SUMMARY (Cutting Edge) 6/13/25 ciT1zen science

Novel Immunomodulatory Therapy for Type 1 Diabetes Using Exosomes ciT1zen science summary

Source: Basak, P., Badal, D., Dayal, D., Bhadada, S., Kumar, R., et al. (2025). Preproinsulin-specific regulatory T cell-derived exosomes loaded with immune-checkpoint ligands can suppress autoimmune responses in type 1 diabetes. *International Immunopharmacology, 161*, 115045.

I. Executive Summary

This research proposes a novel and promising immunomodulatory therapeutic strategy for Type 1 Diabetes (T1D) by combining the advantages of beta cell-specific regulatory T cells (Tregs) and immune-checkpoint ligands (ICLs) delivered via exosomes. The study successfully demonstrated that exosomes derived from preproinsulin (PPI)-specific Tregs, loaded with ICLs (PPI-T-EXOL), can effectively suppress autoimmune responses in T1D. This approach offers a "cell-free, HLA unrestricted, and scalable as an off-the-shelf therapy that is less expensive," addressing key limitations of existing cell-based therapies. The treatment significantly delayed the onset of hyperglycemia and prevented beta cell destruction in a mouse model, highlighting its potential as a targeted and efficient intervention.

II. Main Themes and Key Concepts

1. **Targeting Autoimmune Destruction in T1D:**

- T1D is characterized by the selective destruction of pancreatic beta (β) cells, primarily by infiltrating CD8+ T cells.
- Existing therapeutic approaches often face challenges such as limited success, generalized immunosuppression, and risks of infection. The focus is shifting towards "antigen-specific approaches especially Tregs specific for islet-associated antigens."

1. **Immune-Checkpoint Molecules (ICMs) and Ligands (ICLs) in Autoimmunity:**

- ICMs play a critical role in maintaining self-tolerance by suppressing undesirable immune activation.
- While ICM inhibitors have revolutionized cancer therapy by *reinvigorating* anti-tumor responses, this research repurposes the concept in *reverse* for autoimmune diseases.
- The hypothesis is that persistent ICMs on activated CD8+ or CD4+ T cells in T1D subjects can be targeted by corresponding agonist ligands to induce a "tolerogenic phenotype in autoreactive CD8+ or CD4+ T cells."
- The study identified PD-1, TIGIT, and BTLA as the "3 most abundant ICMs expressed on the peripheral CD8+ T cells" in recent-onset T1D patients, which were then targeted with their corresponding ICLs.

1. **Exosomes as a Drug Delivery System:**

- Exosomes are "nanosized (30–150 nm), biologically active vesicles secreted by host cells including immune cells."

- They are recognized as "intercellular messengers delivering cargo from host cells" and "possess several favorable features, including low immunogenicity, biocompatibility, low toxicity, and provide strong protection for cargo."
- Crucially, exosomes are "reconfigurable biological nano-platform[s]," allowing for targeted modification and efficient cargo delivery.
- The study leveraged exosomes because they "act as miniature Tregs containing the same immunosuppressive characteristics as their origin albeit with no fear of plasticity and sufficient numbers can be obtained from a continuous culture of Tregs," overcoming the challenge of limited numbers and phenotypic plasticity of Tregs for cellular therapy.

1. **Preproinsulin (PPI)-Specific Regulatory T cells (Tregs):**

- The research builds on prior work demonstrating the role of proinsulin-specific Tregs in T1D cellular therapy.
- Tregs were isolated from T1D and healthy subjects and stimulated with PPI in vitro to derive antigen-specific exosomes. This ensures the therapeutic effect is specifically directed at β-cell autoimmunity.

III. Key Findings and Results

- **ICM Profiling:** Peripheral CD8+ T cells from recent-onset T1D subjects showed abundant expression of PD-1, TIGIT, and BTLA.
- **Exosome Characterization:** Exosomes isolated from PPI-specific Tregs (PPI-Treg-derived exosomes) were thoroughly characterized for size, morphology, and protein content.
- **In Vitro Efficacy (Cellular Level):**The ICL-loaded exosomes (PPI-T-EXOL) effectively "inhibited the proliferation of autologous CD8+ and CD4+ Teff cells."
- PPI-T-EXOL and PPI-Tregs infused with PPI-T-EXOL significantly "downregulated the activation and cytotoxic potential of autologous PPI-pulsed CD8+ T cells."
- These Tregs (and by extension, the exosome treatment) "reduced CD8+ T cell-mediated apoptosis of human 1.1B4 β-cell line."
- **In Vivo Efficacy (Mouse Model):**In STZ-induced diabetic C57BL/6 mice, the "mice-specific ICL-loaded exosomes delayed the onset of hyperglycemia, particularly when administered before the onset of diabetes."
- The treatment "prolonged their survival by inhibiting perivascular lymphocytic intra-islet infiltration," directly addressing the β-cell destruction mechanism.

IV. Significance and Future Implications

- **Novel Therapeutic Intervention:** The study presents a novel and promising "immunomodulatory therapy by combining the beta cell-specific Treg-derived exosomes and ICL to treat T1D."
- **Overcoming Limitations of Cell Therapy:** This strategy offers significant advantages over direct Treg cell therapy by being "cell-free, HLA unrestricted, and scalable as an off-the-shelf therapy that is less expensive." This addresses issues of cell limited numbers, phenotypic plasticity, and the need for HLA matching.
- **Targeted Immunosuppression:** The approach aims to suppress β cell-specific T cell responses "without eliciting global immunosuppression," a critical improvement over broader immunosuppressants that increase infection risk.

- **Potential for Early Intervention:** The observed efficacy in delaying hyperglycemia when administered *before* diabetes onset in mice suggests a potential for preventative or early-stage intervention in human T1D.
- **Translational Potential:** The findings provide a strong foundation for developing a clinically relevant therapeutic agent for T1D. Further research and clinical trials would be necessary to translate these findings into human application.

V. Methodological Highlights

- **Patient Cohort:** 40 recent-onset T1D subjects and 20 age-matched healthy controls were recruited for initial ICM profiling and Treg isolation.
- **ICM Profiling:** Flow cytometry was used to profile ICMs on peripheral CD8+ T cells.
- **Exosome Isolation & Characterization:** Standard techniques (western blotting, TEM, zeta potential, particle size analysis) were used to characterize exosomes.
- **In Vitro Assays:** Proliferation assays, activation and cytotoxicity assessment of CD8+ T cells, and β-cell apoptosis assays were performed.
- **In Vivo Model:** STZ-induced diabetic C57BL/6 mice were used to assess the treatment's impact on hyperglycemia onset, survival, and islet infiltration.