Ischemia-reperfusion inexperienced recipient origin non-classical monocytes are dispensable for the pathogenesis of primary lung allograft dysfunction

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Background

Primary Graft Dysfunction (PGD) is the primary driver for both short- and long-term mortality following lung transplantation. As the pathogenesis of PGD is incompletely understood, its incidence remains around 30% and the outcomes of lung transplantation remain inferior to those of other solid organ transplant.[1,2,3] We have previously shown that PGD is mediated by initiation of recipient neutrophil migration into the lung allograft by donor nonclassical monocytes (NCM) which are retained in the allograft despite lung intravascular vascular flushing prior to implantation and thus experience ischemia-reperfusion.[4]

Donor-derived NCM are dissipated and replaced by recipient-derived NCM following lung transplant. Therefore, we hypothesized that recipient-derived NCM could also propagate the influx of recipient neutrophils and lead to PGD.

Research Objectives

1. Analyze the role of recipient and donor NCM in the pathogenesis of PGD
2. Characterize how recipient NCM replace donor NCM following lung transplantation
3. Investigate the transcriptional differences between donor- and recipient- derived NCM following lung transplant

Methods

- Allogeneic and syngeneic murine single lung transplants were performed
  - Donor and Recipient NCM were distinguished using a CD45.1 and CD45.2 isoform system
- Multi-color flow cytometry was used to characterize donor vs. recipient NCM cell percentages at various timepoints following post-reperfusion as well as assess lung allograft neutrophil infiltration to gauge PGD
- Bulk RNAseq was used for transcriptional profiling of florescence-activated cell sorted donor and recipient NCM following lung transplant and pathway analyses were performed

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Results

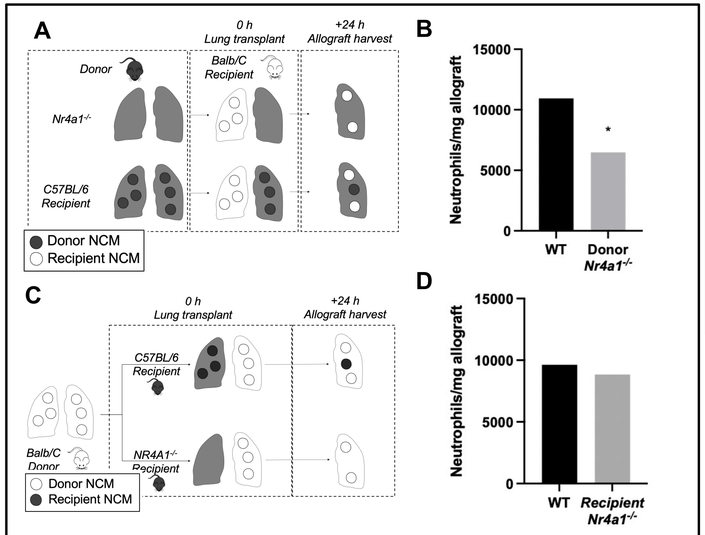


Figure 1. Genetic depletion of donor NCM, and not recipient NCM, abrogates PGD in allogeneic lung transplantation. (**A**) Experimental design for donor NR4A1 knockout transplants. (**B**) Neutrophil infiltration was determined using flow cytometry. * p = 0.05 by unpaired student's *t* test.(**C**) Experimental design for recipient NR4A1 knockout transplants. (**D**) Neutrophil infiltration was determined using flow cytometry, and there was no significant difference between the populations by unpaired student's *t* test.

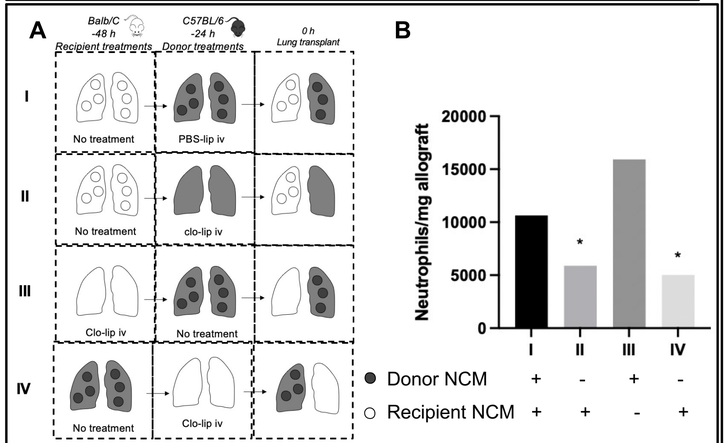


Figure 2. Pharmacologic depletion of donor NCM mitigates PGD while depletion of recipient NCM has no effect in allogeneic lung transplantation. **(A)** Experimental design using different NCM depletion strategies to selectively deplete NCM in donors and recipients using clodronate loaded liposomes (clo-lip). (**B**) Allografts were harvested at 24 hours after transplants, and neutrophil influx was determined by flow cytometry. *p <0.01, compared to group I by unpaired student's *t* test.

