Streamlined Production of Tumor-Associated Antigen-Specific T Cells (TAAT) from Antigen-Naïve Donors in a GREX Device Using Autologous Monocyte-Derived Dendritic Cells (MoDC) Manufactured in the Same Device as Antigen Presenting Cells (APC)

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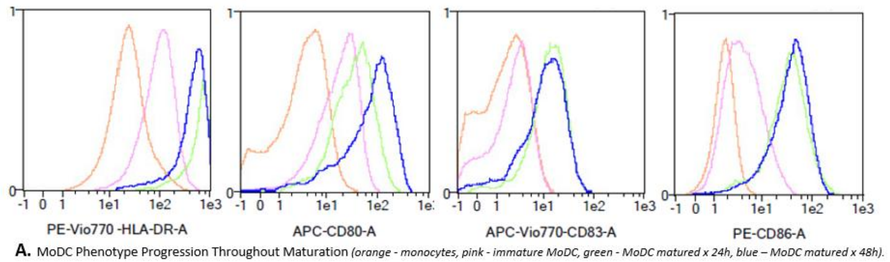
Background & Aim

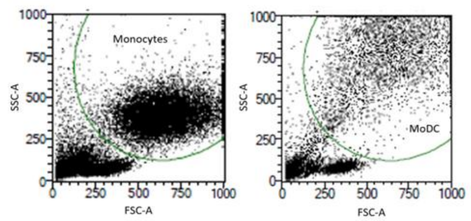
TAAT production requires priming of naïve T cells with high-quality APC, such as peptide loaded MoDC. Generating enough MoDC is a limiting factor, because physical or chemical manipulation needed to overcome cell adherence causes cell damage during harvest. Restimulation prompts a secondary immune response, but increases manufacturing duration, cost and a chance of failure. Our aims were to develop a streamline process which combines MoDC and TAAT production in the same GREX device, assess the need for restimulation, and explore the feasibility of manufacturing clinical doses of TAAT (0.5e4 cells/kg) from phlebotomy collections.

Methods

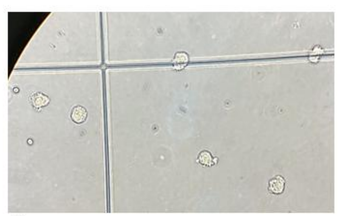
Monocytes isolated from peripheral blood by Ficoll/CD14 selection were seeded in GREX at 0.25-0.5e6 cells/cm^2, differentiated with GM-CSF/IL-4 x3-5 days, loaded with PRAME, Survivin and WT1 peptides, and matured x24-48 hours with TNF-a/IL-6/IL-1b/PGE2. PBMC were added directly to MoDC culture at 1:5 – 1:10 ratio, eliminating the need for MoDC harvest, and expanded for 14 days with IL-7/IL-15/IL-12/IL-6 (x3 runs). A second batch of MoDC was made in another GREX, to which some TAAT were transferred on day 14 (1:10 ratio, fed with IL-7/IL-2). TAAT were challenged on days 14, 21 and 28 with antigen loaded MoDC, PBMC or PHA blasts. Cells manufactured without antigen loading served as a negative control.



A. MoDC Phenotype Progression Throughout Maturation *(orange - monocytes, pink - immature MoDC, green - MoDC matured x 24h, blue – MoDC matured x 48h).*

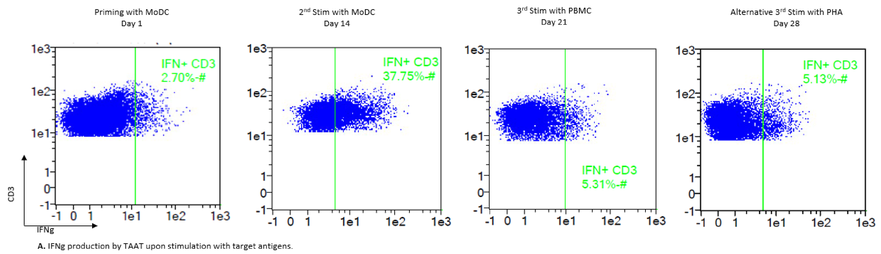


B. MoDC Morphology Progression Throughout Maturation.

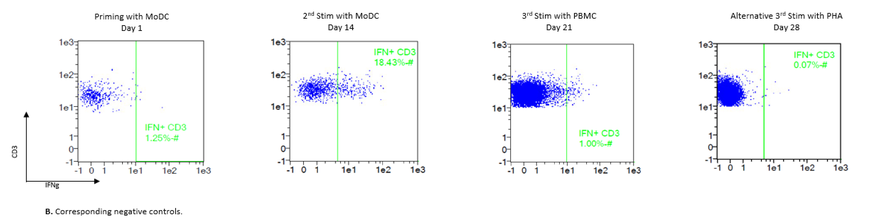


C. MoDC in Hemocytometer, 40x.

Figure 1. A. MoDC Phenotype Progression Throughout 7-Day Maturation (orange: monocytes, pink: immature MoDC, green: MoDC after 24 hours of maturation, blue: MoDC after 48 hours of maturation). B. MoDC Morphology Progression Throughout Maturation. C. MoDC in Hemocytometer, 40x.



A. IFNg production by TAAT upon stimulation with target antigens.



B. Corresponding negative controls.

Figure 2. TAAT response to antigen-loaded APC. A. IFNg production by TAAT upon stimulation with peptide-loaded APC. B: Corresponding negative controls.

Results

MoDC yield was 43.1% of starting monos (SD 11.6), MoDC showed mature phenotype (Figure 1). Priming elicited IFNg response in 2.25% of T cells (SD 0.57; neg control 1.88%, SD 0.63) and resulted in 11.33-fold expansion by day 14 (SD 7.85). 27.49% of T cells were IFNg+ on day 14 restimulation (SD 6.81), compared to 11.98% neg control (SD 5.86). TAAT contracted and re-expanded by day 21 (35.07-fold expansion, variability of physiological process reflected by SD 36.74), with 4.20% IFNg+ cells upon restimulation (SD 0.82; neg control 1.90%, SD 0.79). At day 28 expansion was 257.85-fold (SD 60.25) and 3.27% cells were IFNg+ on restimulation (SD 1.52; neg control 0.90%, SD 0.72, Figure 2 – representative run). Each 1e6 of starting PBMC yielded 10.26e6 antigen-specific T cells by day 14 (SD 5.82e6), which fell to 1.82e6 by day 21 (SD 1.63e6), and expanded to 11.4e6 by day 28 (SD 4.90). 30 mL phlebotomy yielded 50.83e6 of TAAT (SD 5.14e6) by day 14 of co-culture.

Conclusions

Clinically relevant doses of TAAT may be manufactured from 30 mL of blood by combining MoDC production and a single PBMC stimulation in a GREX device in <3 weeks.

Disclosures

None