HIGHLIGHTS

PHAGOCYTOSIS

'ER we go

During phagocytosis, it has generally been accepted that phagosomes form by invagination of the plasma membrane (PM). So, why did a proteomics study by Michel Desjardins and colleagues show that phagosomes contain several endoplasmic reticulum (ER) proteins? They addressed this question in work now published in *Cell*, and found that fusion of the ER with the PM underneath phagocytic cups — membrane structures that surround partially engulfed particles — provides a source of membrane for phagosome formation in macrophages.

Pronase digestion of purified intact phagosomes — a method that degrades all protein moieties that are exposed on the cytoplasmic side of these organelles — allowed the authors to confirm that the ER membrane protein calnexin is a genuine component of the phagosome membrane, and that it is orientated as would be expected if the ER directly fuses with phagosomes. This result was confirmed using pre-embedding immunogold labelling at the electron-microscopy (EM) level with antibodies against the cytoplasmic domain of calnexin.

Desjardins and co-workers then monitored the relative abundance of calnexin in phagosomes during phagocytosis, and found that it is present in several phagocytic structures, ranging from phagocytic cups to mature phagosomes. Using immunoblotting to monitor the kinetics of calnexin association with phagocytic structures, they confirmed that the ER associates with phagosomes at a very early stage of phagosome formation, and also showed that successive waves of ER are incorporated during phagosome maturation.

Phosphatidylinositol 3-kinase (PI3K) is known to be important for phagocytosis and the authors found that PI3K inhibitors decrease ER-mediated phagocytosis in macrophages. When they used EM to visualize the inhibited cells, they saw direct contacts between the ER and PM at sites of internalization, which indicates that the ER is directly recruited to the PM to form phagosomes. Using an immunocytochemical approach to localize the activity of the ER enzyme glucose-6-phosphatase, they were able to confirm the recruitment of the ER to the PM.

ER-mediated phagocytosis seems to be a widely used mechanism in macrophages, as the authors found that it is used for the internalization of inert particles through various receptors (for example, Fc and complement receptors). They also showed that it is used for the internalization of the intracellular pathogens *Leishmania donovani* and *Salmonella typhimurium*.

The discovery of ER-mediated phagocytosis in macrophages by Desjardins and colleagues has altered our long-held view of phagosome formation, and has provided explanations for previous observations (for example, how antigens from intracellular pathogens can be presented by MHC class I molecules). As the authors found that ER-mediated phagocytosis is not the preferred mechanism in neutrophils (in which pathogens are killed rapidly), they propose that intracellular pathogens have evolved to exploit ER-mediated phagocytosis to evade destruction by macrophages.

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References and links

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WEB SITES

Michel Desjardins' laboratory:
http://www.patho.umontreal.ca/mdesjardins.htm

Encyclopedia of Life Sciences:
http://www.els.net
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