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Stem Cell Transplantation, immunotherapy and cell therapy

MONOCYTE ACTIVATION BY CAR-T CELLS: ROLE OF EXTRACELLULAR VESICLES AND SECRETOME IN IMMUNE MODULATION

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Chimeric Antigen Receptor-T (CAR-T) cell therapy has transformed treatment of CD19⁺ B cell malignancies, often causing immune-related toxicities with unclear mechanisms. The monocyte-macrophage lineage appears key, but CAR-T effects on monocytes are not fully known. We investigated for the first time how CAR-T cells interact with monocytes and trigger immune activation.

CAR-T cells (axicabtagene ciloleucel, axi-cel, and tisagenlecleucel, tisa-cel) were isolated from infusion bag washes of eight non-Hodgkin Lymphoma patients with low-grade CRS/ICANS (1-2). To investigate the role of CAR-T–released particles in monocyte activation, cells were co-cultured for 1-3h with CD19⁺ SU-DHL-8 lymphoma cells. Extracellular vesicles (EVs) and the complete secretome (SEC) were collected and used to treat THP-1 monocytes for 1, 3, and 24h. Monocyte-to-macrophage transition (MMt) was analyzed morphologically and molecularly. EV uptake was assessed by fluorescence microscopy, and cytokine expression quantified by digital PCR.

Neither EVs nor SEC significantly affected THP-1 proliferation in either axi-cel or tisa-cel products. When treated for 1-3h with both EVs and SEC, monocytes adopted an irregular shape, with pseudopods formation. This aspect was more evident in cells treated with axi-cel-derived EVs and SEC (Figure 1A) and wasn't appreciable after 24h. To assess if these changes were actually related to the monocyte's differentiation, the *PPM1A* transcript was quantified via dPCR. EVs and SEC obtained after 1h of co-culture of axi-cel products induced *PPM1A* overexpression after 24h of treatment, while EVs and SEC obtained after 3h of co-culture induced the overexpression also after 1-3h post-treatment. Surprisingly, cells treated with tisa-cel-derived EVs and SEC showed an opposite trend, with an increased expression after 1h of treatment with EVs and SEC, not observed after 24h. Collectively, these findings suggest that CAR-T-derived factors promote monocyte differentiation toward a macrophage-like phenotype rather than proliferation. Fluorescence microscopy confirmed EVs uptake by monocytes, indicating their key role in cells activation (Figure 1B). Cytokine analysis revealed significant over-expression of *IL-8* after 1-3h with axi-cel-derived EVs and SEC, but not after 24h. In tisa-cel context just the treatment with EVs and SEC obtained after 3h of co-culture induce cytokines overexpression after 1-3h, but not after 24h (Figure 1C). *TNF-α*, *ICAM1*, and *IL-1β* transcripts show the same trend. No effects were observed using EVs or SEC from naive SU-DHL-8 or non-activated CAR-T cells.

For the first time, we show that CAR-T cells release factors capable of inducing MMt and transient pro-inflammatory activation. These findings highlight a key role of CAR-T-derived EVs/SEC in modulating innate immune responses. Understanding their mechanisms may help identify potential targets to modulate immune activation and improve clinical outcomes in CAR-T-treated patients.

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