Engineered Regulatory T cells ameliorate lung injury in the LPS model of acute lung injury

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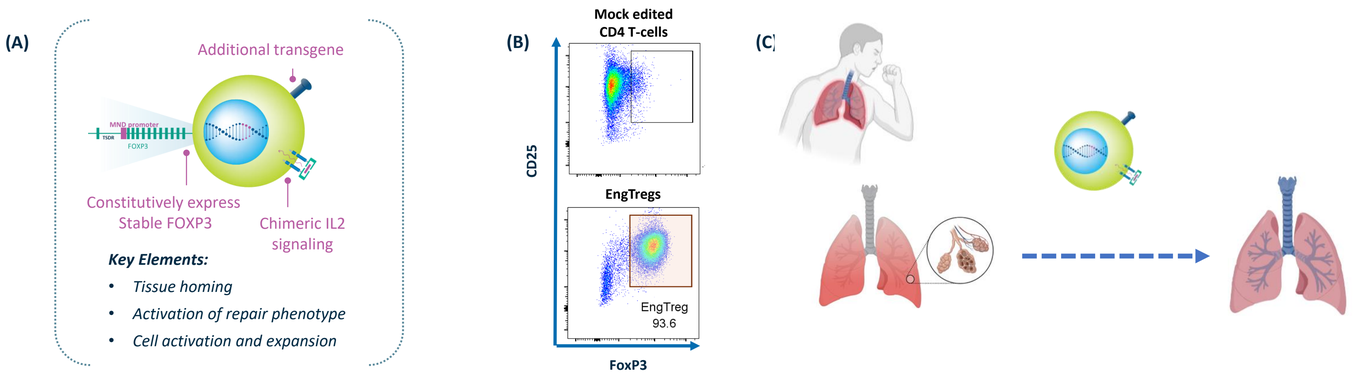
ABSTRACT

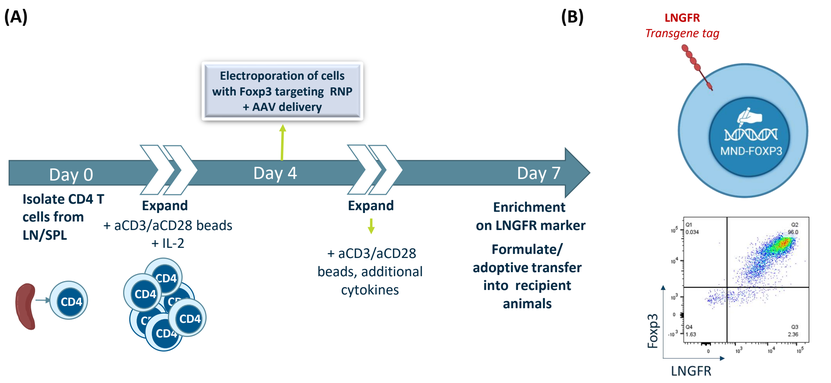
Acute respiratory distress syndrome (ARDS), is marked by acute pulmonary inflammation and life-threatening hypoxia, resulting from different etiologies including viral infections such as SARS-CoV2. Although patient outcomes have been improved by implementation of prone positioning, mechanical ventilation and corticosteroid administration, ARDS continues to have few therapeutic options and a high rate of morbidity and mortality. A key driver of tissue damage in ARDS remains the widespread dysregulation of the pulmonary immune response, ultimately resulting in tissue damage and fluid buildup in the lung parenchyma. Regulatory T cells (Tregs) are known to play a key role in tissue repair and establishing peripheral immune tolerance and their accumulation in the lung is associated with improved clinical outcomes in the context of SARS-CoV2 associated ARDS. We evaluate the efficacy of polyclonal murine Engineered Tregs (mEngTregs), generated by gene editing from splenic CD4 T cells, in an LPS induced model of acute lung injury (ALI) selected due to similarities with viral ARDS kinetics and inflammatory manifestation. We find that mEngTreg treated animals show significantly improved disease outcome as assessed by measurements of body weight, pulse oximetry and lung volume. mEngTregs were found to effectively home to the site of pulmonary inflammation and resemble endogenous Tregs in expression of key markers such as FOXP3, CD25, ST2 and CCR4. Importantly, the presence of the mEngTreg in the lung interstitium correlated with significantly reduced immune infiltrates such as neutrophils as well as lower levels of inflammatory cytokines such as IFN-g and TNF-a in the bronchoalveolar lavage (BAL). Finally, histopathology analysis of lung tissue from LPS ALI mice treated with mEngTregs appeared to show lower tissue damage or potential repair when compared to LPS ALI mice. Overall, our work lends supports to CD4 derived Engineered Tregs as a therapeutic approach for acute pulmonary diseases.

