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Modulation of CD27/CD70 Co-Stimulatory Pathway may Allow for the Generation of a More Potent Human Regulatory T Cell Product for Cell Therapy

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Introduction: Regulatory T cell (Treg) therapy is a promising strategy to promote transplant tolerance and aid in the reduction of immunosuppression^[1]. This study aims to identify new strategies to increase potency, stability and specificity of human Tregs to produce an effective clinical therapy. Ligation of CD70 to CD27, a costimulatory molecule member of the tumour necrosis factor superfamily, is known to be sufficient and indispensable for the generation of effective and long-term antigen-specific Tcell immunity^{[2][3][4][5]}. In human Tregs, CD27 correlates with suppressive potency after *in vitro* expansion^{[6][7]}. We hypothesised that CD27/CD70 pathway may have a direct role in the activity of Tregs, and modulation of CD27/CD70 may allow for the generation of Tregs with enhanced suppressive properties.

Methods: TotalTregs (CD4⁺CD127^{low/-}CD25⁺) and CD27Tregs (CD4⁺CD127^{low/-}CD25⁺CD27⁺) were sorted from healthy donor PBMCs and expanded *in vitro* for 14 days using αCD3/αCD28 coated beads in the presence of rhIL-2. Cells were then flow sorted according to CD27 expression and expanded for a further 14 days. Treg phenotype, suppressive function and cytokine production were analysed after expansion by flow cytometry and ELISA.

Results: CD27 expression identified *in vitro*-expanded human Tregs with regulatory cytokine profile and epigenetically stable Foxp3 expression, studied by demethylation of the TSDR region. Suppressive activity was confined to the CD27⁺ expanded Treg population, and blocking the CD27/CD70 pathway within an *in vitro* suppression assay potentiated Tcell proliferation and Treg suppression.

Figure 1

■ No antibodies
 ■ αCD27 blocking mAb
 ■ αCD70 blocking mAb
 ■ αCD27 and αCD70 blocking mAbs
 ■ αCD27 isotype mAb
 ■ αCD70 isotype mAb
 ■ αCD27 and αCD70 isotype mAbs

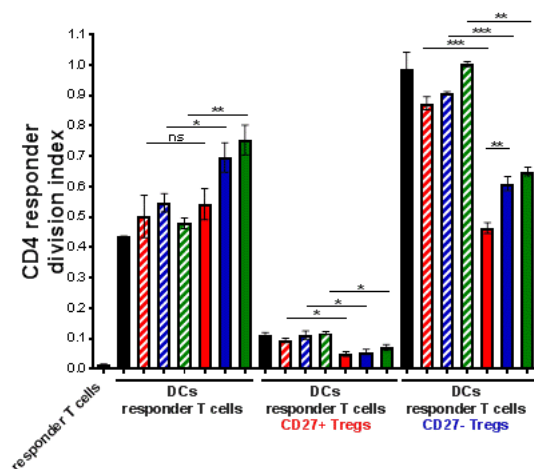


Figure 1. Blocking the CD27/CD70 pathway potentiates T cell proliferation and Treg suppression.

Suppressive capacity of expanded CD27⁺ Tregs and CD27⁻ Tregs was analysed in the absence (black) and in the presence of 10ug/ml of isotype (striped) or αCD27 (red), αCD70 (blue) or αCD27+αCD70 (green) blocking mAbs. 10x10⁴ CD27⁺ Tregs or 10x10⁴ CD27⁻ Tregs were incubated with 10x10⁴ autologous CD3⁺ cells (responder T cells) labelled with VPD and 2x10⁴ dendritic cells (DCs) for 80-96h. The graph represents the division index of responder cells; the average number of cell divisions that a cell in the original population has undergone. Mean with SEM is represented and statistical significance was assessed by a Mann-Whitney test (*p<0.05, **p<0.01, ***p<0.001). No statistical differences between different isotype mAbs and control media were observed. 1 representative donor is displayed.

On ligation of CD27, there was a robust activation and proliferation response in conventional Tcells but high death levels in Tregs, suggesting that CD27 costimulation activates distinct intracellular pathways in these two cell populations.

Figure 2

Unstimulated
 α CD3mAb shadow
 α CD3mAb + α CD28mAb
 α CD3mAb + sCD27L

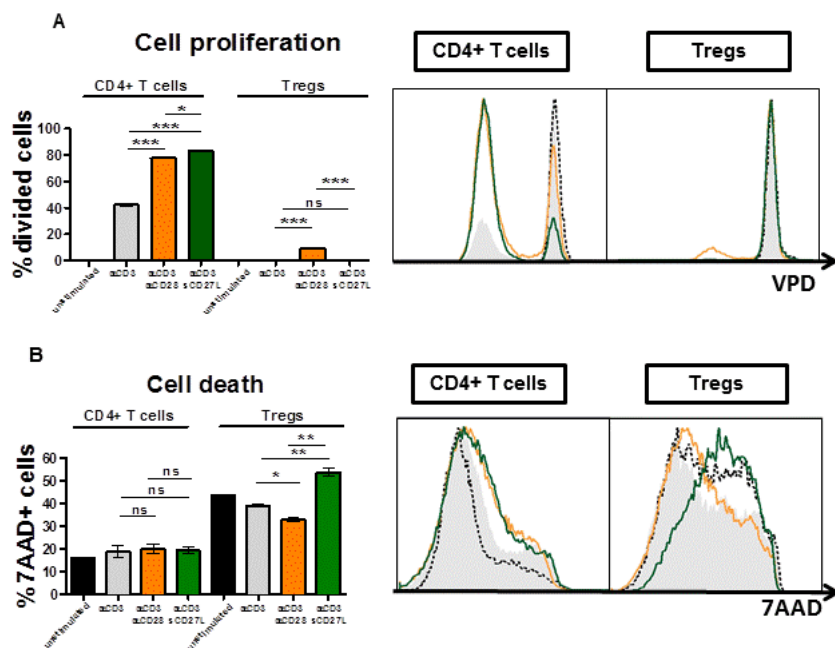


Figure 2. CD27 co-stimulation promotes proliferation of CD4+ T cells and cell death in Tregs.

Total Tregs and CD4+ T cells were isolated from PBMCs from healthy donors, stained with VPD and stimulated *in vitro* for 7 days in round-bottom plates coated with 1ug/ml of α CD3mAb and 5ug/ml soluble α CD28mAb or 5ug/ml soluble CD27L protein (sCD27L). After 7 days of culture A) cell proliferation was studied by VPD dilution and B) cell death was analysed by 7AAD expression using flow cytometry. Mean with SEM is represented (Mann-Whitney test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). 1 representative donor is displayed.

Conclusion: Isolation of Tregs by expression of CD27 may facilitate the identification of a more potent and stable Treg product for cellular therapy. All indicates that CD27/CD70 pathway has a direct role in the activity of human Tregs, and its modulation may facilitate the generation of Tregs with enhanced suppressive properties. CD27 costimulation may activate distinct intracellular pathways in conventional and regulatory T cells, and therefore CD27/CD70 axis could be exploited for regulating the balance between conventional and regulatory T cell responses.

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