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LARGE-SCALE MYELOID DENDRITIC CELLS GENERATION FROM UMBILICAL CD34+ CELLS USING G-REX BIOREACTORS

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Introduction: Dendritic cells (DCs) have the unique ability to prime naïve T-cells, thus representing a fundamental tool in cancer therapy and especially in vaccine- or antitumor specific T cell–based immunotherapy. However, the laborious and costly methods to generate and expand DCs from the peripheral blood (PB) or monocytes, limit their potential for broad clinical applications. In addition, although DCs can be produced from cord blood (CB)-derived CD34+ cells, the final DC yields are low to be clinically translated. New methods and sources are needed in order to obtain sufficient numbers of DCs (10^6 -10^8 DCs).

Materials (or patients) and methods: In this study we sought to investigate the potential of large scale generation of DCs from CB-derived CD34+ cells, using the G-Rex10 bioreactors (Wilson Wolf Corporation) instead of the standard culture dishes or flasks. We additionally asked for the optimal culture conditions and tested the capability of produced DCs to induce Th1 responses upon stimulation with a mixture of Toll Like Receptor ligands (TLR-Ls). Non transplantable CB units were used after a signed informed consent from the parents. CD34+cells were enriched to more than 90% purity from CB units (n=5) by an immunomagnetic separation method. The CD34+ cells were cultured in the presence of AB-blood group serum (ABS) and culture media supplemented with SCF (50ng/ml) and GM-CSF (100ng/ml) for 2, 3, 4, or 5 weeks (wks) and with IL-4 (50ng/ml) plus GM-CSF (100ng/ml) for 1 additional wk. The cells were initially plated in a 6-well plate (10^5 cells/well) and once the cells expanded up to $5x10^6$, half were transferred into G-Rex10 bioreactor and half were cultured in "conventional" culture plates in the presence of the above mentioned conditioned medium. In culture plates the cells were splited at confluency while in G-Rex10 every 3 days.

Results: The highest median absolute number of myeloid DCs (CD33+/CD11+) in the bioreactor was obtained by 5-wks culture over the 3-, 4-, and 6-wks culture ($1.5x10^9$ vs $0.017x10^9$ vs $0.8x10^9$ vs $1.2x10^9$, respectively). The number of myeloid DCs that were conventionally cultured under the same conditions did not exceed a median number of $10x10^6$ cells. To evaluate the impact of the serum origin in DCs expansion, we cultured CB-derived CD34+ cells into G-Rex10 bioreactor, in the presence of either autologous CB serum (ACBS) or ABS. At the end of the culture, we identified $1.5x10^9$ DCs with myeloid characteristics in the ABS culture while only $0.011x10^9$ in the ACBS culture ($p<0.05$). To address whether DCs expanded from CB-derived CD34+ cells have the ability to produce Th1 responses, we stimulated DCs with a mixture of TLR-L 3 (Poly I:C: 20μg/ml) and TLR-L 7/8 (R848: 4μg/ml) for 48 hours and measured the levels of IL-12p70, TNF-α, IL-6 and IL-10 by ELISA. Increased levels of IL-12p70, TNF-α and IL-6 were detected, while the IL-10 levels were low to undetectable, indicating that the produced DCs with myeloid features have a strong potentiality for Th1 responses.

Discussion: Overall, we report for first time, over a 10^4 fold myeloid DCs expansion from CB-derived CD34+cells, by using the new generation G-Rex10 bioreactors and describe optimal culture conditions. This large scale myeloid DC generation could significantly contribute to the clinical applicability of DCs in cancer immunotherapy.

Disclosure of Interest: None Declared

Keywords: dendritic cells, cancer immunotherapy, Tumor specific T-cells, Cord Blood, CD34+ cells