Activation of Human CD4$^+$CD25$^+$CD127^{lo}Treg with Alloantigen and rIL-2 Induces IFNGR

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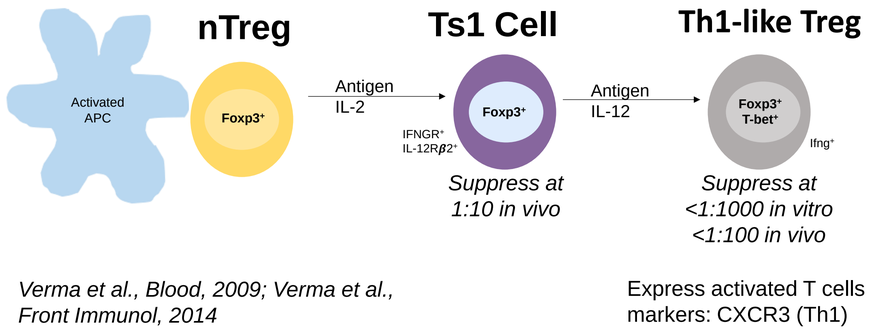
INTRODUCTION

Activation of human alloantigen specific CD4$^+$CD25$^+$CD127^{lo}Foxp3$^+$ Treg that induce tolerance is a key goal. In rats, we have shown that CD4$^+$ CD25$^+$ Treg activated with alloantigen and rIL-2 express more Foxp3 and CD25 and receptors for IFN-γ (IFNGR) and IL-12 (IL-12Rβ2). These cells are more potent suppressors of specific alloactivation (Verma et al, 2009).



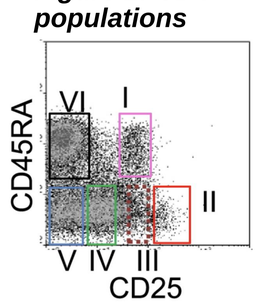
Verma et al., Blood, 2009; Verma et al., Front Immunol, 2014

Express activated T cells markers: CXCR3 (Th1)

***Figure 1**. Proposed pathway for activation/proliferation of alloantigen-specific nTreg in rats. Initial co-culture of Foxp3+ nTreg and alloantigen and IL-2 gives rise to Ts1 cells that can suppress T effector cells (Teff) at a ratio of 1:10 Ts1:Teff in vivo. Reculturing Ts1 cells with alloantigen and IL-12 induces Th1-like Treg that have further enhanced suppressive capacity.*

Human CD4$^+$ cells based on CD45RA and Foxp3/CD25 are shown to comprise of 5 populations (Figure 2); Pop I (Foxp3$^+$CD25$^+$CD45RA$^+$) as resting Treg; Pop II (Foxp3^{hi}CD25^{hi}CD45RA$^-$) as activated Treg and Pop III (Foxp3$^+$CD25$^+$CD45RA$^-$) including both Treg and activated effector T cells (Miyara, 2009). Pop II and III express chemokine receptors of activated T cells, including CXCR3 (Th1), CCR6 (Th17), CCR4 (Th2) which promote migration to inflammatory site.

Figure 2: CD4$^+$ populations



Here, we examined activation of human CD4$^+$CD25$^+$CD127^{lo}Treg and subpopulations and studied their FACS profile for Treg markers, cytokine receptor and chemokine receptors.

METHODS

CD4$^+$CD127^{lo}CD25$^+$ Treg isolated by FACS from blood of healthy volunteers were cultured for 4 days with rIL-2 and irradiated allostimulators (alloS). Cells were examined using multicolour flow cytometry. Data was acquired on BD FACSCanto II using BD FACSDiva software (v8.0) and analysed using FlowJo for shifts in Pop I, II, III and chemokine receptors.

Some cells were stained for *FOXP3* and *IFNGR1* using RNAscope.

