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Life & Health Sciences

Phagocyte chemotaxis to apoptotic bodies – a role for transglutaminase-2?

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INTRODUCTION: As cells die by apoptosis they undergo dramatic surface changes that include the generation and release of membrane blebs known as apoptotic bodies (fig 1A). The phagocytic clearance of apoptotic cells and bodies marks the conclusion of the apoptosis programme and is regarded as its definitive stage[1] (fig 1B). Failure to clear apoptotic cells can result in cell lysis and subsequent autoimmunity and inflammation.

Effective clearance of apoptotic cells by professional phagocytes (e.g. macrophages, MØ) requires recognition and removal of apoptotic rather than viable cells, a process dependent on so called "eat me" signals exposed on the apoptotic cell and phagocyte receptors including innate immune receptors. However prior to this, professional phagocytes must migrate to sites of apoptosis along a chemotactic gradient of "find me" signals - factors which enhance the migration of phagocytes to sites of apoptosis. The identity of these signals remains largely unclear although the chemokine fraktalkine and externalisation of lysophosphatidylcholine has been associated with phagocyte chemotaxis to supernatants of apoptotic cells[2,3].

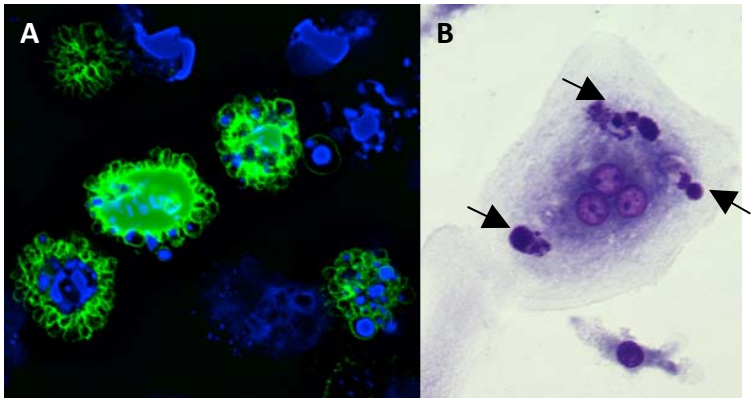

Figure 1: Macrophage interaction with apoptotic cells & bodies. A) HeLa cells with fluorescently-labelled membrane (green, GFP) and nuclear material (blue, DAPI) demonstrating membrane blebbing during apoptosis, B) Jenner-Giesma stained macrophage interacting with apoptotic cells (arrows).

Transglutaminase (TG)-2, a multi-functional enzyme with Ca^{2+}-dependent protein cross linking activity, functions in cell signalling and as an integrin binding adhesion co-receptor in the context of extracellular matrix (ECM) components[4]. Acting within these capacities TG2 plays multiple roles associated with cell adhesion and migration. This project aimed to test the hypothesis that TG-2 is released from dying cells in apoptotic bodies which diffuse from apoptotic cells to promote TG-2-dependent attraction of phagocytes.

RESULTS: TG-2 is released from apoptotic cells in apoptotic bodies: TG-2 is actively externalised by cells in response to stress[5]. Here, we show TG-2 is also relocated to the extracellular environment during apoptosis by membrane blebbing and release of apoptotic bodies. Briefly, proteins from viable and apoptotic B and T cells and released apoptotic bodies (prepared by centrifugation of culture supernatants (fig 2A)) were resolved by SDS-PAGE, electro-blotted to nitrocellulose and probed with a rabbit polyclonal anti-TG-2 antibody. Visualisation of the blot with enhanced chemoluminescence (ECL) indicates the presence of TG-2 within released blebs of apoptotic cells (fig 2B).

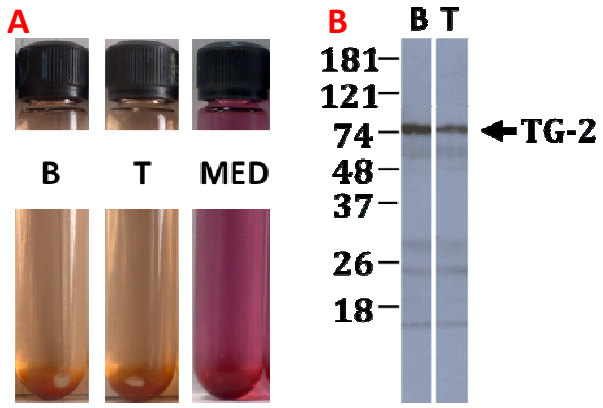

Figure 2: Apoptotic bodies released form dying cells contain TG-2. A) Culture supernatants from apoptotic B & T cells (but not medium alone 'Med') contained apoptotic bodies that could be pelleted following (15,000xg; 30 min). B) TG-2 was present in purified apoptotic bodies from B and T cells, as detected by immunoblotting. TG-2 has a molecular weight of 77-85kDa.

Apoptotic cell supernatants are chemoattractive for macrophages: Human B or T cell lines were induced to apoptosis by UV. Cell-free supernatants from these cultures and the apoptotic body fraction of these supernatants were tested for chemoattractive capacity for a human MØ cell line. Using chemotaxis chambers, supernatants were separated from MØ by a polycarbonate (PVP-free) membrane incorporating 5μm pores. Following 4h incubation MØ chemotaxis was established by light microscopy through counting transmigrated cells per high power (x400) field of view (HPF).

