Physical and functional characterisation of apoptotic cell-derived extracellular vesicles

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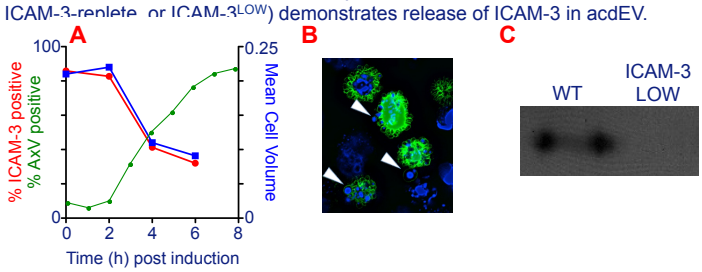
INTRODUCTION

Apoptosis culminates in rapid, non-phlogistic removal of cell corpses by recruited and resident phagocytes of the innate immune system[1-3]. Relatively little is known of the molecular mechanisms underlying phagocyte recruitment to sites of cell death *in vivo*. However defective clearance of dying cells is known to contribute to autoimmunity and the development of atherosclerosis[1].

We have shown previously that ICAM-3 (a human leukocyte-restricted IgSF member) acts as a molecular 'flag' to mediate corpse removal[4,5]. Here we show that ICAM-3 on apoptotic cell-derived extracellular vesicles (acdEV) mediates phagocyte recruitment. Furthermore we demonstrate that ICAM-3 promotes migration to apoptotic foam cells and mediates *in vitro* transendothelial migration of monocytes, suggesting ICAM-3 on acdEV may have a role to play in the pathogenesis of atherosclerosis.

Apoptotic cell ICAM-3 is shed in Extracellular Vesicles

(A) UV-induced apoptosis in human B cells proceeds rapidly, as detected by annexin V (AxV) staining. Alterations in ICAM-3 levels (detected with mAb staining) are closely associated with changes in cell size (as assessed by flow cytometry – electronic volume) and occur very early in apoptosis. (B) Apoptotic HeLa cells expressing ICAM-3-GFP (green) reveal ICAM-3 localisation to apoptotic bodies. (C) Western blot analysis of EV of apoptotic human B cells (WT: ICAM-3-replete, or ICAM-3^{LOW}) demonstrates release of ICAM-3 in acdEV.

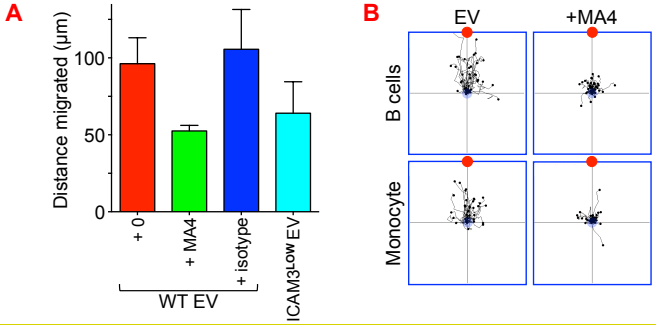


Using IZON's Tunable Resistive Pulse Sensing technology (qNano), acdEV from human B cells were analysed after depletion of cells and large apoptotic bodies by centrifugation (2000x*g*, 20 min). Particle size was 250nm ± 14 with a modal size of 160nm. Approximately 50% of all acdEV were released within the first 5 hours of apoptosis.

EV promote monocyte attraction via ICAM-3

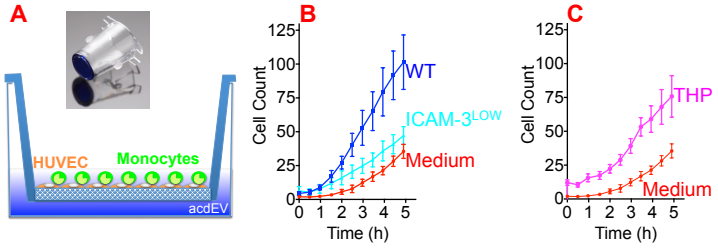
(A) EV from apoptotic B cells (WT & ICAM-3^{LOW}) were assayed for their chemoattractive capacity using Dunn chambers. WT, but not ICAM-3^{LOW}, EV are potent monocyte attractants. This migration is inhibited by anti-ICAM-3 mAb (MA4) but not by an isotype control mAb. Data shown are mean ± SEM (n=3).

