DENDRITIC CELLS

Actin a dangerous part

> "filamentous actin … is a damage-associated molecular pattern that is sensed by DCs"

The exact mechanisms by which dendritic cells (DCs) sense damaged cells have attracted much interest, as a better understanding of these pathways could facilitate the development of more effective vaccines. Now, two studies in *Immunity* have shown that filamentous actin (F-actin) is a damage-associated molecular pattern that is sensed by DCs via the receptor C-type lectin domain family 9 member A (CLEC9A; also known as DNGR1).

Certain DC subsets specialize in the cross-presentation of antigens from dead cells. Although CLEC9A was thought to be important for these particular DC functions, the ligand for this receptor had remained elusive. To address this, Zhang *et al.* generated tagged forms of the extracellular domain of mouse and human CLEC9A. Using these ectodomain reagents, they confirmed that CLEC9A binds to late-stage apoptotic cells undergoing secondary necrosis, but not to early-stage apoptotic cells. The CLEC9A ectodomain reagents recognized various types of dead cells (including platelets and red blood cells) from mice and humans, and also other freeze-thawed mammalian and insect cells, but not freeze-thawed bacteria. This suggested that the CLEC9A ligand is highly conserved in evolutionary terms.

Immunohistochemistry analyses using fixed and permeabilized fibroblasts showed that CLEC9A binding activity colocalized with F-actin, but not with all cellular actin. The authors found that CLEC9A recognizes F-actin complexed to the actin-binding domain of certain cytoskeletal molecules, including spectrin and actinin. They propose that the role of the cytoskeletal molecules is to maintain actin in a filamentous conformation, which is necessary for recognition by CLEC9A. Zhang *et al.* also determined the crystal structure of the C-type lectin domain of CLEC9A; these studies support the idea that CLEC9A molecules dimerize. Furthermore, their structural work identified two conserved tryptophan residues that are crucial for the recognition of F-actin by CLEC9A.

Likewise, Reis e Sousa and colleagues used reagents comprising the extracellular domain of DNGR1 to search for a ligand for this receptor. They found that DNGR1 ligands were present in lysed cells from a wide range of species, from insects to humans. In affinity-purification studies, DNGR1 precipitated with multiple actin-associated proteins, but none of these actin-binding proteins alone served as a ligand for DNGR1. Instead, and consistent with the study by Zhang *et al.*, Ahrens *et al.* showed by immunohistochemistry that DNGR1 binding activity colocalized with F-actin in a variety of cell types; they went on to formally demonstrate that DNGR1 binds to F-actin but not to monomeric actin. Finally, they used a DNGR1-expressing reporter cell line to show that the binding of F-actin by DNGR1 leads to downstream signalling via the tyrosine kinase SYK. SYK activation occurred when these reporter cells were cultured with F-actin or freeze-thawed cells, but not when the freeze-thawed cells were first treated with an actin-depolymerizing agent. The authors suggest that the identification of F-actin as the ligand for DNGR1 indicates that cytoskeletal exposure is a universal sign of cell damage that can be targeted by the innate immune system.

Interestingly, a related study in *Blood* by Reis e Sousa and colleagues has found that DNGR1 expression can be used to specifically identify mouse and human DCs that depend on the transcription factor BATF3 for their development. This finding is notable, as BATF3-dependent DCs are thought to have enhanced cross-presenting activity. In support of this, two other new studies by the Reis e Sousa and Sancho groups show that DNGR1 promotes DC cross-priming of cytotoxic T lymphocytes during virus infection in mice by diverting necrotic cargo away from lysosomes.

Yvonne Bordon

ORIGINAL RESEARCH PAPERS Zhang, J.-G. *et al.* The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. *Immunity* 5 Apr 2012 (doi:10.1016/j.immuni.2012.03.009) | Ahrens, S. *et al.* F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. *Immunity* 5 Apr 2012 (doi:10.1016/j.immuni.2012.03.008) | Poulin, L. F. *et al.* DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and non-lymphoid tissues. *Blood* 22 Mar 2012 (doi:10.1182/blood-2012-01-406967) | Zelenay, S. *et al.* The dendritic cell receptor DNGR-1 controls endocytic handling of necrotic cell antigens to favor cross-priming of CTLs in virus-infected mice. *J. Clin. Invest.* 16 Apr 2012 (doi:10.1172/JCI60644) | Iborra, S. *et al.* The DC receptor DNGR-1 mediates cross-priming of CTLs during vaccinia virus infection in mice. *J. Clin. Invest.* 16 Apr 2012 (doi:10.1172/JCI60660)