TOLEROGENIC POTENTIAL OF FOXP3+ EXOSOMES DERIVED FROM ALLOANTIGEN SPECIFICALLY EXPANDED REGULATORY T CELLS

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1 INTRODUCTION

We and others are utilizing *ex vivo* expanded CD4+CD127-CD25highFOXP3+ "donor specific" regulatory T cells (Ds-Tregs) for the induction of immune tolerance in transplant patients. However, the exact immunomodulatory mechanisms employed by Ds-Tregs remain unclear. Here we studied if exosomes/ extracellular vesicles (Evs) secreted by human Ds-Tregs during expansion are immunomodulatory.

2 EXPERIMENTAL SCHEMA

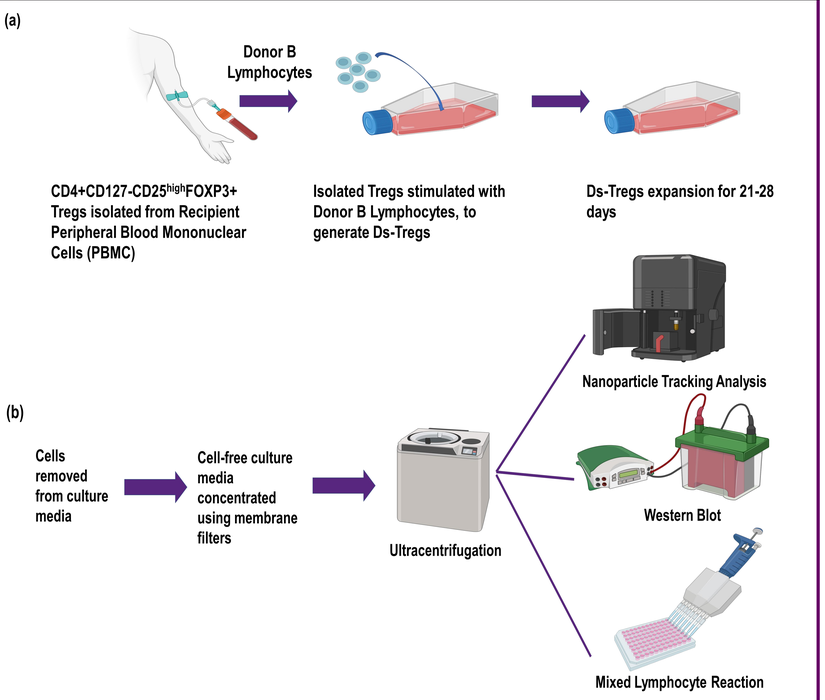


Figure 1(a-b). ***Generation, isolation and characterization of Ds-Tregs derived EVs***. Ds-Tregs were generated and cultured post-stimulation with allogenic B-lymphocytes. After 28 days, culture media were filtered, concentrated, and ultracentrifuged to isolate EVs. EVs were then characterized using Nanoparticle Tracking Analysis, Western Blot, and functionally assessed using a 5-day Mixed Lymphocyte Reaction (MLR) culture.

3 CHARACTERISTICS OF ISOLATED DS-TREG EVs

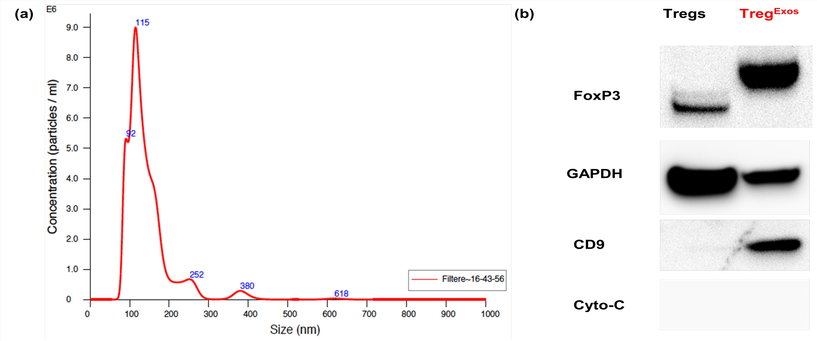
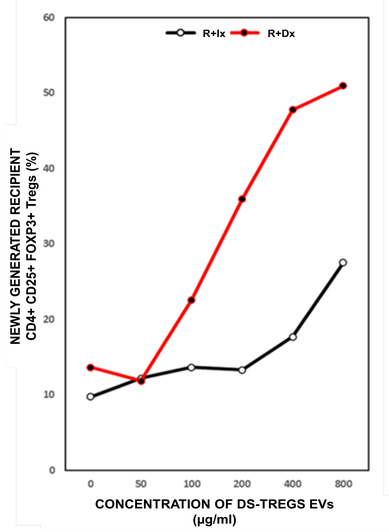


