Improving Organ Transplants:

The Role of Exosomes in the Maintenance of Transplant Tolerance

Jacob Wieland, Parthasarathy Rangarajan and Sabarinathan Ramachandran
University of Minnesota, Department of Surgery, Schultz Diabetes Institute

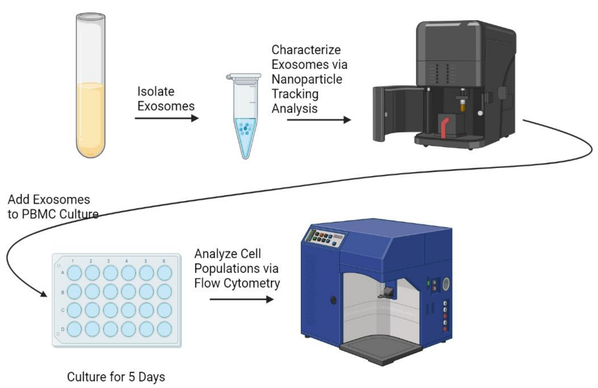
ABSTRACT

Goal: Examine the role of exosomes in transplant tolerance post-administration of Apoptotic Donor Leukocytes (ADL)
Question: Do exosomes isolated from non-human primate serum following ADL administration induce an immunosuppressive effect?

INTRODUCTION

- To avoid transplant rejection, current strategies involve the use of immunosuppressive drugs
- Administration of a "cellular vaccine" of apoptotic donor leukocytes (ADLs) represents a way to potentially induce transplant tolerance without the use of immunosuppressants (1)
- Recently, it was demonstrated that the administration of ADL to non-human primates induced tolerance of islet allografts for >1 year (2)
- The aim of this study is to identify the role of circulating exosomes in transplant tolerance.

METHODOLOGY



- Isolate exosomes via ExoQuick isolation kit from serum
  - Exo1: Pre transplant stage
  - Exo2: NHPs rejecting islet allogarfts (no ADL)
  - Exo3: Animal did receive ADL treatment, 93 days post-transplant, transplant tolerance established
- Analyze exosome size via nanoparticle tracking analysis (NTA)
- Coculture of NHP PBMCs with 50 µg exosome
- After 5 days, analyze T-cell populations via flow cytometry

RESULTS

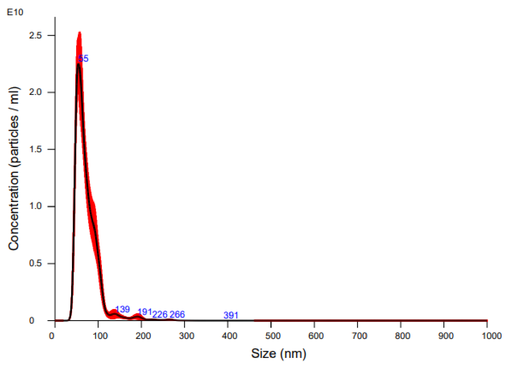


Figure 1. Exosome Nanoparticle Tracking Analysis. Exosomes are typically between 50-150 nm in diameter. Exosome group 2 is shown here as an example. All groups fell within the expected size range for exosomes (3)

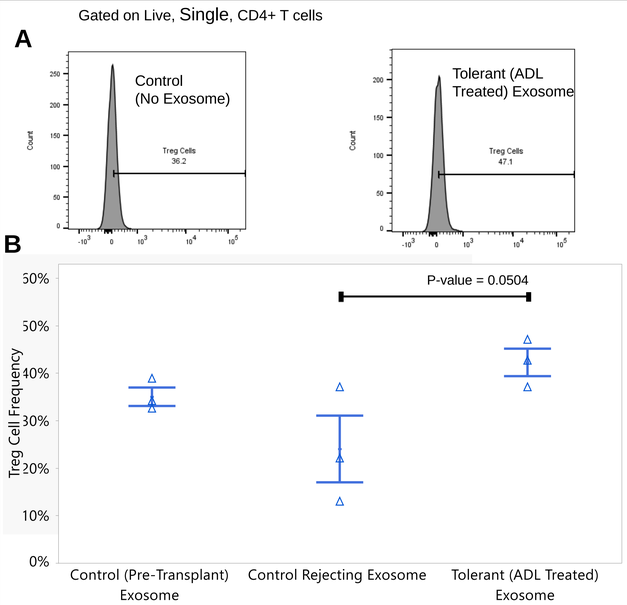


Figure 2. Exosome from tolerant NHPs induces expansion of Tregs. Flow cytometry analysis of CD4+ T cell expression of FoxP3 on control and exosome treated PBMCs **(A).** Comparison of Treg frequency within CD4+ T cells treated with no exosomes (control), pre-transplant exosomes, non-ADL sac exosomes, and exosomes from ADL treated animals **(B)**

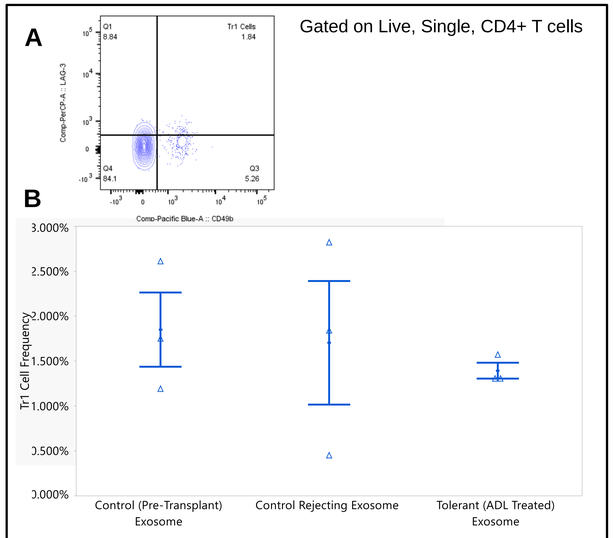


Figure 3. Effect of Exosome on Tr1 expansion. Flow cytometry analysis of CD4+ T cell expression of Lag3 and CD49b **(A).** Comparison of Tr1 cell frequency within CD4+ T cells treated with no exosomes (control), pre-transplant exosomes, non-ADL sac exosomes, and exosomes from ADL treated animals **(B)**

CONCLUSION

- Exosomes isolated from the tolerant NHPs treated with ADLs demonstrated an immunosuppressive effect.
- Exosomes from the ADL treated NHPs induced a significant expansion of Tregs when compared to exosomes from control rejecting NHPs (p= 0.0504)
- Exosomes did not have a significant impact on the expansion of Tr1 cells (Tolerant vs rejecting).

FUTURE DIRECTIONS

Proliferation of T cell subpopulations

RNA-seq of Exosome RNA
- Look for mi-RNAs that correspond with Treg differentiation, proliferation, and apoptosis (4)

Profile Cytokines carried by exosomes
- Anti-inflammatory cytokines
- Cytokines associated with Treg and Tr1 differentiation
  - IL-10 and TGF-β

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