

# Characterisation of ICAM-3 and extracellular vesicles in the clearance of apoptotic cells

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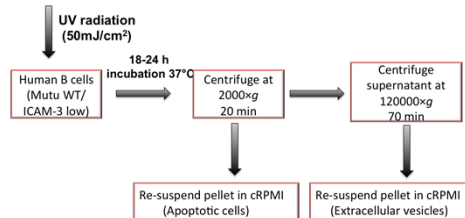
## Introduction

Apoptotic cell clearance is a vital mechanism that is part of programmed cell death that prevents dying cells from undergoing necrosis which may lead to inflammatory and autoimmune disorders<sup>1-3</sup>. Apoptotic cells are removed by phagocytes, in a process that involves 'find me' and 'eat me' signals that facilitate the synapsing and engulfing of cell corpses<sup>4</sup>. Extracellular vesicles (EV) are shed by dying cells to promote their removal<sup>5</sup>. The tethering (binding) synapse is achieved by many ligands on the apoptotic cell and counter receptors on the phagocytes<sup>3-4</sup>. One interesting apoptotic cell associated ligand is ICAM-3, which is a highly glycosylated adhesion molecule and is expressed exclusively on human leukocytes.

This project aims to characterise the role of ICAM3 and EV in the clearance of apoptotic cells and to identify the mechanism that underlie their function in apoptotic cell clearance.

## Methodology

### Generating Apoptotic cells & Extracellular vesicles:

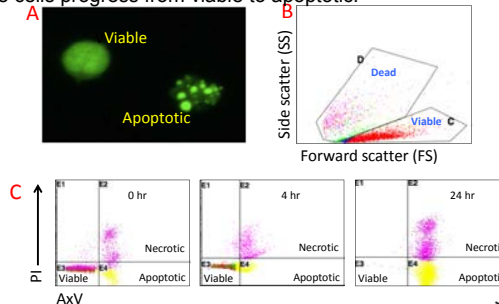


- Analysis of apoptosis:** Apoptotic cells were stained with AxV/PI and cell progression from viable to apoptotic analysed by flow cytometry.
- Macrophage (MØ)-AC interaction assay:** AC (Mutu WT/Low) were co-cultured for 1 hr at 37°C with MØ (THP-1 stimulated with dihydroxyvitamin D3 for 48hrs). The interaction (tethering & engulfment) of AC with MØ was scored using light microscopy following Jenner/Giemsa staining.
- Analysis of inflammatory responses:** TNF- $\alpha$  production from LPS-stimulated MØ in the presence or absence of AC or EV was assessed using a capture ELISA.
- Horizontal Chemotaxis Assays:** A Dunn chamber slide was used to observe MØ migration towards EV. MØ were differentiated on coverslips and exposed on the slide to EV.
- Vertical Chemotaxis Assays:** Transwells containing MØ (upper well) were exposed to EV (lower well). The Cell IQ automated cell tracking (CM Technologies) system was used to observe MØ migration from the upper chamber to the lower chamber containing EV.

## RESULTS AND DISCUSSION

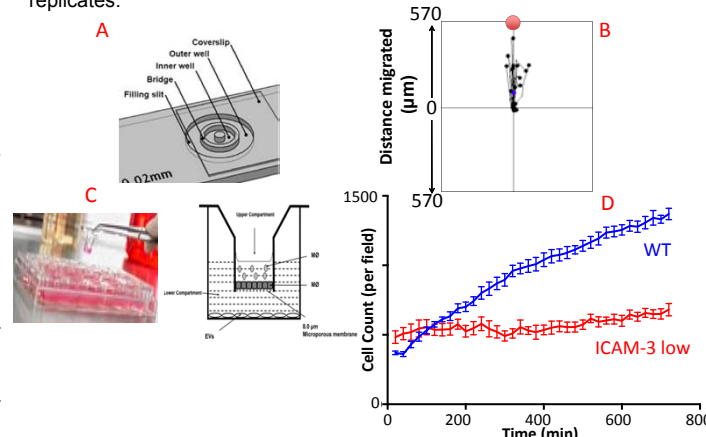
### Induction of B cell apoptosis

**Figure 1.** The human B cell line (Mutu) was induced to apoptosis by UV radiation (50mJ/cm<sup>2</sup>). A) Micrograph of viable and apoptotic acridine orange stained B cells, showing typical nuclear morphology; B) Flow cytometry data showing FS/SS of a B cell culture at 0h post UV; C) Flow cytometry data showing AxV/PI stained B cells post UV radiation 0-24 hr, where the cells progress from viable to apoptotic.



### MØ migrate towards EV bearing ICAM3

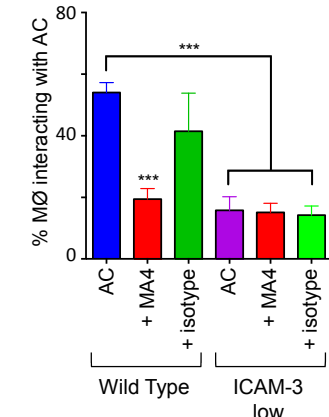
**Figure 3.** EV from apoptotic B cells ( $\pm$  ICAM-3) were used as putative attractants for MØ. A) Dunn chambers are used to observe horizontal migration across a bridge. B) Horizontal chemotaxis data showing MØ migration to EV from apoptotic B cells. The red dot is the chemoattractant source. C) diagram of assay set up showing the lower compartment where EV are situated and the upper transwell compartment where MØ were placed. D) vertical chemotaxis data (Cell IQ tracking system) showing the migration of MØ from an upper to a lower chamber containing EV from WT and ICAM-3 low B cells. Data shown are mean  $\pm$  SD of 5 technical replicates.



### ICAM3 promotes MØ interaction with AC

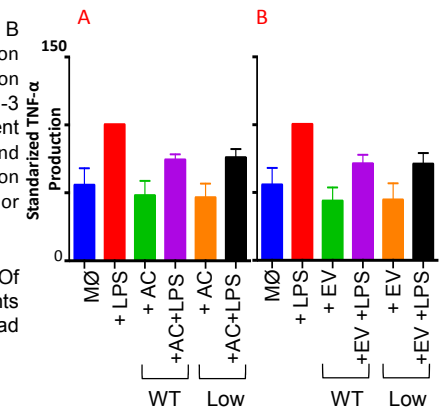
**Figure 2.** Interaction of apoptotic B cells (AC) with MØ is reduced by the anti-ICAM-3 monoclonal antibody (MA4) or by the absence of ICAM-3 (ICAM-3 low).

Data shown are mean  $\pm$  SEM of independent experiments (n=3). Each experiment had 4 technical replicates. \*\*\*P<0.001 ANOVA with Dunnett's test.



### Extracellular vesicles are potentially anti-inflammatory

**Figure 4.** (A) Apoptotic B cells show a trend on reduced TNF- $\alpha$  detection regardless of their ICAM-3 expression. (B) Treatment of MØ with EV shows a trend to reduced TNF- $\alpha$  detection regardless of presence or absence of ICAM-3.



Data shown are SEM  $\pm$  of independent experiments (n=3). Each experiment had 3 technical replicates.

## CONCLUSIONS & FUTURE WORK

- ICAM3 enables interaction between MØ and AC and is chemoattractive for MØ when released on apoptotic cell-derived EV.
- EV show potential to have anti-inflammatory effects.
- Future work will assess**
  - the interaction of EV ( $\pm$  ICAM-3) with phagocytes to dissect the mechanism of EV action; and
  - the ability of the anti-ICAM-3 mAb to block MØ recruitment to sites of cell death *in vivo*.