

## PT21 Lipid Analysis of Phagosomal Membranes

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Lipid rafts may influence phagosomal lipid accumulation and composition

**Introduction.** In phagocytosis, uptake of particles into cells is mediated by Fc receptors [1] whose engagement leads to formation and closure of the phagocytic cup around the phagocytic particle [2]. It has been suggested that specialized membrane domains, called lipid rafts (rich in cholesterol and sphingolipids), play a crucial role in membrane restructuring during phagocytosis [3]. We aim to determine the role lipid rafts play in phagocytosis by isolating phagosomal membranes and analysing their lipid composition by nano-ESI-QTOF-MS.

**Experimental.** Magnetic beads coated with IgG antibodies are presented to activate Fc receptors on J774 mouse macrophages for phagocytosis. After incubation to trigger phagocytosis, cells were disrupted by nitrogen cavitation and the phagosomal membranes attached to the magnetic beads isolated recovered with a magnet. Lipids were extracted by Folch extraction and analysed using nano-ESI-QTOF-MS/MS for phospholipid and sphingomyelin and DI-EI-MS for cholesterol.

**Results and Discussion.** Late phagosomes display a significant increase of phosphatidylcholine and sphingomyelin compared to early phagosomes, whereas their composition did not change significantly, suggesting late phagosomes accumulate more lipids without changing composition. Cells enriched with cholesterol or 7-ketocholesterol prior to phagocytosis displayed decreased lipid accumulation and changes in relative composition, including an increase in the proportion of sphingomyelin from 9% to 27% in 7-ketocholesterol samples. This suggests that lipid rafts may play a role in phagocytosis.

[1] E.Garcia-Garcia, C. Rosales, JLB 2002, 72: 1092-1108.

[2] K.K. Huyhn, J.G. Kay, J.L. Stow, S. Grinstein, Physiology 2007, 22: 366-372

[3] Simons, K. and E. Ikonen, Nature, 1997. 387: 569-572.