Utilizing mRNA as a source of antigens for the expansion of virus-specific T cells

Maryam A. Pasdar[1], Sandeep K. Srivastava[1], Anushree Datar[1], Emily K. Reynolds[1], Gage Paul[1], Patrick J. Hanley[1,2]

[1] Center for Cancer and Immunology Research, Children's National Hospital, Washington, DC, USA
[2] Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, Washington, DC, USA

INTRODUCTION

Overlapping peptide libraries (Pepmixes) have transformed adoptive immunotherapy and enabled rapid expansion of antigen-specific T-cells such as Virus-specific T cells(VSTs), which have been used successfully to treat patients with viral infections after hematopoietic stem cell transplant. These Pepmixes present challenges when used for clinical manufacture due to cost, potential for impurities, and increasing scrutiny by regulatory agencies. Another option is the use of mRNA representing the peptide(s) of interest. The mRNA can be electroporated into the antigen-presenting cell, translated, and ultimately used by an antigen-presenting cell to stimulate T-cells.

METHODS

To determine if mRNA is a viable alternative to Pepmixes, we expanded VSTs from 3 HLA-A2 CMV-seropositive donors using either mRNA or the peptide NLVMPVATV from the CMV antigen pp65. In preliminary experiments, dendritic cells(DC) were generated from PBMC and cultured for 5 days, matured with a cytokine cocktail, harvested on day 6, electroporated or peptide-pulsed, and irradiated. Following irradiation, 2 conditions were plated: electroporated or pulsed DCs were cultured with T-cells and remaining DCs were frozen to use at later stimulations. Later, we used a 10-day rapid VST expansion protocol. PBMC were plated to isolate monocytes from the rest of the cell population. These monocytes underwent either electroporation or pulsing and were then co-cultured with the non-adherent cells that remained after monocyte isolation. Cells were fed on day 6, and the final VST harvest occurred on day 10, with no stimulation following the initial plating.

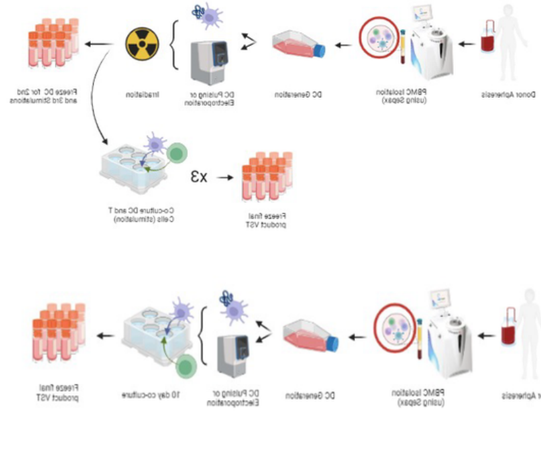

Figure 1. *Schematic representation of both VST cell manufacturing methods. Top: multi-stim DC manufacturing protocol. Bottom: 10-day rapid expansion protocol.*

RESULTS

Figure 2. Electroporated and peptide-pulsed DC had similar phenotypes and viabilities

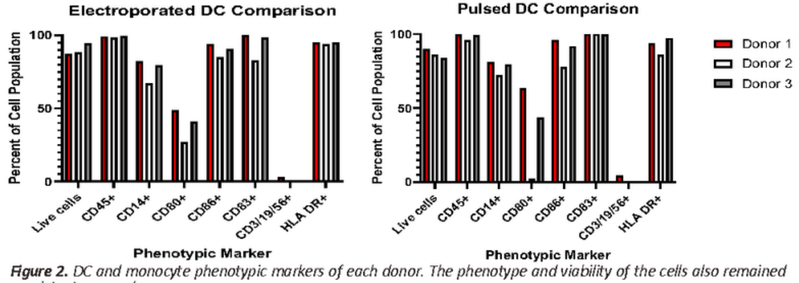

Figure 2. DC and monocyte phenotypic markers of each donor. The phenotype and viability of the cells also remained consistent across donors.