PREMISE



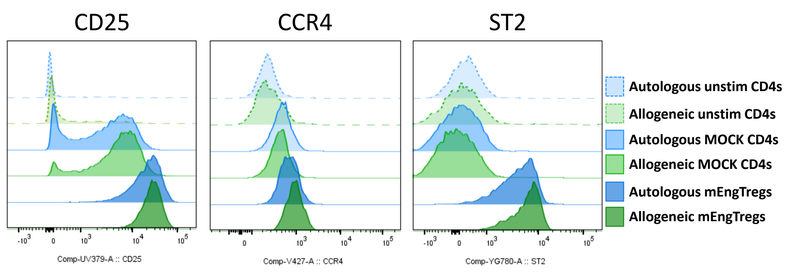
Overview of human Engineered Tregs as a therapeutic approach for acute pulmonary inflammation. (A) Briefly, gene editing approach of PBMC isolated CD4+ T cells, leads to stable FOXP3 expression and expression of a rapamycin-activated signaling complex that provides tunable IL-2 signal, thereby effectively divorcing FOXP3 expression from existing regulatory elements known to promote Treg instability under inflammatory conditions (Honaker S, *Science Translational Medicine*, 2020). Additional key elements obtained through manufacturing process and expression of additional transgenes would enable effective tissue homing and mediation of tissue Treg capabilities including enhanced proliferation / survival in response to signals from the inflammatory microenvironment. (B) EngTregs but not mock edited PBMC derived CD4 T cells express high levels of CD25 and FoxP3. (C) Building on significant preclinical support showing efficacy of Tregs in treating acute lung injury, an off the shelf allogeneic Treg product would offer a promising therapeutic approach for acute inflammatory pulmonary diseases such as ARDS.

1. Methods: GNTI murine polyclonal Engineered Treg surrogate process



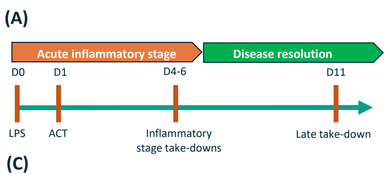
GNTI murine polyclonal Engineered Treg surrogate production process. (A) Protocol outline detailing stimulation and gene editing timelines prior to magnetic enrichment using LNGFR surface protein tag edited into the *foxp3* gene locus. (B) Day 7 enriched murine surrogate polyclonal murine Engineered Tregs (mEngTregs) co-express FOXP3 and LNGFR.

2. Results: Expression of key Treg markers by GNTI mouse Engineered Tregs



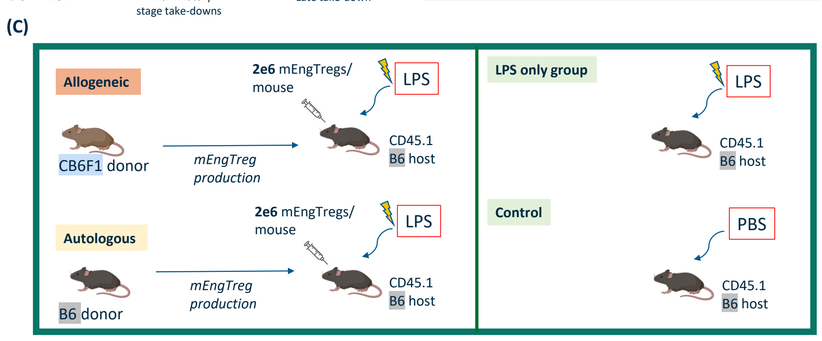
GNTI mouse Engineered Tregs express higher levels of key homing, tissue and effector markers compared to mock edited splenic T conventional cells (Tcons). Day 7 GNTI surrogate polyclonal mEngTregs produced for the acute lung injury experiments expressed higher levels of key Treg effector markers (e.g. CD25), homing receptors (e.g. CCR4) and tissue Treg markers (e.g. ST2) compared to unstimulated and mock edited Tcons.

