Adoptive Transfer of Murine Engineered T Regulatory Cells Ameliorates Disease in a Model of Lipopolysaccharide Induced Acute Lung Injury

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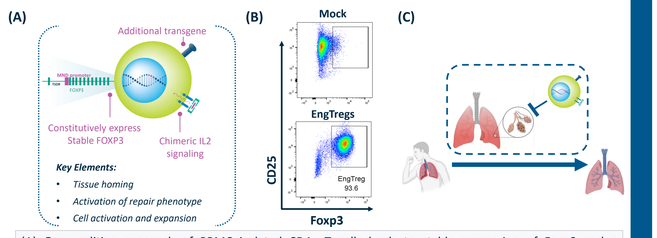
INTRODUCTION & PREMISE OF GNTI PLATFORM

Introduction

- Acute Respiratory Distress Syndrome (ARDS) is a life-threatening condition characterized by diffuse alveolar tissue damage, pulmonary edema, severe hypoxemia, increased inflammatory cytokine response, and immune cell infiltration.
- A major driver of ARDS is the widespread dysregulation of the pulmonary immune response.
- Despite medical advancements, treatment of ARDS still primarily relies on managing symptoms, stabilizing disease progression, and relying on the patient's intrinsic healing processes.
- Foxp3+ T regulatory cells play a key role in lung homeostasis and tolerance, controlling inflammation and promoting tissue repair responses during ARDS resolution.
- GentiBio's platform allows for the generation of stable, tissue targeted, selectively activated T regulatory cells from PBMC isolated bulk CD4+ T cells.
- The production of a murine polyclonal surrogate engineered T regulatory cell (poly-mEngTreg) allows for the evaluation of the GNTI platform in murine preclinical models.
- Intratracheal instillation of lipopolysaccharides (LPS) triggers acute lung injury (ALI) in mice, recapitulating many symptoms visible throughout the course of human ARDS.
- **It is the purpose of the current work to assess the ability of murine polyclonal engineered T regulatory cells to ameliorate disease in a mouse model of LPS induced acute lung injury.**

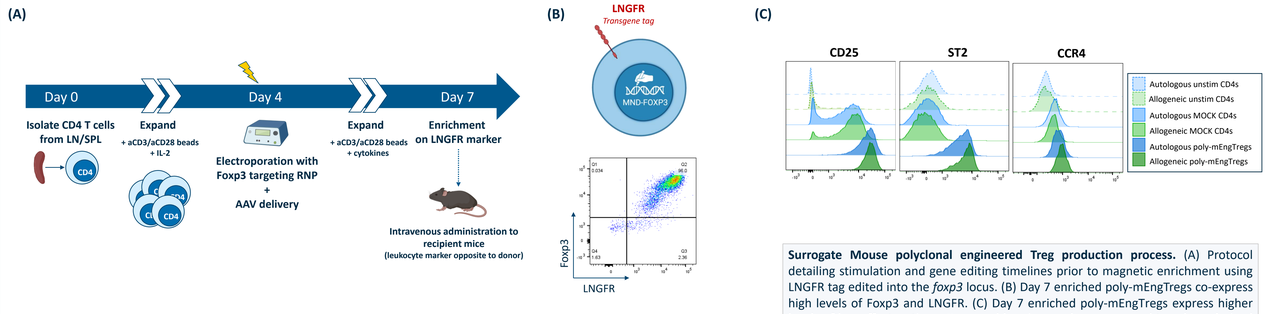
Premise of GNTI Treg Platform



(A) Gene editing approach of PBMC isolated CD4+ T cells leads to stable expression of Foxp3 and a rapamycin-activated signaling complex that provides tunable IL-2 signal, divorcing Foxp3 expression from regulatory elements known to promote Treg instability under inflammatory conditions[1]. Additional transgenes could enable tissue homing and mediation of tissue Treg capabilities. (B) Engineered T regulatory cells (EngTregs) express high levels of CD25 and Foxp3. (C) Building on preclinical support of Treg efficacy in the treatment of acute lung injury, an off the shelf allogeneic Treg product could offer a promising therapeutic approach for acute inflammatory pulmonary diseases such as ARDS.

