Optimization of Treg cell-based therapies by expression of T-bet

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BACKGROUND

Adoptive Cell Therapies

Over recent years, the therapeutic potential of regulatory T cells (T_{reg}) as adoptive cell therapies (ACT) has been demonstrated in models of graft-versus-host disease (GVHD), solid organ transplantation, type 1 diabetes (T1D), and inflammatory bowel disease. There are now over 50 active and completed clinical trials. However, target-tissue specificity, persistence and functional activity remain major limitations.

Regulatory T cells

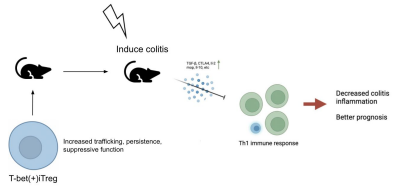
Anomalies or variations in T_{reg} populations are largely responsible for the onset of autoimmune diseases. These cells are indispensable for the maintenance of immunological tolerance and bear the potential to re-establish self-tolerance in treating autoimmune diseases. T_{reg} cells are able to adapt to their environment and optimize suppression of ongoing tissue-specific immune responses by acquiring various different transcriptional programs.

RATIONALE

Aim: Tailor T_{reg} cell therapy against T helper 1 (T_H1) mediated autoimmune complications.

Hypothesis: Inducing the expression of T-bet in induced T_{reg}(iT_{reg}) generated *in vitro* enhances T_H1 suppressive ability, trafficking, and persistence in tissue targeted during T_H1 autoimmunity.

Graphical Abstract



METHODS

Generation of iT_{reg} cells

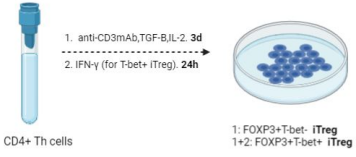


Figure 1. Generation of iT_{reg} cells. TNBS-specific naive CD4+ T_H cells will be isolated from PBMCs and then converted into T-bet(-) iT_{reg} or T-bet(+) iT_{reg} under iT_{reg}-polarizing conditions.

Quality control assays

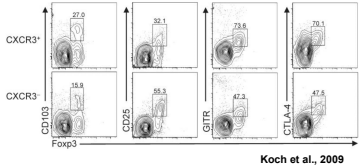


Figure 2. Validation of iT_{reg} cells generated. Expression of indicated markers on CD4+CXCR3+ or CD4+CXCR3- iT_{reg} generated in the experiment.

METHODS

Quality control assays

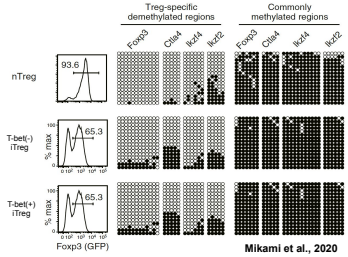


Figure 3. Verification of CpG methylation status. CpG methylation status of T-bet(+) and T-bet(-) iT_{reg} at Treg-specific demethylated regions (Treg-DRs) located in signature T_{reg} genes.

In vivo colitis mouse model

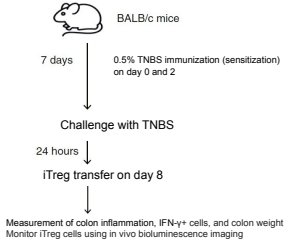


Figure 4. Proposed *in vivo* colitis mouse model. A colitis mouse model induced by trinitrobenzene sulfonic acid (TNBS) will be used.

In vivo bioluminescence imaging

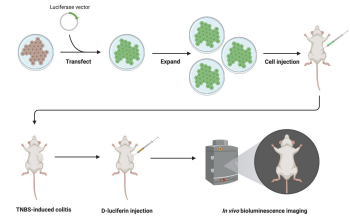


Figure 5. Protocol for in vivo bioluminescence imaging. Luciferase and D-luciferin will be used.

EXPECTED RESULTS

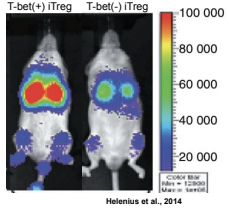


Figure 6. In vivo bioluminescence imaging of mice (expected). T-bet(+) iT_{reg} show more efficacious trafficking to sites of inflammation (colon)

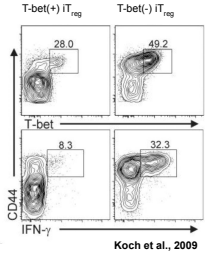


Figure 7 (left). Expected CD44 and T-bet expression or IFN-γ production. Expression measured in splenocytes isolated from T-bet(+) or T-bet(-) ACT mice, as measured by flow cytometry. Plots are gated on CD4+Foxp3- cells. Numbers in plots indicate the percent of T-bet+ or IFN-γ+ cells among total CD44hi CD4+Foxp3- cells.

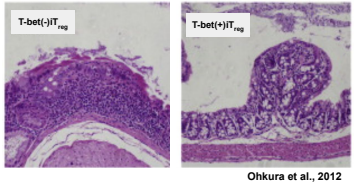


Figure 8. Histology of colon in mice with iT_{reg} ACT. T-bet(-) iT_{reg} is expected to demonstrate greater inflammation compared to T-bet(+) iT_{reg}.

CONCLUSION

(Expected) Mice treated with T-bet+ iT_{reg} cells will show:
- Unchanged colon weight, less colon inflammation
- Less IFN-γ+CD4+ effector T_H
- More efficient trafficking, greater persistence, and improved functional activity

Expression of T-bet by iT_{reg} cells improves efficacy of Treg ACT against T_H1 autoimmune disorders.

FUTURE DIRECTION

- Further validation of this experiment required
- Provide rationale for other similar T_{reg} ACT autoimmune therapies optimized towards a specific T_H subset

e.g., expression of GATA3 (T_H2) and RORγt (T_H17)

ACKNOWLEDGEMENT

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