Recipient Emphysema Differentially Affects Immunologic Responses During Acute Lung Allograft Rejection in a Pre-Clinical Model

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BACKGROUND

Immune-modifying disease processes such as IPF and COPD result in a recipient environment that can be hostile to allografts by as of yet, ill-defined mechanisms. In an era driving towards personalized medicine, a better understanding of the potentially differing immune mechanisms present in different patient populations may well shed light on novel therapeutic strategies to modulate immunity and promote long term graft survival. Here we utilize a systems biology approach to determine the impact of recipient pre-transplant emphysema on post transplant graft survival.

METHODS

- C57BL/6 male mice were **exposed to cigarette smoke (CS)** for 5 hours a day, 5 days a week for 6 months, to induce the development of emphysema.
- Emphysematous C57Bl/6 and non-smoke exposed age matched controls underwent **allogeneic orthotopic left lung transplant** from age-matched BALB/c donors.
- Allografts were **harvested at 7 days Post-Ltx for analysis** of graft injury, using:
  - **µ-CT** imaging with 3D reconstruction and automated left lung volume measurement
  - **H&E** staining of formalin fixed paraffin embedded sections
  - **Pathology scored** using ISHLT acute rejection criteria
- In a separate series of transplants, **RNA** was extracted from 7d allografts and analyzed using **Nanostring nCounter Analysis** System using 561 gene Mouse Immunology Codeset. Analysis and normalization of the raw Nanostring data was conducted using nSolver Analysis Software v1.1.
  - Clustergrams and principle component analysis (PCA) were performed in MATLAB 2015, and analysis of gene ontology (GO) biological processes was performed using PANTHER.
- **Mixed Lymphocyte Reaction** was performed using post-LTx Splenocytes co-cultured with naïve Balb/c stimulator cells.
  - GZB, IL-6, IL-17, IFNγ
- **Mass Cytometric** analysis of immune cell populations of post-LTx lungs