3. Methods: Overview of LPS induced ALI approach and study groups



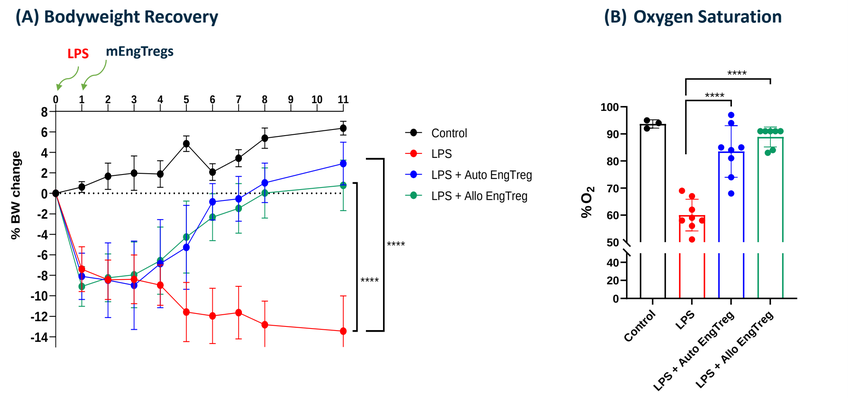
(B)

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



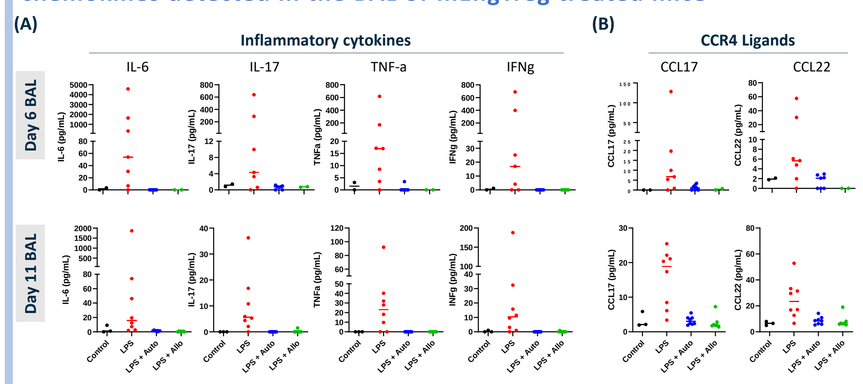
Overview of LPS induced ALI approach and study groups. (A) Graphic depiction of study outline. Adoptive cell transfer (ACT) of mEngTregs occurs 24 hours following intratracheal (i.t.) administration of LPS. (B) CB6F1 cells were used as allogeneic cells to model non-Natural Killer cell mediated rejection of transferred cells. CD45.1$^+$ C57BL/6 (CD45.1 B6) congenic hosts were used as recipients to enable tracking of mEngTregs. (C) Graphic schematic of study groups and controls.

4. Results: Reduced ALI disease severity in autologous and allogeneic mEngTreg treated mice



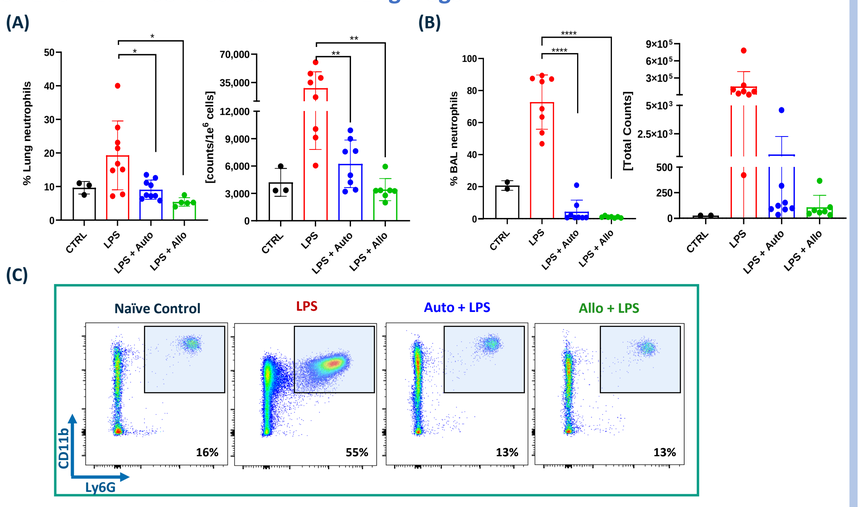
Reduced disease severity in mEngTreg treated animals. (A) Significant improvement in body weight of autologous or allogeneic mEngTreg treated LPS ALI group. (B) Significantly improved D11 O_2 saturation in mEngTreg treated LPS ALI animals. (A)(B) No significant differences observed between autologous and allogeneic mEngTreg treated groups.