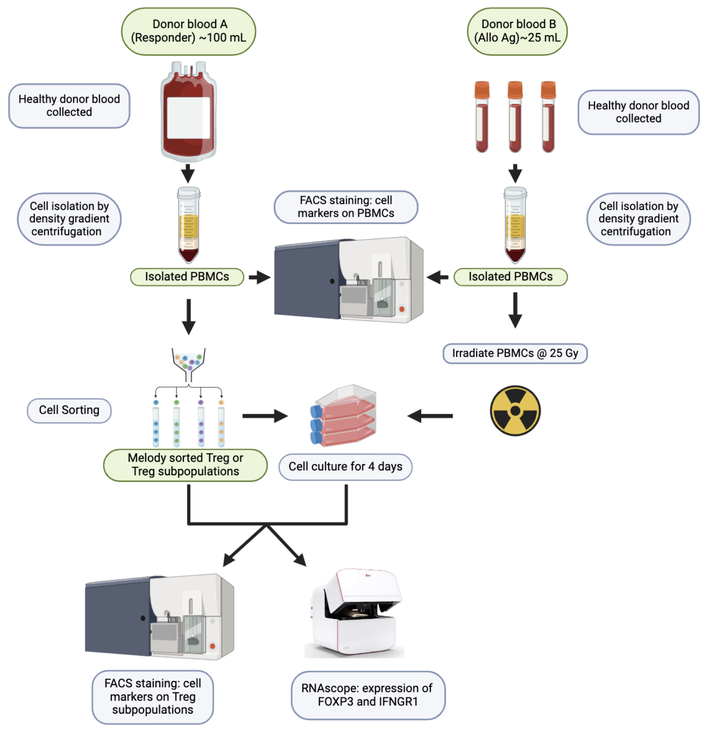


***Figure 3.** Experimental flowchart*

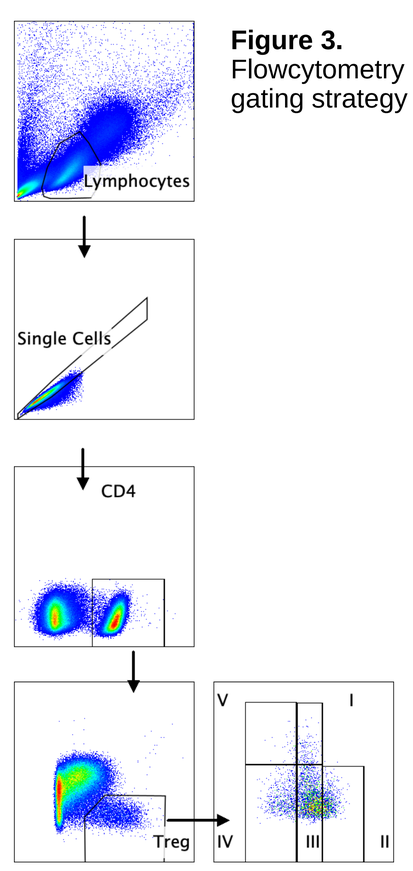
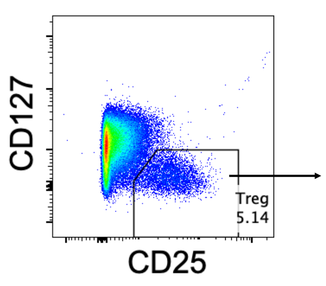


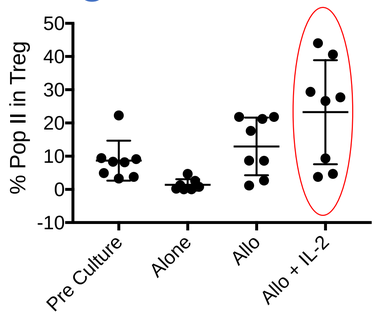
Figure 3. Flowcytometry gating strategy

RESULTS

Culture with rIL-2 and alloantigen increases proportion of Pop II within Treg



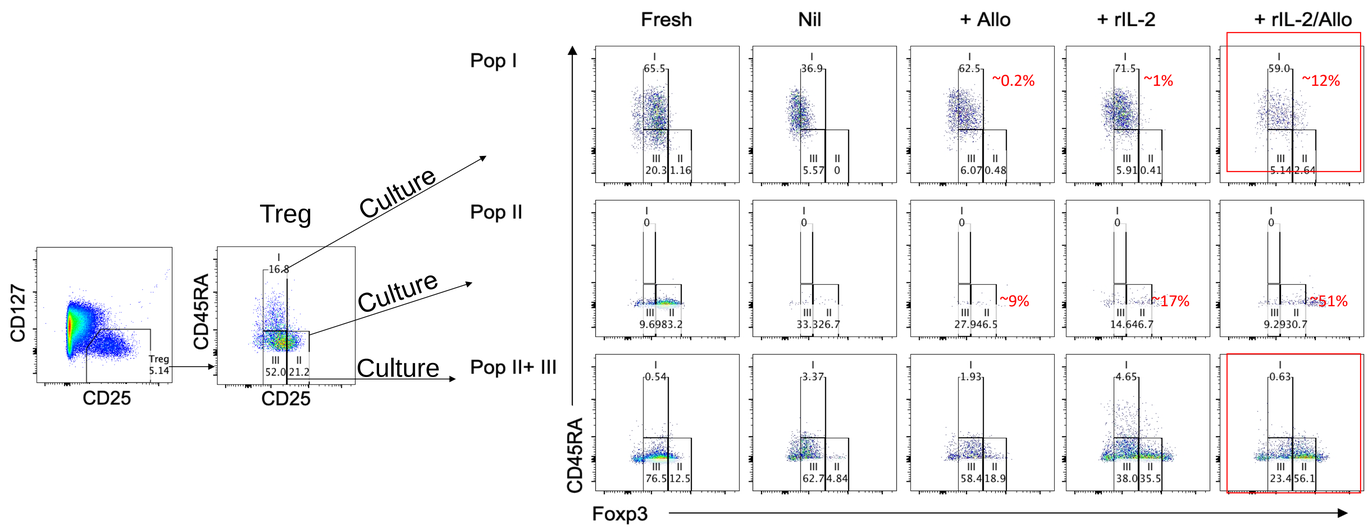
Enrich by FACS Sort (>90%)
4d Culture (rIL-2/AlloAg)



Alone: ↓ Pop II (1.3% vs 8.6%).
With Allo: ↔ Pop II (12% vs 8.6%).
With Allo/rIL-2: ↑ Pop II increased (6/8 exp)
Similar CXCR3 expression

Figure 4. tTreg cultured alone had reduced Pop II compared to the fresh starting population (1.3% vs 8.6%). Culture with alloS preserved Pop II (12% vs 8.6%). IL-2 alone increased Pop II in 5/8 of experiments, similar to the culture with alloS and rIL-2 (6/8 experiments). CXCR3 expression in Pop II was similar in unfractionated Treg to those cultured with IL-2 or alloS alone or IL-2 with alloS (data not shown).