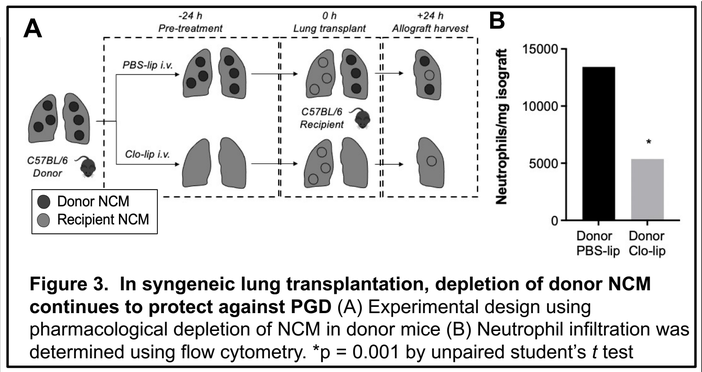
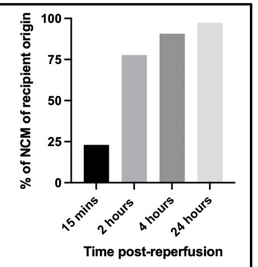


Figure 3. In syngeneic lung transplantation, depletion of donor NCM continues to protect against PGD (A) Experimental design using pharmacological depletion of NCM in donor mice (B) Neutrophil infiltration was determined using flow cytometry. *p = 0.001 by unpaired student's *t* test

Figure 4. Recipient NCM replace nearly all donor NCM by 4 hours. Following allogeneic lung transplantation, allografts were harvested at +15 min, 2 hours, 4 hours or 24 hours following reperfusion. Flow cytometry was then used to determine the composition of recipient and donor NCM.



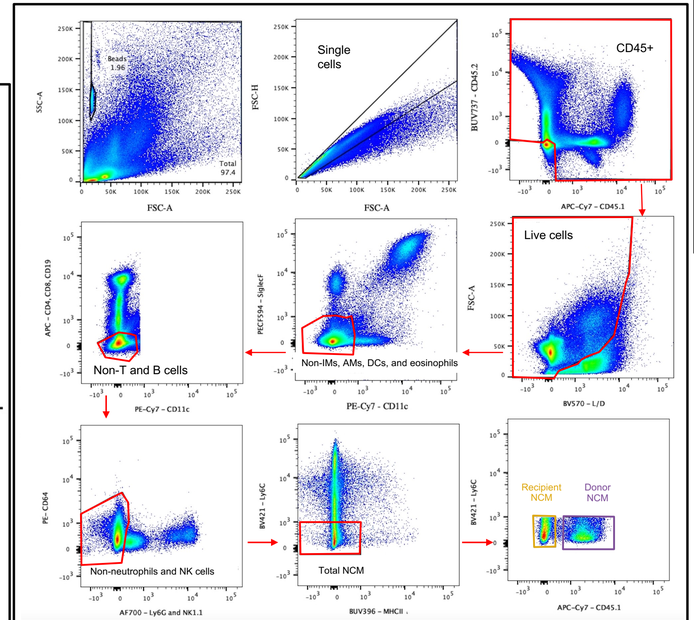


Figure 5. Sorting strategy for donor and recipient NCM. Using a novel negative selection technique, donor (CD45.1+ NCM) and Recipient (CD45.2+ NCM) were sorted.

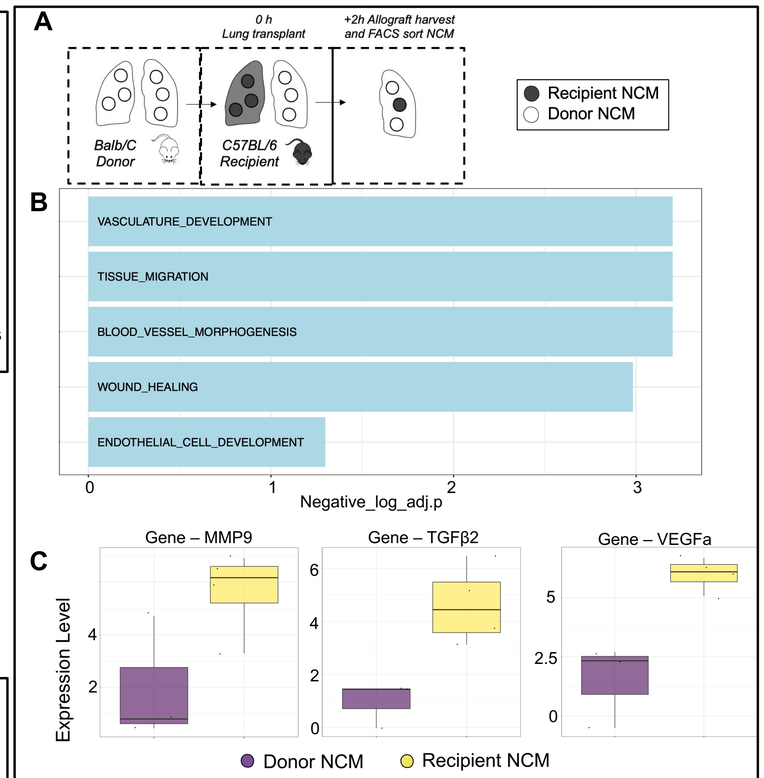


Figure 6. Bulk RNA-seq data from donor or recipient derived NCM. (**A**) Experimental plan to isolate recipient and donor NCM from +2hr posttransplant lung. (**B**) Pathway analysis with GO Biological processes was performed comparing donor and recipient NCM, with upregulated recipient NCM pathways shown. (**C**) Box plots of expression for select genes significantly upregulated in recipient compared to donor NCM

Conclusions

- Neutrophil influx is self-propagating after being initiated by donor NCM, and continues even in the absence of recipient NCM
- Protection against PGD via depletion of donor NCM is not due to species-specific differences in NCM or donor-recipient incompatibility
- Donor NCM are quickly replaced by recipient NCM by 4 hours following lung transplant
- Ischemia-Reperfusion inexperienced recipient NCM upregulate VEGF, MMP, and TGFb2, suggesting a role in vasculature remodeling

References

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