HIGHLIGHTS

APOPTOSIS

Eat me!

Dying cells are a danger to their healthy neighbours, and must be rapidly cleared away. This task is carried out by phagocytes, which recognize and then engulf the apoptotic cells. The 'eat-me' signal for phagocytes has been proposed to be phosphatidylserine (PS), which is exposed on the surface of dying cells. But how is this signal read by phagocytes?

Reporting in *Nature*, Shigekazu Nagata and colleagues now describe a factor that could act as a bridge between the dying cells and the phagocytes. They isolated this protein — the milk fat globule-EGF-factor 8 (MFG-E8) — while searching for factors that mediate the engulfment of apoptotic cells by macrophages (a type of phagocytic cell).

The authors started by using activated mouse macrophages to immunize Armenian hamsters, from which they prepared a series of antibodies. One of these — designated 2422 — increased the percentage of macrophages that engulfed apoptotic cells. Nagata and colleagues then used immunoprecipitation followed by affinity purification to identify the target of 2422, and found it to be two isoforms of MFG-E8. A purified recombinant form of the larger isoform (MFG-E8-L) did not bind to freshly isolated thymocytes, but it could bind to thymocytes if they were first treated with dexamethasone (which induces apoptosis).

How does MFG-E8-L recognize these apoptotic cells? Annexin V is known to bind PS, but pretreatment with MFG-E8-L reduced this interaction (and vice versa), which indicates that MFG-E8-L recognizes PS on the apoptotic cells. But this doesn't explain how the MFG-E8-L then interacts with phagocytes. The authors noticed, however, that MFG-E8-L contains an arginine–glycine–aspartate (RGD) motif, which can be recognized by members of the integrin family. As integrins are found on phagocytes, could MFG-E8-L act as a bridge between them and the PS on apoptotic cells?

To test this, Nagata and colleagues established mouse NIH3T3 transformants that express high levels of $\alpha_v\beta_3$ integrin. They found that MFG-E8-L increased the adherence of these cells to PS-coated microtitre plates. Moreover, MFG-E8-L could stimulate these NIH3T3 cells to engulf apoptotic thymocytes, as the percentage of NIH3T3 cells that internally carried four or more thymocytes increased from 9% to 46% in the presence of MFG-E8-L. Finally, they confirmed these results using a form of MFG-E8-L with a mutation in the RGD domain. This acted in a dominant-negative manner and blocked the phagocytosis of apoptotic cells by macrophages both *in vitro* and *in vivo*.

These results are exciting because they not only show how phagocytes can recognize apoptotic cells, but they also strengthen the case for PS as the 'eat-me' signal.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Hanayama, R. *et al*. Identification of a factor that links apoptotic cells to phagocytes. *Nature* **417**, 182–187 (2002)
FURTHER READING Henson, P. M. *et al*. The phosphatidylserine receptor: a crucial molecular switch? *Nature Rev. Mol. Cell Biol*. **2**, 627–633 (2001)

WEB SITE
Shigekazu Nagata's laboratory: http://www.med.osaka-u.ac.jp/pub/genetic/index.html