Treg Populations enriched and cultured for 4 days with Allo, rIL-2 or Allo/rIL-2



Pooled results

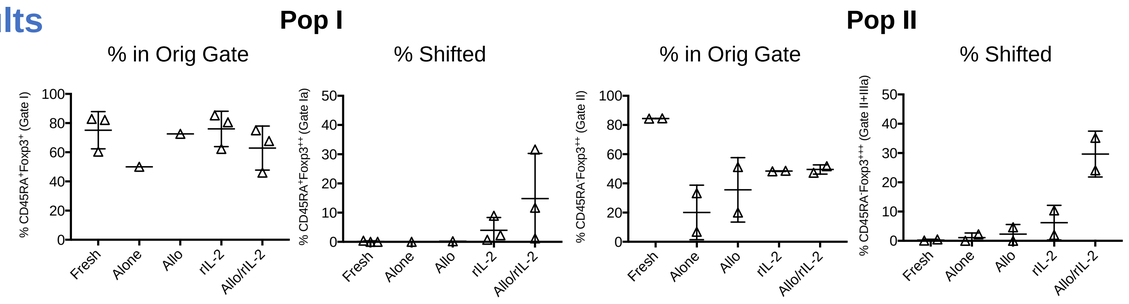
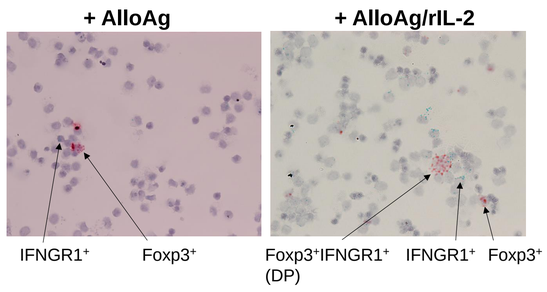


***Figure 6**. Enriched Pop I cells lost Foxp3 in absence of alloS or rIL-2. Culture with both alloS and rIL-2 produced Foxp3^{hi} and CD25$^+$ but remained CD45RA$^+$. CXCR3$^-$ and CCR6$^-$ were not induced (data not shown). A new subpopulation of CD45RA$^+$Foxp3^{hi}CD25^{hi} cells appeared.*
Cells in Pop II died when cultured alone. Activation with rIL-2 alone or with alloS increased expression of Foxp3, CD25, CCR6 and maintained CCR4 and CXCR3 expression (chemokine data not shown).
Pop III showed two shifts, some expressed less Foxp3 while others increased Foxp3 and CD25 expression, shifting to Pop II.

rIL-2/alloantigen induces expression of IFNGR on Treg



Culture with	% Foxp3$^+$/IFNGR$^+$ Treg
Pre-Cult	0
Nil	0
rIL-2	0
Allo	4.2
Allo/rIL-2	10.8

Figure 5. RNAscope showed freshly isolated Treg expressed *FOXP3*$^+$, but not *IFNGR1*. Treg cultured with alloS and rIL-2 had *FOXP3*$^+$*IFNGR1*$^+$ double positive cells but also single *FOXP3*$^+$and *IFNGR1*$^+$ cells. Treg cultured with rIL-2 alone had no *IFNGR1*$^+$ cells whereas Treg cultured with alloS only had single positive *FOXP3*$^+$ and *IFNGR1*$^+$ cells, none were double positive. Treg cultured alone had fewer *FOXP3*$^+$ cells and no *IFNGR1* $^+$ cells.

CONCLUSION

Treg activation can be monitored by changes in expression of CD25, Foxp3, CD45RA and chemokine and cytokine receptors. Human tTreg stimulated with alloS and rIL-2 induced IFNGR, like our rat studies. IFN-γ may induce potent antigen-specific Treg for therapy.

References:
Verma ND, Plain KM, Nomura M, Tran GT, Robinson C, Boyd R, Hodgkinson SJ, Hall BM. CD4+CD25+ T cells alloactivated ex vivo by IL-2 or IL-4 become potent alloantigen-specific inhibitors of rejection with different phenotypes, suggesting separate pathways of activation by Th1 and Th2 responses. Blood. 2009 Jan 8;113(2):479-87. doi: 10.1182/blood-2008-05-156612. Epub 2008 Sep 30. PMID: 18827184.
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ACKNOWLEDGEMENTS: We sincerely appreciate volunteers who generously donated blood for this study. Authors also acknowledge the help provided by nursing staff at the neurology unit, Liverpool hospital and Dr. Jarrad Begg, Cancer Therapy Centre, for helping in cell irradiation.