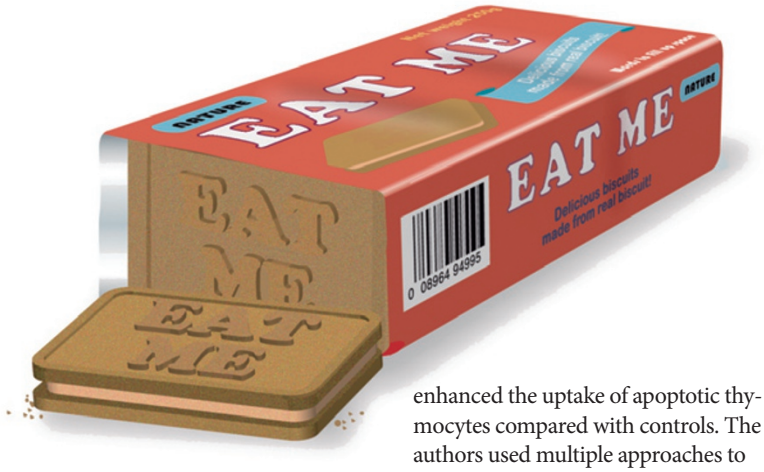
APOPTOSIS

Recognizing 'eat-me' signals



The clearance of apoptotic cells is crucial to avoid the development of autoimmune disease, and phosphatidylserine (PtdSer) on the surface of apoptotic cells is recognized as an engulfment signal by phagocytes. To date the receptors that directly recognize PtdSer had remained elusive, but now, two papers published in *Nature* report the identification of two PtdSer receptors.

Miyanishi *et al.* had previously shown that milk fat globule EGF factor 8 (MFGE8) is a bridging molecule expressed by macrophages that stimulates the engulfment of apoptotic cells. Here, to assay apoptotic-cell engulfment, they used apoptotic thymocytes deficient in caspase-activated DNase (CAD) as the target cells, as the DNA of these cells is only degraded once the cells have been engulfed by phagocytes. Peritoneal macrophages that express very little MFGE8 were still able to efficiently engulf apoptotic cells, so the authors set out to identify the recognition receptor involved. Hybridomas were generated from which a single monoclonal antibody, Kat5-18, was identified that could inhibit the phagocytosis of apoptotic cells by peritoneal macrophages. Expression cloning showed that Kat5-18 recognized TIM4 (T-cell immunoglobulin domain and mucin domain 4). This molecule belongs to a family of eight members in mice and is expressed by peritoneal macrophages and by $MAC1^+$ cells in the spleen, lymph nodes and fetal liver. TIM4 was found to bind directly, via its immunoglobulin domains, to PtdSer exposed on apoptotic cells. Transfection of a fibroblast cell line with TIM4 enhanced the ability of the fibroblasts to engulf apoptotic cells, and this function was confirmed *in vivo* by the ability of Kat5-18 to significantly reduce the engulfment of thymocytes from $CAD^{-/-}$ mice that had been induced to undergo apoptosis. The authors further found that mouse TIM1, but not TIM2 or TIM3, could also mediate this function.

In a second study, Park *et al.* set out to identify the receptor upstream of the ELMO1 (engulfment and cell motility 1)–DOCK180–RAC signalling module, which is known to promote the internalization of apoptotic cells. The authors performed a yeast-two-hybrid screen with the N-terminal portion of ELMO1 as bait, and identified a cytoplasmic fragment of BAI1 (brain-specific angiogenesis factor 1) as a binding partner. Despite its name, BAI1 expression was detected in primary human monocytes and macrophages, in macrophage cell lines and in tissues such as the bone marrow and spleen. To assess the role of BAI1 as an engulfment receptor, a macrophage cell line was stably transfected with haemagglutinin-tagged BAI1. Expression of BAI1 enhanced the uptake of apoptotic thymocytes compared with controls. The authors used multiple approaches to show that the thrombospondin type 1 repeats in the extracellular domain of BAI1 are crucial for binding to PtdSer on apoptotic cells and that BAI1 can compete with annexin-V (which binds to PtdSer on apoptotic cells) for binding. Disrupting the binding of BAI1 to the ELMO1–DOCK180–RAC signalling components reduced the uptake of apoptotic cells. Knockdown of BAI1 expression using short interfering RNA decreased the uptake of apoptotic cells, with the efficiency of knockdown correlating with the degree of inhibition of engulfment.

These papers identify receptors that can directly bind to PtdSer on apoptotic cells and mediate their uptake, and in the case of BAI1, reveal the signalling mechanism used by the receptor.

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ORIGINAL RESEARCH PAPERS Miyanishi, M. *et al.* Identification of Tim4 as a phosphatidylserine receptor. *Nature* 24 October 2007 (doi:10.1038/nature06307) | Park, D. *et al.* BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature* 24 October 2007 (doi:10.1038/nature06329)
FURTHER READING Ravichandran, K. S. & Lorenz, U. Engulfment of apoptotic cells: signals for a good meal. *Nature Rev. Immunol.* **7**, 964–974 (2007)