MURINE SURROGATE SYSTEM

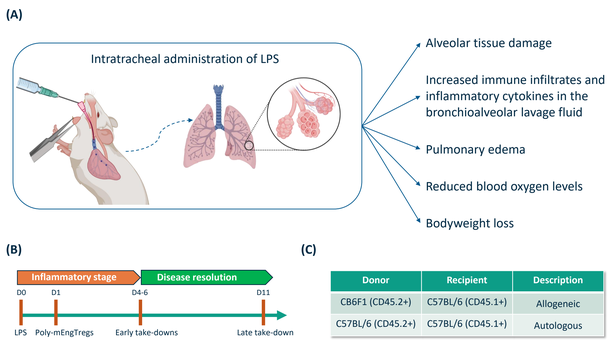
Murine surrogate polyclonal engineered Treg production



Surrogate Mouse polyclonal engineered Treg production process. (A) Protocol detailing stimulation and gene editing timelines prior to magnetic enrichment using LNGFR tag edited into the *foxp3* locus. (B) Day 7 enriched poly-mEngTregs co-express high levels of Foxp3 and LNGFR. (C) Day 7 enriched poly-mEngTregs express higher levels of key effector, tissue Treg, and homing markers compared to spleen-derived T conventional cells.

METHODS

Overview of LPS induced ALI approach and study design



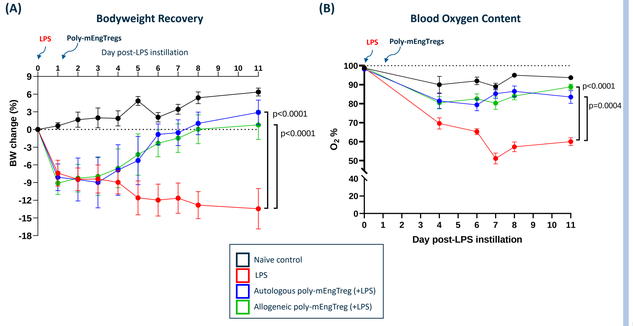
Donor	Recipient	Description
CB6F1 (CD45.2+)	C57BL/6 (CD45.1+)	Allogeneic
C57BL/6 (CD45.2+)	C57BL/6 (CD45.1+)	Autologous

Key Read-outs
✓ Bodyweight change and pulse oximetry
✓ Hematoxylin and eosin (H&E) stain of lung
✓ Lung weight
✓ Engraftment of poly-mEngTregs in spleen, lung, and bronchioalveolar fluid (BAL)
✓ Proliferation of poly-mEngTregs in spleen, lung, and BAL
✓ Neutrophil infiltration and alveolar macrophage population in lung and BAL
✓ Inflammatory cytokine response in BAL

LPS induced ALI introduction and study design. (A) Direct pulmonary insult with LPS via intratracheal instillation triggers acute lung injury (ALI) in mice, recapitulating many pathophysiological conditions visible in the progression of human ARDS. (B) Timeline of study design and disease progression. Adoptive transfer of poly-mEngTregs occurred 24 hours following intratracheal administration of LPS from *E. coli* serotype O55:B5. (C) CB6F1 donors were used in the generation of allogeneic poly-mEngTregs to model non-NK cell rejection of transferred cells. CD45.1 expressing congenic hosts were used as recipients to enable *ex vivo* tracking of CD45.2+ poly-mEngTregs. (D) Graphic schematic of experimental groups and key read-outs.