RESULTS

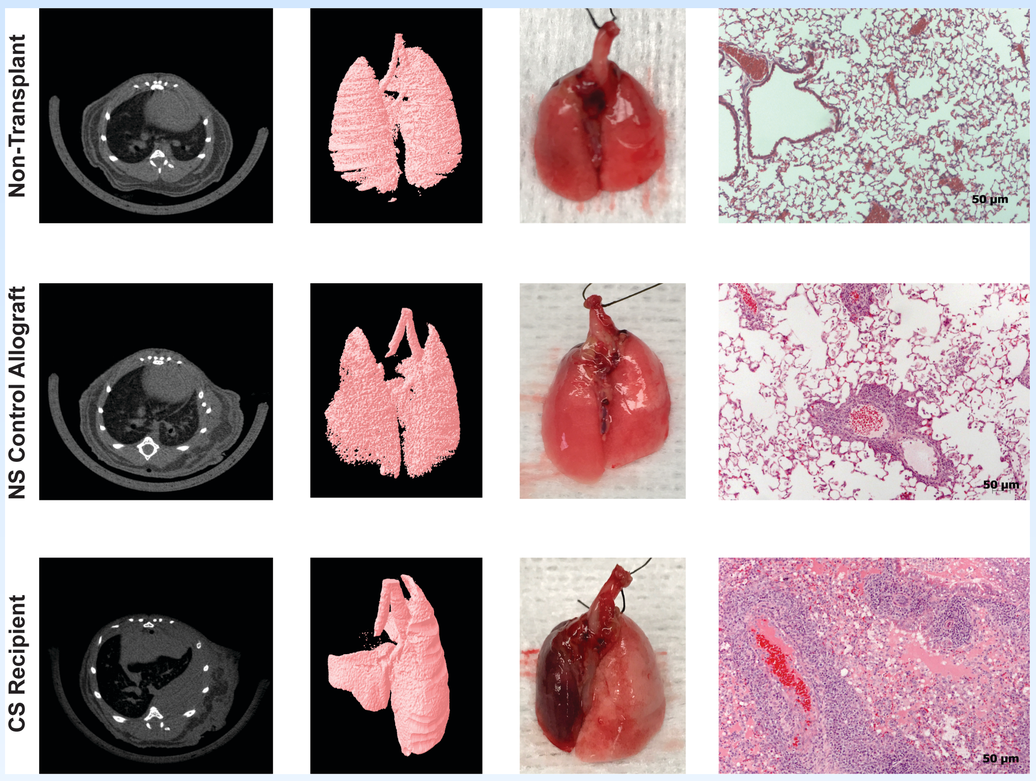

Figure 1. Recipient cigarette smoke (CS) exposure history diminishes post-LTx outcomes. Allografts were imaged at 7 days post-LTx using micro-CT (Skyscan 1272, Bruker) at a pixel resolution of 35µM. 3D reconstructions were performed using NRecon reconstruction software. Mice were then sacrificed, and formalin-inflated lungs were processed, paraffin embedded, and stained with hematoxylin and eosin. CS recipient mice (bottom row) demonstrated no visible radiographic airspace, poorly inflated with formalin at the time of harvest, and had strikingly increased cellular infiltration and mucous and fluid deposition throughout the allograft.

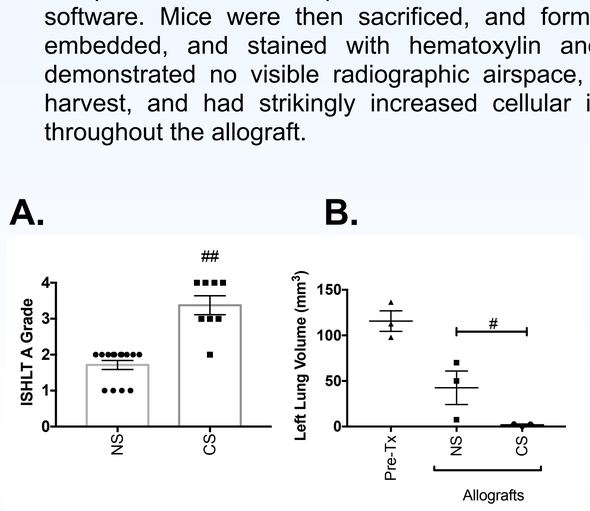

Figure 2. Acute rejection is exacerbated in CS recipients. Left lung volume measurements (A) were made from 3D reconstructions of allografts at 7 days post-LTx, and is an effective measurement of detectable airspace. H&E sections were scored for histologic injury using ISHLT A criteria (B). #, p<0.05; ##, p<0.001.

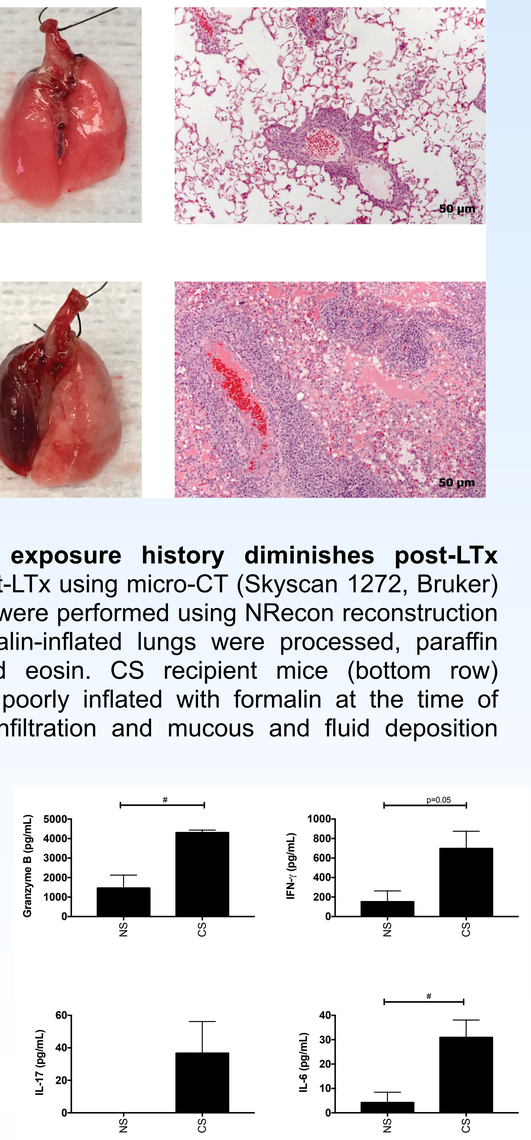

Figure 3. Immune cells in CS recipients develop an increased pro-inflammatory state post-LTx. Splenocytes from recipients 7 days post-Ltx were co-cultured with naïve allogeneic stimulator cells. Pro-inflammatory cytokine production was measured by ELISA. n=3 per group. #, p<0.05.