5. Results: Lower concentration of pro-inflammatory cytokines and chemokines detected in the BAL of mEngTreg treated mice



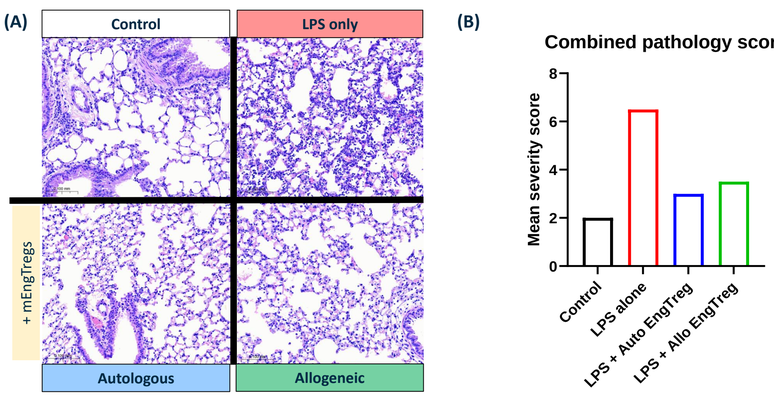
Decreased pro-inflammatory cytokines and chemokines in the BAL of mEngTreg treated animals. (A) Lower levels of key inflammatory cytokines IL-6, TNF-a, IL-17, and IFN-g detected in the BAL of polyclonal mEngTreg treated animals on days 6 and 11. (B) Decreased levels of CCR4 ligands, CCL17 and CCL22 in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

6. Results: Reduced neutrophil infiltrates detected in the lungs and BAL of LPS ALI mice treated with mEngTregs



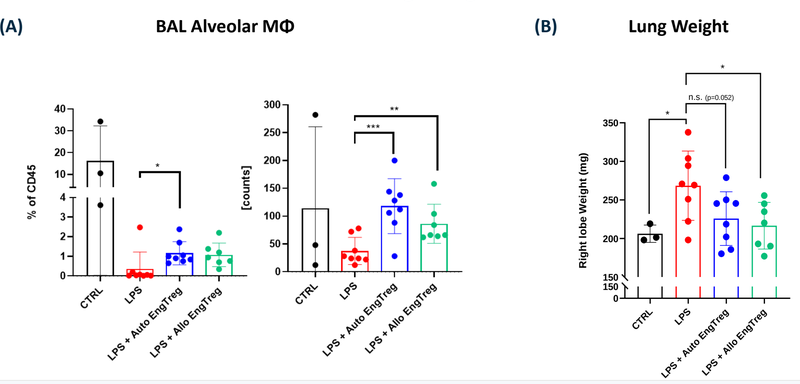
Decreased immune infiltrates in the lung and BAL of mEngTreg treated LPS ALI mice. (A)(B) Decreased neutrophil frequency and counts by day 11 in the lung (A) and BAL (B) of both allogeneic and autologous poly-mEngTreg treated animals. (C) Representative flow cytometry plots of lung neutrophil infiltrates.