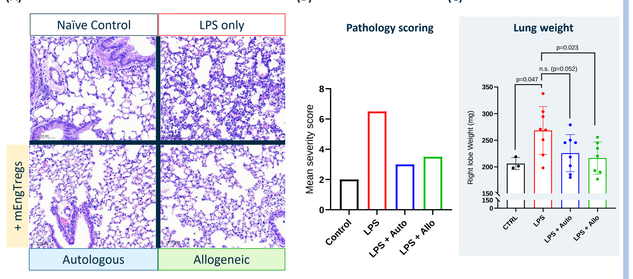
RESULTS

Figure 1. Reduced disease severity in poly-mEngTreg treated mice



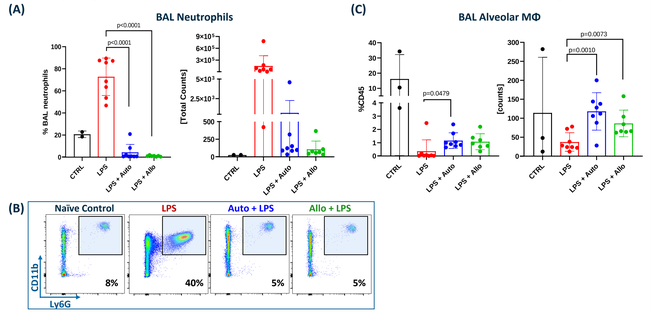
Reduced disease severity in poly-mEngTreg treated mice. Significant improvements in body weight recovery (A) and O_2 saturation (B) of autologous and allogeneic poly-mEngTreg treated animals. Autologous and allogeneic poly-mEngTregs ameliorate disease to an equivalent extent. Statistics by two way repeated measures ANOVA (mixed effects analysis with multiple comparisons); Day 11 statistics reported.

Figure 2. Poly-mEngTregs ameliorate ALI based on histopathology and lung weight



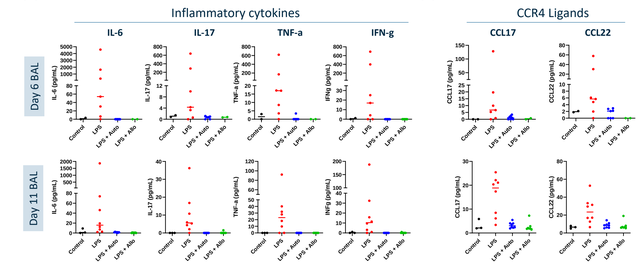
Amelioration of LPS induced lung injury in autologous and allogeneic poly-mEngTreg treated mice. (A) Fewer infiltrating leukocytes observed on Day 6 in lung sections from LPS treated mice receiving allogeneic or autologous poly-mEngTregs. (B) Lower combined lesion score observed (based on H&E and Masson's trichrome staining). (C) Lower lung weight in poly-mEngTreg treated groups at Day 11, suggesting a significant reduction in fluid build-up and inflammation. Statistics by unpaired T test.

Figure 3. Reduced neutrophil infiltration and restoration of alveolar macrophages in the BAL of LPS ALI mice treated with poly-mEngTregs



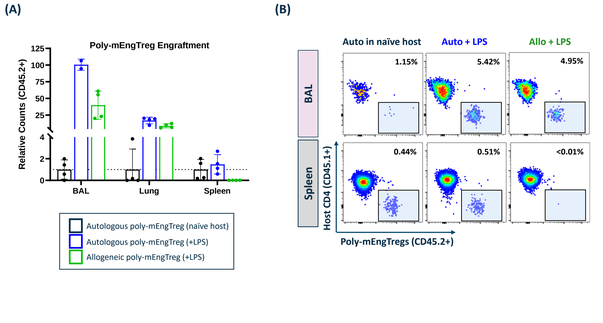
Decreased immune infiltrates and restoration of alveolar macrophage compartment in the BAL of poly-mEngTreg treated LPS ALI mice. (A) Decreased neutrophil frequency and counts at Day 11 in the BAL of both allogeneic and autologous poly-mEngTreg treated animals. (B) Representative flow cytometry plots of lung infiltrating neutrophils. (C) Normalized frequency and counts of alveolar macrophages in the late phase of acute lung injury in LPS treated mice receiving allogeneic or autologous poly-mEngTregs. Statistics by unpaired T test.

