identified and used to quantify donor/recipient expression in the RNA-seq data. The resultant genes were compared to known marker genes from the lung and immune cell types.

Results: Recipient-specific transcripts identified from the RNA-seq data were enriched for immune cell specific transcripts and gene ontology analysis depicted neutrophil activation and degranulation (Fig 1). Donor-specific transcripts were enriched in lung parenchymal cell types. Allele fractions of the recipient specific transcripts had a median between 0.04-0.06, while donor specific transcripts had the expected median of 0.5. This indicates that the majority of gene expression is derived from the donor lung and that there is only a small fraction of recipient derived transcripts as would be expected in the early reperfusion period.

Conclusion: We show that it is possible to deconvolute donor and recipient transcripts at the earliest stages of reperfusion by taking advantage of SNVs. The resultant pathway analysis corresponds with our current knowledge of reperfusion biology. Following validation, we aim to apply this to less well-defined phenomena such as CLAD and to generalize the technique to solid-organ transplantation.