INNATE IMMUNITY

VAMPing up macrophage responses

As crucial players in immediate early defence against infection, macrophages are armed with multiple mechanisms to alert the immune system to an intruder. Two such macrophage activities are phagocytosis of the intruding microorganism and rapid secretion of pro-inflammatory cytokines, such as tumour-necrosis factor (TNF). In a recent report by Jennifer Stow and colleagues, these two activities are shown to be linked by a joint trafficking pathway, which allows macrophages simultaneously to release TNF and to expand their plasma membrane for phagocytosis.

To identify the proteins that are involved in the TNF-secretion pathway, the authors carried out DNA-microarray analysis of macrophages activated with lipopolysaccharide. Vesicle-associated membrane protein 3 (VAMP3) was highly expressed by activated macrophages, and its expression correlated with TNF secretion. Consistent with having a role in the trafficking of TNF, VAMP3 could interact with SNARE proteins (which mediate intracellular membrane-fusion events) in the Golgi (such as syntaxin-6) and the plasma membrane (such as syntaxin-4). Moreover, overexpression of VAMP3 by macrophages increased TNF secretion, whereas knockdown of *Vamp3* expression, using small interfering RNA, blocked the delivery of TNF to the cell surface, leading to its accumulation in the Golgi.

Fluorescently labelled VAMP3 and TNF were both shown to be localized in recycling endosomes, as indicated by colocalization with internalized transferrin and the recycling-endosome protein RAB11, pointing to an unexpected route for TNF exocytosis. Further analysis of this pathway indicated that two fusion events involving VAMP3 and its SNARE-protein partners occurred: the first between TNF carriers exiting the Golgi and recycling endosomes, and the second between recycling endosomes and the plasma membrane.

Because SNARE proteins and VAMP3 have previously been shown to be required for phagocytosis of large microorganisms, such as yeast, the authors next investigated the possibility that the TNF-secretion pathway and phagocytosis converged. Imaging of activated macrophages that were incubated with the yeast *Candida albicans* indicated that VAMP3 and TNF were present in the phagocytic cup — the initial stage of phagocytosis — but TNF was not detected after internalization or in mature phagosomes. Addition of an inhibitor of TNF-converting enzyme (TACE) to the cells, to block proteolytic release of cell-surface TNF, led to the accumulation of TNF in the phagocytic cups.

So, the delivery of TNF to the macrophage surface, mediated by a series of membrane-fusion events that involve VAMP3 and SNARE proteins, seems to be targeted to sites of phagocytic-cup formation. After it has reached the cell surface, TNF is rapidly cleaved by TACE and released, before closure of the phagocytic cup. This provides the macrophage with an elegant means to release TNF while simultaneously expanding the plasma membrane for formation of the phagosome.

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References and links

ORIGINAL RESEARCH PAPER Murray, R. Z., Kay, J. G., Sangermani, D. G. & Stow, J. L. A role for the phagosome in cytokine secretion. *Science* 10 Nov 2005 (doi:10.1126/science.1120225)