Figure 4. Lower concentration of pro-inflammatory cytokines and chemokines in the BAL of poly-mEngTreg treated mice



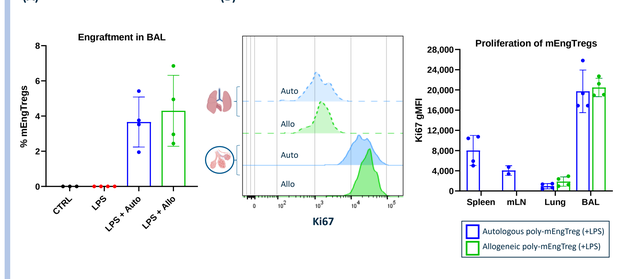
Decreased pro-inflammatory cytokines and chemokines in the BAL of poly-mEngTreg treated animals. (A) Lower levels of key inflammatory cytokines IL-6, IL-17, TNF-a, and IFN-g detected in the BAL of poly-mEngTreg treated animals on Day 6 and 11. (B) Reduction of CCR4 ligands, CCL17 and CCL22, in the BAL of autologous and allogeneic poly-mEngTreg treated ALI mice compared to LPS controls.

Figure 5. Specific homing of poly-mEngTregs to the site of inflammation



Inflammation specific homing and persistence of polyclonal mEngTregs in ALI mice. (A) Increased counts of autologous and allogeneic poly-mEngTregs in the BAL and lungs, but not spleen, of LPS ALI mice during the inflammatory stage of disease (Day 5) compared to naïve mice receiving autologous cell transfer. (B) Representative flow cytometry plots of poly-mEngTregs detected within the spleen and BAL of LPS treated or naïve mice.

Figure 6. Tissue-specific proliferation of poly-mEngTregs at the site of inflammation



Inflammation driven proliferation of polyclonal mEngTregs (A) Autologous and allogeneic poly-mEngTregs engraft to a comparable extent in the BAL at the height of inflammation. (B) Proliferation of poly-mEngTregs is increased at the site of inflammation and there is no difference in the proliferative status of autologous and allogeneic cells. Mesenteric lymph nodes (mLN) included as an example of non-inflammatory distal mucosal site.

CONCLUSIONS

- **GentiBio's platform overcomes the scaling limitations of sorted patient Treg cells by starting with more abundant T cell sources and specifically enriching edited cells with an engineered IL-2 signaling receptor.**
- **Genti surrogate murine engineered Treg cells express key markers of pulmonary thymic Tregs including Foxp3, CD25, CCR4 and ST2.**
- **Poly-mEngTreg treatment significantly improves disease outcome in a model of LPS induced acute lung injury.**
- **Allogeneic and autologous poly-mEngTregs show equivalent efficacy based on body weight and blood oxygen saturation.**
- **Fewer day 11 inflammatory infiltrates observed in the lung and BAL of autologous and allogeneic poly-mEngTreg treated mice, with normalized counts of alveolar macrophages, reduced BAL inflammatory cytokines, and normalized lung weight suggesting a return towards pulmonary immune homeostasis.**
- **High frequency of poly-mEngTregs are detected during the inflammatory phase of disease at the site of inflammation, while lower persistence is observed at distal sites with lower inflammation during ALI.**
- **Poly-mEngTregs are actively proliferation at the site of inflammation.**
- **These data lend support to the use of allogeneic CD4 derived engineered Tregs as a powerful off-the-shelf therapeutic approach for acute onset inflammatory diseases such as ARDS.**

*These authors contributed equally to this work

REFERENCES

- [1] Honaker S, *Science Translational Medicine*, 2020
- Introductory and methods figures created with BioRender.com

ACKNOWLEDGEMENTS

- The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs.
- The research groups of Dr. Jason Mock and Dr. Heth Turnquist contributed to the process of setting up models of acute lung injury.

DISCLOSURES

- All authors are employed by GentiBio.