TREGS GENERATED BY MESENCHYMAL STROMAL CELLS VERSUS TREGS INDUCED BY TGF-BETA, IL-2 AND ATRA: DIFFERENCES IN GENE EXPRESSION OF IMMUNOREGULATORY-RELATED MOLECULES

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INTRODUCTION

Recently, *ex vivo* generation of regulatory T cells (Tregs) has been attractive because its potential use for the development of new therapies of autoimmune diseases. Tregs $CD4^+$ $CD25^+$ $CD127^-$ can be generated by co-culture of $CD3^+$ T lymphocytes and Mesenchymal Stromal cells (MSCs). Also, $CD4^+$ $CD25^+$ $CD127^-$ induced Tregs (iTregs) can be generated in vitro by addition of TGF-beta, IL-2 and ATRA in a naïve $CD4^+$ cells culture.

AIM

The aim of this study was to compare the expression of immunoregulatory-related molecules between Tregs generated in MSCs cocultures and Tregs induced by TGF-beta, IL-2 and ATRA.

METHODS

Immunomagnetically purified $CD3^+$ T lymphocytes were activated with anti-CD2/CD3/CD28 beads and then cultured in the presence of MSCs previously adhered to the plate. In addition, immunomagnetically purified $CD4^+$ $CD25^-$ $CD45RA^+$ naïve T cells from umbilical cord blood were activated with anti-CD2/CD3/CD28 beads and cultured in the presence of TGF-beta, IL-2 and ATRA. After 5 days, $CD4^+$ $CD25^+$ $CD127^-$ Tregs of both cultures were purified by immunomagnetic column and their total RNAs were extracted. Real–time PCR was performed for FOXP3, GITR, CTLA-4, TGF-beta and IL-10 genes. Human β-actin gene was used as endogenous control. The experiments were performed in duplicate. The experimental strategy is represented in figure 1.

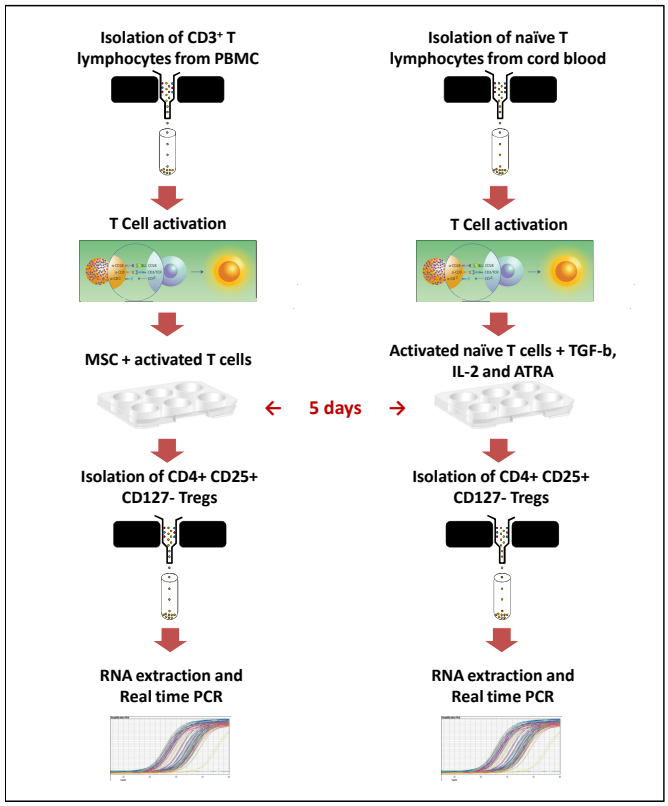

Figure 1. Experimental strategy. The CD4+ CD25+ CD127- cells were isolated from both cultures after 5 days and Real–time PCR was performed for FOXP3, GITR, CTLA-4, TGF-beta and IL-10 genes .

RESULTS

Tregs induced by TGF-beta, IL-2 and ATRA expressed significantly higher levels of FOXP3 than Tregs generated by co-culture with MSCs (p = 0.0205) (**Figure 2 A**). On the other hand, the expression of TGF-beta, CTLA-4 and GITR were higher in Tregs generated by co-culture with MSCs (p = 0.0014, p = 0.0162 and p = 0.0238 respectively) (**Figure 2 B-D**) . Although not statistically significant, IL-10 expression was higher in Tregs generated by co-culture with MSCs (**Figure 2 E**) .

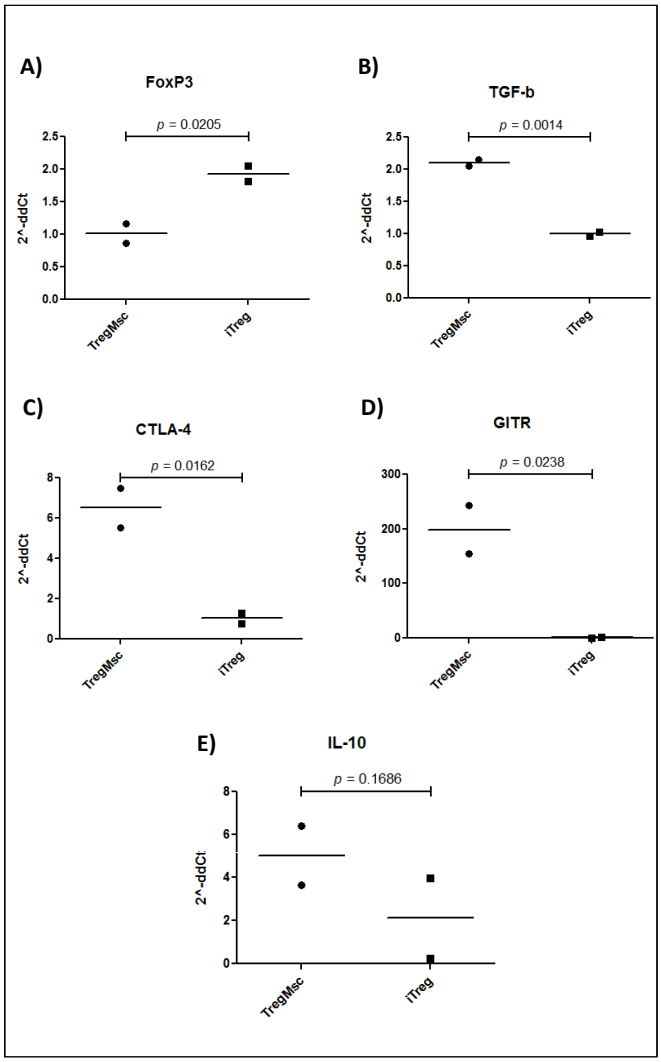

Figure 2. Gene expression of immunoregulatory related molecules. A) FOXp3 mRNA levels; B) TGF-b mRNA levels; C) CTLA-4 mRNA levels; D) GITR mRNA levels; E) IL-10 mRNA levels.

CONCLUSIONS

Despite both MSCs-generated Tregs and iTregs share a similar phenotype ($CD4^+$ $CD25^+$ $CD127^-$), the gene expression of immune regulatory-related molecules differs between them. Studies must be done to check if these differences may alter the functional properties of these Tregs, such as the immunosuppressive action.

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