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.

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.

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).

CONCLUSION

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.

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.