SUMMARY

Clearance of dying cells is of great importance for maintaining balanced tissue homeostasis in mammalian organisms. Failure to do so may lead to inflammation and autoimmune diseases. Although we know quite a lot about the elimination of apoptotic and necrotic cells, there are still open questions on how cells dying through or with autophagy get eliminated.

MCF-7 cells undergo autophagic death upon tamoxifen treatment; this we consider here death through autophagy or *de novo* autophagy, since death is trigered by autophagy. When plated on non-adhesive substratum these cells die by anoikis while inducing autophagy - what is considered here death with autophagy (cells' death is not caused by autophagy) or anoikic-autophagy to distinguish it from the *de novo* one. In each case autophagy was revealed by monodansylcadaverine staining, elevated LC3II protein levels and electron microscopy examination.

In living cells autophagy takes place usually unnoticed to the neighbours. However, its co-ocurrance with cell death may contribute to the clearance of these dying cells by recruited phagocytes. Both *de novo* and anoikis-derived autophagic dying cells were engulfed by human macrophages and MCF-7 cells. Inhibition of autophagy by 3-methyladenine (3-MA) abolished engulfment of cells dying through *de novo* autophagy, but not those dying through anoikis with autophagy. Blocking exposure of phosphatidylserine on both dying cell types inhibited phagocytosis by MCF-7 but not by macrophages. This means that when autophagy induces cell death it also contributes to the clearance of dying cells.

Gene expression profiling of 95 genes involved in the apopto-phagocytic system by TaqMan Low Density Array (TLDA) showed that while both types of phagocytes expressed full repertoire of the phosphatidylserine recognition and signaling pathway, macrophages could evolve during engulfment of *de novo* autophagic cells the potential of calreticulin-mediated recognition, tethering, tickling and engulfment processes. Different sets of genes (e.g. the phagocytosis receptors for asialoglycoprotein and oxidized LDL, the bridging molecule PTX3, the engulfment molecules GULP1 and RAC1) were

upregulated in macrophages engulfing *de novo* autophagic as compared to anoikis-derived autophagic dying cells (e.g. the bridging molecule MFGE8, the engulfment gene RAC1). In MCF-7 cells, *de novo* autophagic cells induced the oxidized LDL receptor and ELMO1 engulfment gene, while anoikic-autophagic ones lead to elevated AXL receptor.

LPS-induced production of pro-inflammatory cytokines in macrophages could be prevented by the dying autophagic cells similarly to anoikic-autophagic and apoptotic cells. However, we also observed that phagocytosis of cells dying through autophagy leads to a pro-inflammatory response in macrophages characterized by the induction and secretion of IL-1β as well as IL-6, TNFα, IL-8 and the anti-inflammatory cytokine IL-10. The IL-1β secretion could be inhibited by preventing autophagy with 3-MA or blocking caspase-1 activation. The results suggest that inside macrophages cells dying through autophagy can activate NLR family protein(s) during the phagocytosis process.

Our data show that cells dying through autophagy and those committing anoikis with autophagy may engage overlapping but distinct sets of clearance mechanisms in professional and non-professional phagocytes. Better understanding of the molecular mechanism of these phenomena may lead to more rational design of autophagy-based therapeutic interventions.