7. Results: Reduced disease severity based on histopathology in polyclonal mEngTreg treated ALI mice



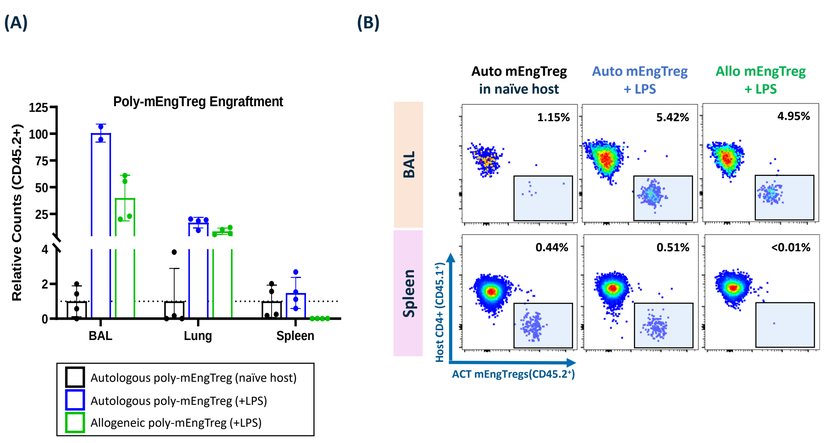
Amelioration of LPS induced ALI in autologous and allogeneic mEngTreg treated mice. (A) Fewer infiltrating leukocytes observed in H&E stained lung sections from LPS treated mice receiving allogeneic or autologous polyclonal mEngTregs. (B) Lower combined lesion score observed by day 6 following mEngTreg treatment (based on H&E and trichrome staining).

8. Results: Restoration of alveolar macrophages in mEngTreg treated ALI mice correlates with lower lung weight



Restoration of alveolar macrophages correlates with reduced lung weight following LPS induced ALI in allogeneic mEngTreg treated mice. (A) Normalized frequency and counts of pro-repair alveolar macrophages in the late phase of acute lung injury in i.t. LPS treated mice receiving allogeneic or autologous polyclonal mEngTregs. (B) Normalized (lower) lung weight observed by day 11 in mEngTreg treated LPS ALI mice.

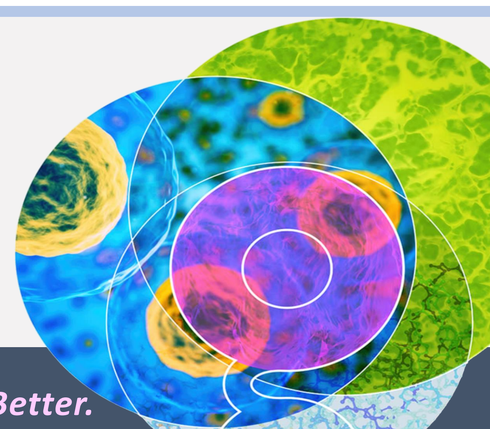
9. Results: Specific homing and persistence of allogeneic mEngTregs to site of tissue damage in LPS induced ALI model



Inflammation tuned homing and persistence of polyclonal mEngTregs in ALI treated mice. (A) Increased counts of autologous and allogeneic mEngTregs in the BAL and lungs but not spleen of LPS ALI mice (D5-6) compared to naïve mice receiving autologous polyclonal mEngTregs. (B) Representative flow cytometry plots of autologous and allogeneic ACT mEngTregs collected from the spleen and BAL of LPS treated or naïve mice (D5).

CONCLUSIONS

- GentiBio's Engineered Treg platform overcomes the scaling limitations of sorted donor 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.
- Equivalent efficacy and improved disease outcome observed in LPS induced acute lung injury mice treated with allogeneic or autologous mEngTregs based on body weight and pulse oximetry measurements.
- Fewer day 11 inflammatory infiltrates observed in the lung and BAL of autologous and allogeneic mEngTreg treated mice, with normalized counts of alveolar macrophages, reduced BAL inflammatory cytokines and normalized lung weight suggesting return towards pulmonary immune homeostasis.
- High frequency of allogeneic mEngTregs detected during the inflammatory phase of disease and at the site of inflammation while lower persistence is observed in distal sites with lower inflammation during ALI.
- This data lends support to the use of allogeneic CD4 derived Engineered Tregs as a powerful off-the-shelf therapeutic approach for acute onset inflammatory diseases including vARDS.



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We make Tregs. *Better.*