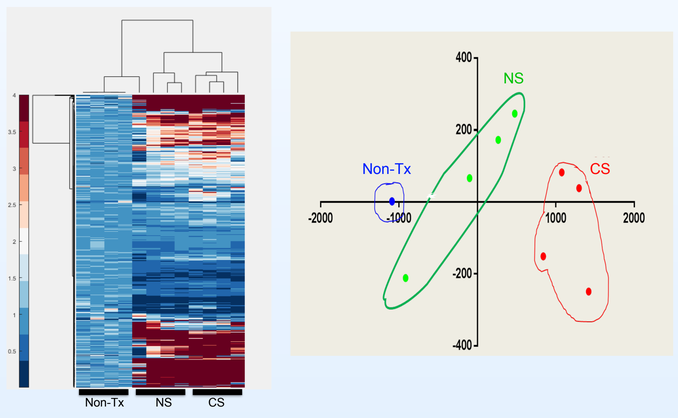

Figure 4. Nanostring analysis of mRNA revealed significantly different inflammatory gene expression among CS Recipients, NS recipients, and non-transplant controls. Principle component analysis (PCA) and heat map visualization of gene expression demonstrates distinctly different patterns among the three analyzed groups.

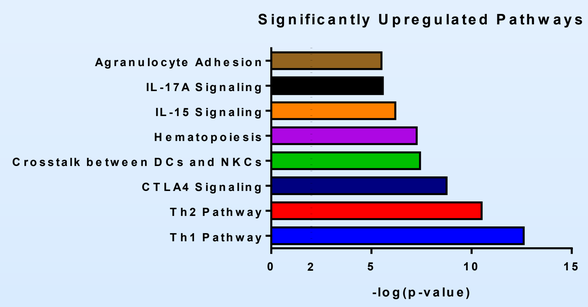

Figure 5. Nanostring analysis of mRNA expression revealed significantly altered immune pathways in CS recipients versus NS transplants. Results of pathway analysis using IPA for pathways significantly enriched in genes upregulated by CS compared to NS showing a significant increase in genes related to cell mediated response and cross talk between innate and adaptive immunity.

RESULTS

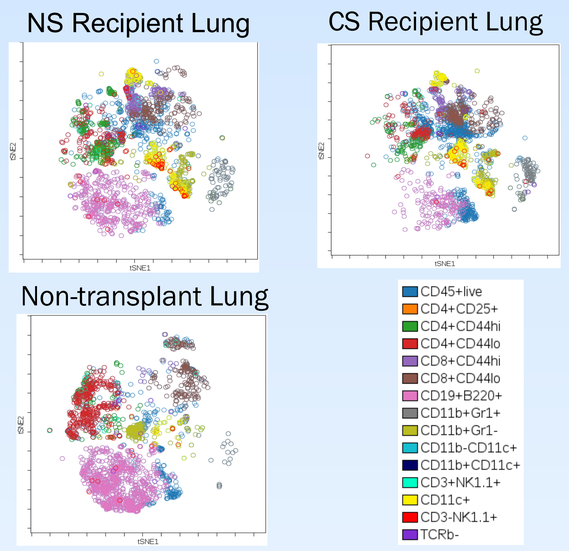

Figure 6. High-dimensional mass cytometric analysis of 7 day post-LTx lungs demonstrated distinctly altered immunocellular infiltration into CS recipient allografts. Single cell suspensions of allografts were purified for CD45+ cells and tagged with heavy-metal labeled antibodies. Analysis was performed using Helios CyTOF mass cytometry. Initial analysis demonstrated alterations in CD8/CD4 memory cell populations and frequencies in CS recipients. B cell and myeloid cell populations in CS were also altered as compared to NS and non-Tx controls.

CONCLUSIONS

- Recipient CS exposure results in acute rejection and graft failure with distinctly altered immune responses from age-matched NS controls.
- Using high-throughput techniques we have identified pathways that markedly differ in CS-recipient populations.
- Personalized immunotherapy specifically tailored to this patient population may be necessary to achieve substantive improvements in long-term outcomes.
- Moving forward, we plan to utilize the results of these analyses to probe the differential immune responses in these populations in an attempt to identify novel therapeutic approaches to LTX care.

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