Investigating the role of the CD97 cell surface receptor in efferocytosis

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1. Introduction

- Efferocytosis is the process by which certain immune cells find, engulf and clear away dead cells in the body (**Figure 1**). It plays a vital role in tissue repair and maintaining immune balance, with impaired efferocytosis linked to various diseases[1].

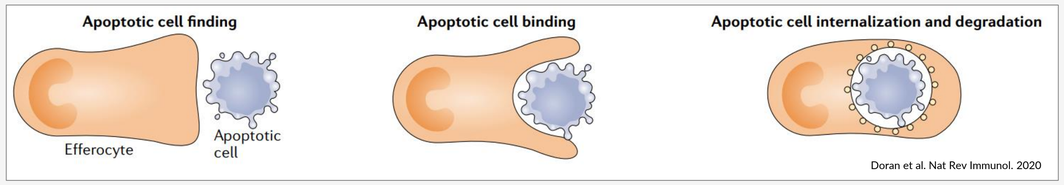

Figure 1: An illustration of the stages of efferocytosis.

- The cell surface receptor CD97 was recently identified as an efferocytosis-associated protein. In mice, its abundance significantly increased following efferocytosis, indicating it likely plays an important role in the process[2].

2. Aims

- Evaluate the role of CD97 in efferocytosis & examine the extent of its involvement.

3. Methodology

- We genetically modified human macrophages (THP-1 cells) to remove the gene that produces the CD97 protein, to generate CD97-knockout clones. The knockouts were confirmed by immunofluorescence microscopy (**Figure 2**), reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (**Figure 3**), and flow cytometry (not shown).

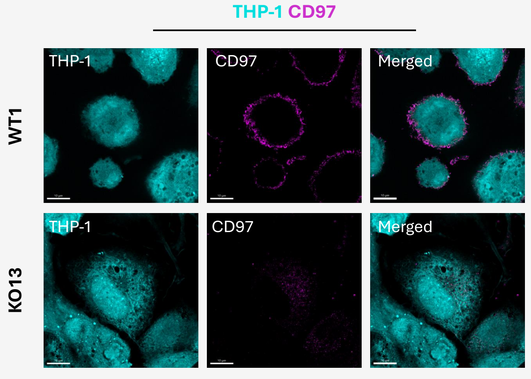

Figure 2: Immunofluorescence microscopy images of a wildtype THP-1 macrophage clone (WT1) and a CD97-knockout clone (KO13). The macrophages are shown in blue, while CD97 protein is shown in pink.

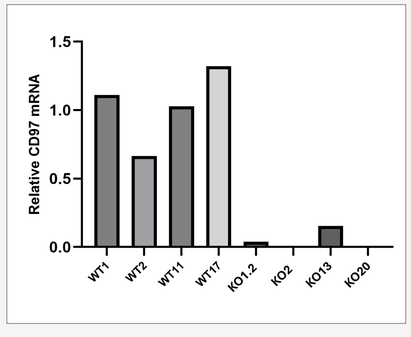

Figure 3: Relative CD97 mRNA transcripts produced by the different wildtype (WT1, WT2, WT11, WT17) and knockout (KO1.2, KO2, KO13, KO20) clones.

- We compared CD97-knockout macrophages to wildtype cells to study how the absence of CD97 affects macrophage function.

4. Results

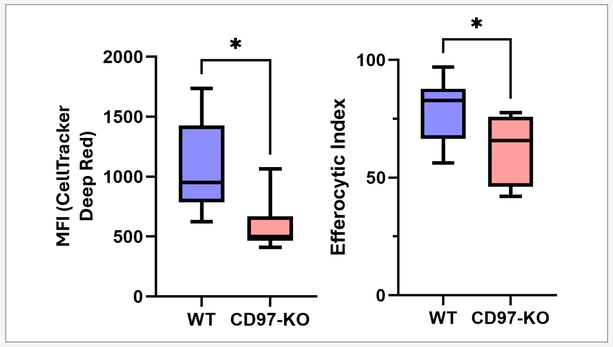

Figure 4: Efferocytosis assay by flow cytometry. A) Mean fluorescence intensity (MFI) emitted from apoptotic cells attached or engulfed by THP-1 macrophages is significantly higher in the wildtype clones in comparison to the CD97-knockout cells. B) The efferocytic index, defined as the percentage of macrophages undergoing efferocytosis of one or more apoptotic cells relative to the total macrophage population in each sample, is significantly higher in wildtype clones compared to CD97-knockout clones. Data was obtained from three independent repeats.