Figure 3. Final 10-day VST exhibited similar CD3+ phenotype and CD4+/CD8+ ratio in both conditions

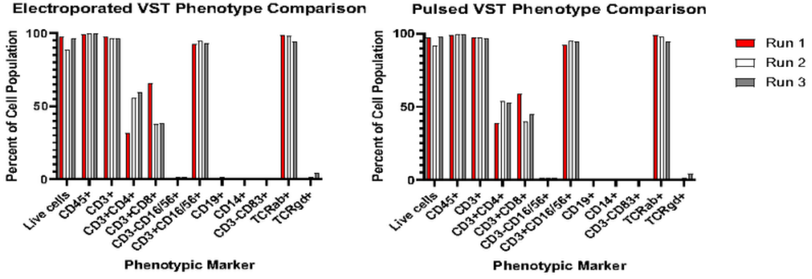

Figure 3. Cellular phenotyping by flow cytometry performed on final product VST harvested on day 10 of the rapid expansion protocol. These cells were stimulated only once on the day of initiation.

Figure 4. Using a modified rapid expansion protocol, the fold expansion trended higher when using electroporated monocytes

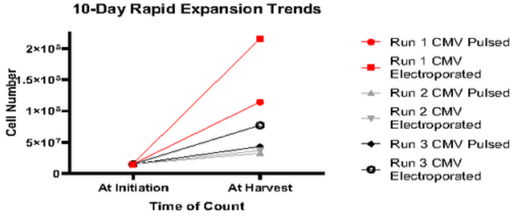

Figure 4. *VST were generated using a modified rapid culture method. Monocytes were isolated and either electroporated or pulsed with overlapping peptide libraries of NLVMPVATV from the CMV antigen pp65. VST cocultured with electroporated monocytes had larger cell counts at harvest on day 10.*

Figure 5. Cells produced similar reactivity to CMV antigen when analyzed by INFg Elispot.

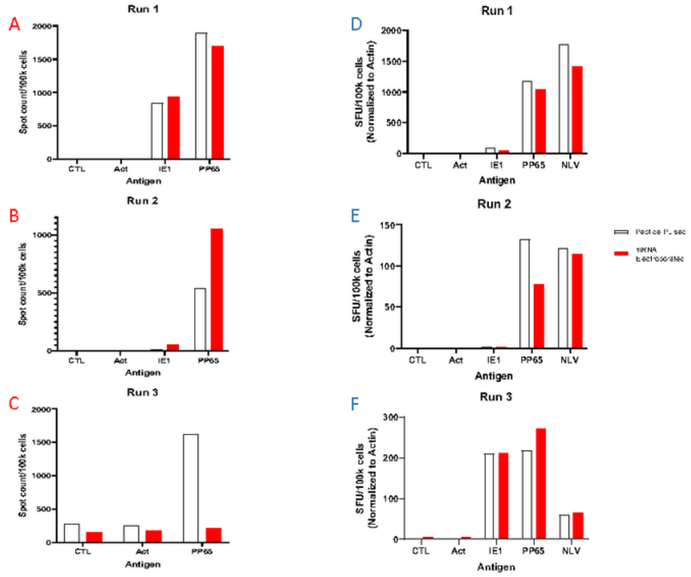

Figure 5. *VST harvested post-stim 2 in the first round of experiments using DC yielded minimal differences in specificity when analyzed with INFg Elispots (A-C). Rapidly expanded VST that were initiated with monocytes and cultured for 10 days also had similar specificity in both the electroporated and pulsed conditions (D-F).*

CONCLUSIONS

- Electroporation of mRNA into DCs and monocytes for initiation of VST is a suitable alternative to peptides.
- More cell characterization is needed at the gene expression level to compare VST generated using peptide pulsing or APC electroporation.
- This concept could be used in future experiments for the generation of other peptide dependent generation protocols, such as Tumor Antigen Associated T-Cells (TAA-T).

Acknowledgements/COI
This study was conducted in collaboration with MaxCyte. COI for Dr. Patrick Hanley: Co-founder, board of directors, Mana Therapeutics; Advisory board: Cellevolve, Cellenkos, Discovery Life Sciences, Capsida, MicrofluidX

Co-Host
Sponsors
Children's National
NIH National Institute of Allergy and Infectious Diseases