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Identification of a Type 1 Regulatory T Cell Master Regulator

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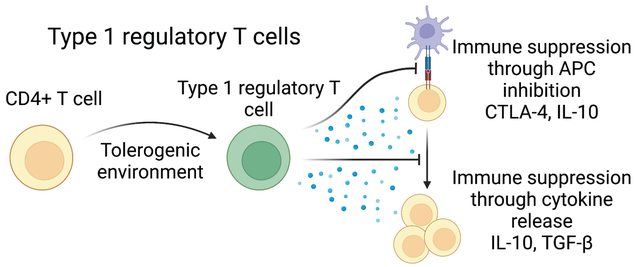
INTRODUCTION

Type 1 regulatory T cells (Tr1s) are a CD4⁺ T cell type with critical roles in transplant acceptance and prevention of autoimmunity.



MASTER REGULATOR:
A transcription factor specific to a T cell subset that is critical for differentiation and function of that subset.

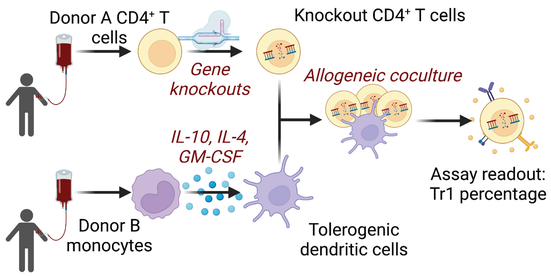
No Tr1 master regulator has been identified.
Parallel ATAC-seq and RNA-seq of Tr1s by Alma-Martina Cepika has revealed a list of transcription factors that could contain a Tr1 master regulator.

Subset:	Master Regulator:
Th1	T-bet
Th2	GATA3
Th9	PU.1
Th17	RORγt
Th22	AHR
T_{FH}	BCL-6
Treg	FOXP3
Tr1	???

METHODS

CRISPR-Cas9 was used to knock out potential Tr1 master regulators, and the cells were differentiated into Tr1s *in vitro*. Gene knockouts that impair Tr1 differentiation are candidates for further testing.
Flow cytometry was used to characterize the culture product and determine Tr1 percentage.

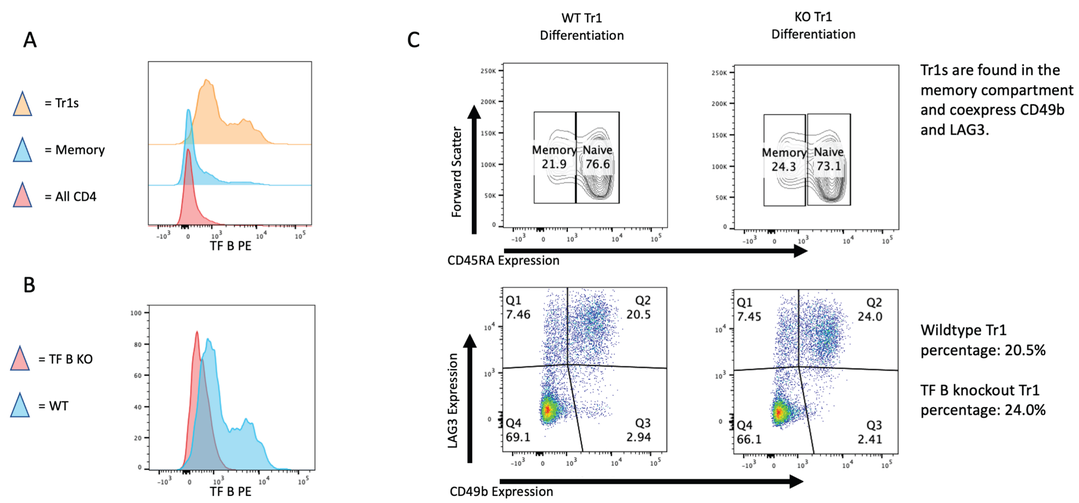
Type 1 Regulatory T Cell Differentiation



Tr1 differentiation protocol (Tallo10) by Chen, Cepika et al., Science and Translational Medicine, 2021.

RESULTS

Knockout of TF B does not affect Tr1 induction efficiency



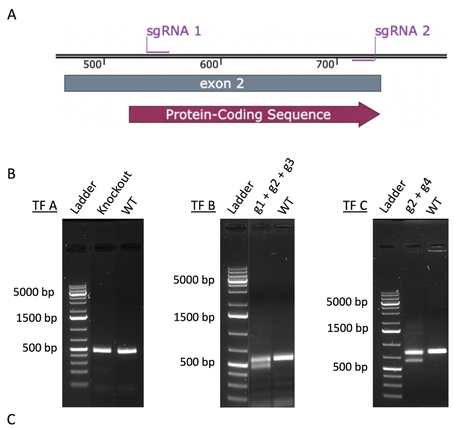
Tr1s are found in the memory compartment and coexpress CD49b and LAG3.

Wildtype Tr1 percentage: 20.5%

TF B knockout Tr1 percentage: 24.0%

Effect of TF B knockout on Tr1 differentiation. A: TF B is only expressed in Tr1s. B: TF B knockout cells express no TF B. C: knockout of TF B has no effect on Tr1 differentiation as determined by expression of CD49b and LAG3 at day 10 of Tr1 differentiation.

Knockout strategy and efficiency

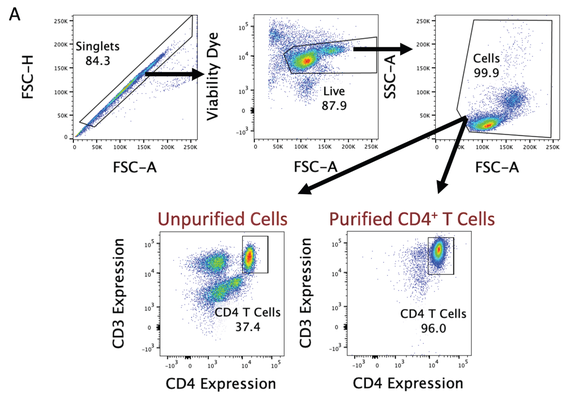


C

Gene	Mean Editing Efficiency	Mean KO Score	Mean Model R^2
A (n=2)	74	72	0.97
B (n=3)	78	75	0.92
C (n=1)	95	94	0.88

Knockout strategies and knockout efficiency. A: example knockout strategy where Cas9 sgRNs are targeted to either end of the first protein-coding exon of a gene of interest. B: example DNA gels showing the effect of knockout strategies for genes TF A, B, and C, on PCR product length. TF A was knocked out with a single guide and shows no major difference in amplicon length due to knockout. C: Synthego ICE analysis output predicting knockout efficiency from Sanger sequencing data.

Cell type enrichment



B

Cell Type Isolated	Mean Purity (%)
CD4	95.0 (n = 16)
CD14	98.6 (n = 3)

Purity of cell types enriched for using magnetic cell isolation. A: representative fluorescence-assisted cell sorter plots showing gating strategy and the content of purified and unpurified cell suspensions. B: mean purity of CD4+ and CD14+ cell enrichments.

CONCLUSION

We have shown preliminarily that a type 1 regulatory T cell--specific transcription factor (TF B) is, surprisingly, not necessary for the induction of Tr1s. This is based on only one donor and limited protein expression data and warrants more detailed investigation. Working knockouts have been determined for 2 other genes and are awaiting testing.

We are expanding this experimental approach to include more donors and more genes. We are also testing alternative methods of teasing out the roles of each transcription factor in Tr1 differentiation, such as CRISPRa.

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