(B) Representative chemotaxis plots show monocyte migration to acdEV from WT B cells or monocytes in the presence or absence of a blocking anti-ICAM-3 mAb (MA4). The red spot indicates the chemoattractant source and cells begin their migration from the blue spot. The plots show a scale of ± 470µm.



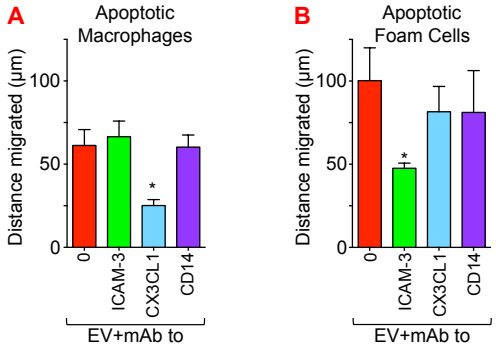
EV and ICAM-3 induce monocyte transendothelial migration

HUVEC cells were grown on 8µm transwells (BD) until a fluid-tight barrier was formed. acdEV from B cells (WT or ICAM-3^{LOW}) or THP-1 monocytes) were placed in the lower chamber whilst THP cells stimulated with dihydroxyvitamin D3 were placed in the upper chamber (A). Migration of monocytes to the lower chamber was assessed using the Cell IQ automated cell tracking system (CM Technologies). Monocytes migrated more quickly towards B cell EV that were replete for ICAM-3 (B) and showed strong migration to EVfrom ICAM-3-bearing apoptotic THP-1 monocytes (C).



Phagocyte recruitment to acdEV from MØ and foam cells

EV from apoptotic THP-1-derived macrophages (A) or oxidised LDL-laden macrophages (foam cells; B) were assessed for their capacity to recruit THP-1 monocytes using a horizontal Dunn chemotaxis chamber method. Data shown are mean ± SEM (n>3). Addition of blocking mAbs to: ICAM-3, CX3CL1 or CD14 (an important MØ receptor for apoptotic cells) highlights the importance of ICAM-3 in recruitment of phagocytes to apoptotic foam cells. $P<0.05$.



CONCLUSIONS

ICAM-3 is shed with acdEV during leukocyte apoptosis. Such acdEV are released early in apoptosis and are potent chemoattractants for monocytes.

ICAM-3 on acdEV promotes monocyte recruitment to a range of apoptotic cells, including lymphocytes and monocytes. ICAM-3 and acdEV are capable of recruiting monocytes across an endothelial barrier.

CD14, an important phagocyte receptor for apoptotic cells, does not appear to be required for phagocyte migration to acdEV.

ICAM-3 and CX3CL1 are involved in migration to apoptotic foam cells and macrophages respectively.

Taken together these data suggest acdEV and ICAM-3 may play an important role in the recruitment of macrophages to sites of cell death. Such sites may include tumours and atherosclerotic plaques. Furthermore, the inhibition of monocyte migration in the presence of anti-ICAM-3 mAbs suggests ICAM-3 may be a useful target for modulation of monocyte recruitment for therapeutic gain.

REFERENCES [1] Devitt, A. & Marshall, L. J. (2011) *J. Leuk Biol*, 90: 447-457. [2] Devitt, A., *et al.* (1998): *Nature*, 392: 505-9. [3] Devitt, A., *et al.* (2004). *JCB* 167: 1161-70. [4] Moffatt, O. D. *et al.* (1999): *JI*, 162:6800-10 [5] Torr *et al.* (2011). *Cell Death & Diff* 19: 671-679.

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