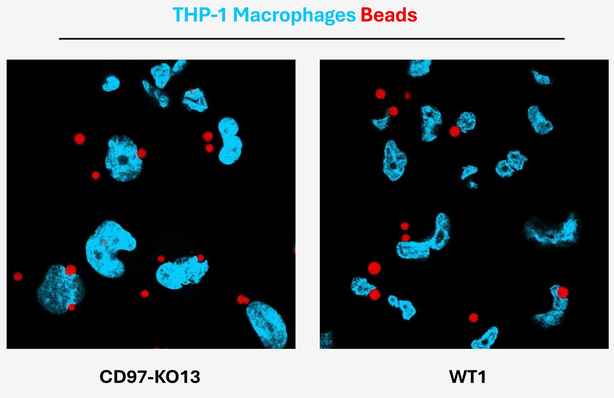

Figure 5: Assessment of phagocytic capacity using immunofluorescence microscopy. Wildtype and CD97-knockout macrophage clones were incubated with Alexa Fluor-594-coated 3µm silica beads for 1 hour. The resulting images showed comparable phagocytic capacity between the clones, suggesting that CD97 does not play a role in phagocytosis of inert particles.

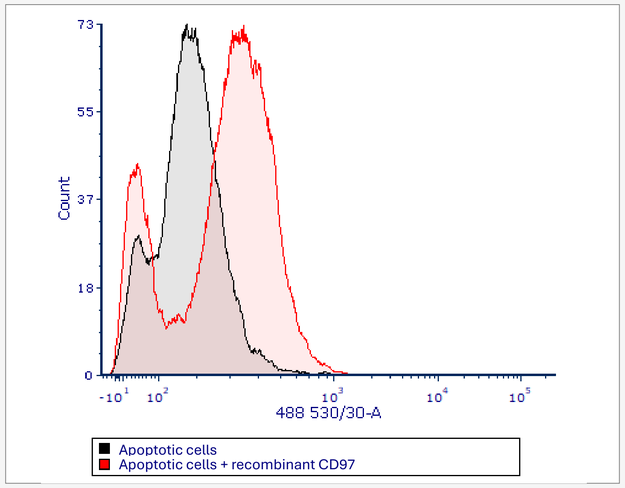

Figure 6: Flow cytometry analysis shows CD97 protein binding to apoptotic cells. The graph represents thousands of individual cells measured using flow cytometry. The y axis shows how many cells were detected at each fluorescence intensity level, which is displayed on the x-axis. The black curve shows the baseline fluorescence of stained apoptotic cells on their own (PE Annexin V stain). The red curve shows what happened when these apoptotic cells were exposed for 30 minutes to lab-made CD97 protein (stained with Fluorescein isothiocyanate) and then washed 3 times. The curve shifts to the right, indicating that CD97 is successfully binding to the apoptotic cells. This rightward shift occurs because cells with bound CD97 give off more fluorescent signal compared to cells without CD97. The more CD97 binding occurs, the more the curve shifts right. This experiment demonstrates that CD97 specifically recognises and attaches to apoptotic cells.

5. Conclusions

- CD97 function enhances the efferocytic capacity of macrophages.
- CD97 selectively mediates efferocytosis without affecting general phagocytic functions.
- CD97 likely exerts its effect by promoting the binding of macrophages to apoptotic cells.

References:
1. Doran AC, Yurdagul A, Tabas I. Efferocytosis in health and disease. Nature Reviews Immunology. 2020;20(4):254-67.
2. Raymond BBA, Frey AM, Trost M. Proteomic mapping of macrophages in response to the clearance of apoptotic cells reveals a unique reprogramming profile. bioRxiv. 2023:2023.11.23.567533.