Results indicate that apoptotic cell supernatants are chemoattractive for MØ migration. Figure 3A highlights a dose-dependent relationship between apoptotic B cell supernatants and MØ transmigration. Isolation of blebs from a T cell apoptotic cell supernatant by centrifugation (15000xg, 30 min) shows that both the apoptotic body fraction and the supernatant itself show a detectable chemoattractive capacity (fig 3B).

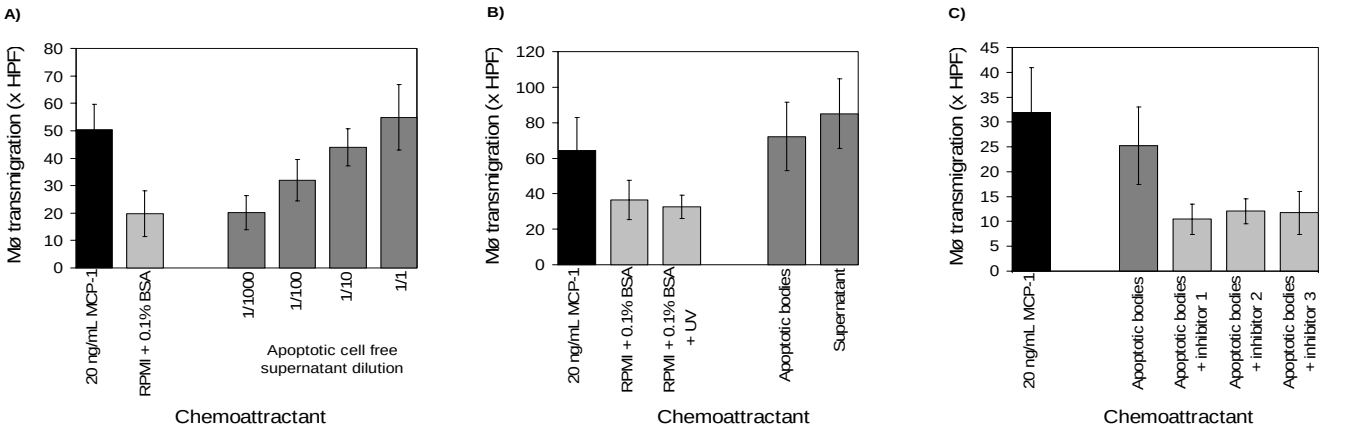

Figure 3: MØ migration towards apoptotic cell supernatants and apoptotic bodies is TG-2-dependent. A) Apoptotic B cell supernatants were centrifuged (200 xg, 10 min) to remove cells. This cell-free supernatant induced MØ cell migration in a dose-dependent manner. MCP-1 was included as a positive control. B) Medium from T cell cultures undergoing apoptosis were centrifuged (15,000 xg, 30 min) to isolate apoptotic bodies (the pellet). Apoptotic bodies and body-free supernatants both induced MØ chemotaxis. C) Chemoattraction to apoptotic bodies could be inhibited with three separate TG-2 inhibitors. Results shown are mean ±SD (n=15).

Inhibition of TG-2 reduces MØ chemotaxis to apoptotic bodies: The protein cross linking activity of TG-2 is involved in the regulation of the cytoskeleton[2] and may therefore be associated with cell migration and adhesion. To test this three separate TG-2 inhibitors were tested for their ability to inhibit MØ chemoattraction to apoptotic bodies purified by centrifugation. Preliminary results indicate that the chemotactic response of MØ to apoptotic bodies is limited by the presence of any of three TG-2 inhibitors. An inhibition of MØ transmigration in excess of 50% was noted in the presence of these inhibitors relative to non-inhibited apoptotic bodies (fig 3C).

DISCUSSION: TG-2 plays a considerable role in the modulation of cell adhesion[4]. The multi-functional nature of this enzyme means that this function may be associated with its different activities. For example, TG-2 may crosslink and stabilise ECM groups thereby directing phagocyte migration to sites of apoptosis. Alternatively, functioning as an integrin co-receptor, it may increase phagocyte adhesion and therefore motility.

Inhibition of TG-2 enzyme activity reduces phagocyte recruitment to apoptotic bodies. This may suggest a role for TG-2 in generating a “find-me signal” associated with apoptotic bodies. However additional work is required to definitively demonstrate that TG-2 inhibition within apoptotic bodies rather than within phagocytes is responsible for the results presented here.

References: **1. Savill, J. & Fadok, V.** (2000) *Nature*. **407**, 784-788. **2. Lauber, K., *et al*.** (2003) *Cell*. **113**, 717-730. **3. Truman, L.A., *et al.*** (2008) *Blood*. **112**, 5026-5036. **4. Fésüs, L. & Piacentini, M.** (2002) *Trends in Biochemical Sciences*. **27,** 534-539. **5. Beninati, S. & Piacentini, M.** (2004) *Amino Acids*. **26**, 367-372.