Figure 2(a-b). ***Characterization of Ds-Tregs derived EVs.*** (a) Ds-Tregs derived EVs were of the correct size (b) and showed presence of exosome specific CD9 protein. Ds-Treg EVs had significantly higher expression of FOXP3, the transcription factor associated with Treg function, when compared to Treg cells at the protein and GAPDH levels ($p<0.05$, paired t-test).

4 DS-TREGS DERIVED EVs ENHANCED THE GENERATION OF RECIPIENT TREGS

Figure 3. ***Ds-Tregs derived EVs enhanced the generation of newly developed Tregs in Recipient (R) PBMCs in a dose dependent and antigen-specific manner.*** "Recipient" PBMC were stimulated with irradiated "donor" cells with and without the addition of Ds-Tregs derived EVs at different concentrations. After 5 days, the MLR cell culture were assessed using flow cytometry. Ds-Treg derived EVs increased the (%) of Tregs in R-PBMC in a dose dependent and donor-specific manner. Treg generation were observed when specific donor cells used to expand the Ds-Tregs were used as stimulators (Dx) (R+Dx) as opposed to third-party stimulators (Ix) (R+Ix) in the MLR.



5 DS-TREGS DERIVED EVs REDUCED RECIPIENT CD4+ PROLIFERATION

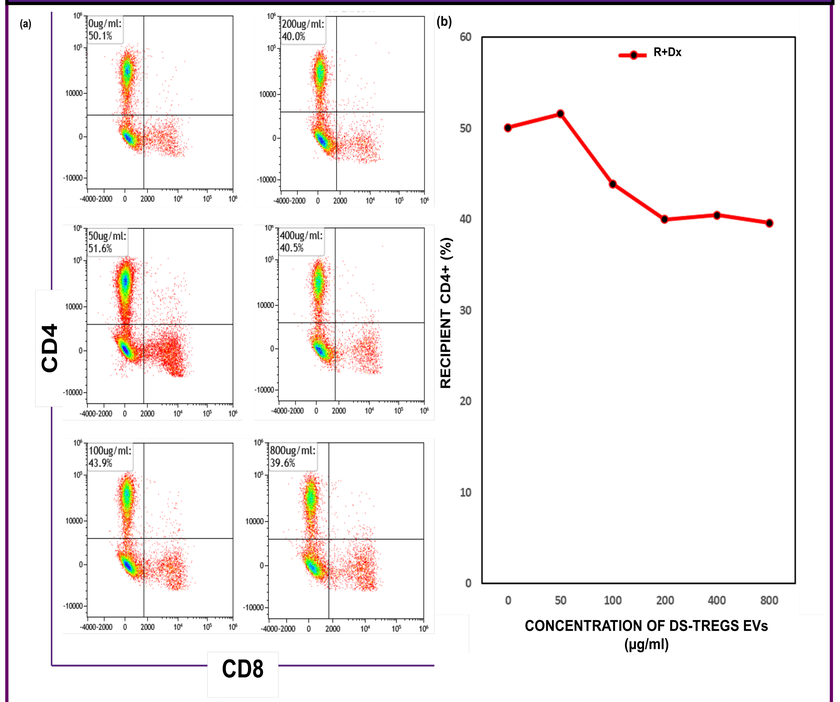


Figure 4(a-b). ***Ds-Treg derived EVs reduced the proliferation of CD4+ T cells of Recipient (R) PBMCs in a dose dependent manner. "***Recipient" PBMC were stimulated with irradiated "donor" cells with and without the addition of Ds-Tregs derived EVs at different concentrations. After 5 days, the MLR cell culture were assessed using flow cytometry. Flow charts in diagram are pre-gated on proliferated non-CD4+ FOXP3 Recipient cells.

6 CONCLUSION

EVs secreted during the expansion of high quality CD4+CD127-CD25highFOXP3+ Ds-Tregs can induce the generation of new Tregs in autologous naïve Recipient PBMC in an antigen specific manner. Ds-Tregs derived EVs also reduced the proliferation of new CD4+ T cells in Recipient PBMC. The presented data suggests that EVs derived from Ds-Tregs can possibly be used to complement Treg therapy to promote immune tolerance.

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