Editorial

Engulfing by lipids: a matter of taste?

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Getting rid of dead corpses is a tantalising effort. A continuous and conspicuous cell turnover is imposed by the apoptotic clock and challenges phagocytes both during embryonic development and in adult life.^{1,2} Macrophages, by far the most zealous phagocytes are typically in charge of the clearance of dying cells in vertebrates.³ This is not the case in the nematode *C. elegans*, where professional phagocytes do not exist and engulfment is the task of randomly selected bystander somatic cells.^{4,5} However, also in mammals, amateur phagocytes can substitute for professionals. The long term outcome of an amateur's take over has not been established so far although it is likely that deleterious immunological consequences may arise from the elusion of the 'safe clearance' orchestrated by macrophages.^{6–8} The study of perturbed engulfment patterns and their consequences in 'in vivo' models such as the macrophage less PU.1 null embryos^{9–12} or mice lacking engulfment receptors^{13–19} will be precious to elucidate the pathophysiological impact of an impaired clearance of cellular debris generated by the apoptotic program.

Phosphatidylserine driven recognition and professionals

The recognition and ingestion of apoptotic preys in mammals appears to be a complex process only hazily understood so far. Experimental evidence, mainly based on *in vitro* inhibitory assays, has implicated a number of surface receptors on the phagocyte side. Among them are lectins, scavenger class A receptors (SR-A), the LPS receptor CD14, the integrins $\alpha V \beta 3$ or $\beta 5$ acting alone or in concert with CD36, a scavenger receptor of B class (see for review^{6,20}). Interestingly, functional records concerning all these molecules pre-existed their implication in engulfment^{21–23} and actually in this precise framework their molecular interaction with the apoptotic surface is not at all clear as yet. An exquisite hallmark of engulfment, defined herein as the process of phagocytosis of cells committed to die, is a lipid-mediated recognition system, concerning concomitantly the phagocyte and the prey^{24–26} which is extensively discussed by Schlegel²⁷ and Fadok²⁸ in this issue. The exposure of increased amounts of anionic phospholipids, namely phosphatidylserine on the outer surface of the apoptotic prey was recognised several years ago as one of the earliest signs of membrane modification on committed cells. How the phagocytes instantly sense these modifications, remains yet to be established. In fact, a longstanding search for a specific phosphatidylserine receptor (PSR) has only recently led to the

identification of a novel surface molecule highly conserved across evolution.²⁹ This PSR is expressed by macrophage-like cells and fibroblasts and recognises stereospecifically phosphatidylserine, in contrast to previous candidate receptors, which failed to discriminate among phospholipid species. It is however too early to know how this molecule acts on the cell surface to trigger engulfment. In this context it is interesting to recall that, as elucidated by Tepper and co-workers,³⁰ the exofacial flopping of phosphatidylserine, a phospholipid species usually segregated to the inner membrane leaflet, is only apparently a minor event but actually perturbs dramatically the interactions among the different lipids at the membrane and transiently scrambles the overall lipid arrangement across the bilayer. This dramatic membrane destabilisation may facilitate the profound structural and morphological change consequent to apoptotic commitment.

The ability to expose phosphatidylserine is, in addition, a competence factor for an efficient phagocyte, which like many activated or fusogenic cell types, shows an unscrupulous asymmetric distribution of phospholipids at the membrane in the absence of any apoptotic commitment. This mobility is determinant for engulfment since its inhibition by pharmacological interference pre-empted an optimal phagocytosis.²⁵ ABC1³¹ the putative mammalian homologue of *C. elegans* engulfment gene *ced-7*³² is a key player there. Indeed, ABC1 is essential for membrane cholesterol homeostasis, as demonstrated by studies on Tangier patients^{33–35} and ABC1 null mice,^{13,36,37} and this stems from the ABC1 involvement in the dynamic control of transbilayer lipid turnover. Given that the loss of ABC1 function in mice leads to suboptimal engulfment by professional macrophages,¹³ we may assume that the biophysical properties of the membrane, as dictated by the arrangement of lipids across the bilayer, is crucial for initiating engulfment. This membrane-priming role for the ABC transporter is also supported by the work of R.H. Horvitz and collaborators³⁸ in the nematode model system. They evidence in fact a crucial role for *ced-7* in the membrane redistribution of the *ced-1*³⁸ receptor around the corpse to be engulfed.

The engulfment however can take place in the absence of the phosphatidylserine dependent pathway. Activated macrophages, i.e. professionals, are the only cells that, even if equipped with the complete set of engulfment receptors, select systematically the phosphatidylserine-driven recognition/engulfment pathway. Amateurs, in contrast, make most frequently use of integrins ($\alpha V \beta 3$ or $\beta 5$) as trigger molecules.^{39–45} Dendritic cells, whose profes-

sional status as phagocyte is controversial, ingest slowly apoptotic cells (hours as opposed to minutes), prefer late apoptotic preys, neglect, as mentioned above, the phosphatidylserine-mediated pathway and ingest via an integrin-mediated system.^{46–48} Mesangial cells or retinal pigment cells when forced to ingest apoptotic preys behave similarly. Interestingly the surface occupation of integrins has been recently linked to the sequential membrane recruitment and activation of crkII/DOCK 180 and Rac1 in a signalling cascade activating ingestion.⁴⁰ The crkII/DOCK 180/Rac1 pathway parallels perfectly that of the ancestors in *C. elegans*, respectively ced-2, ced-5 and ced-10.⁴⁹

Whether the cell-specific use of receptors in mammals discloses major differences in further handling of the prey is to date an open and legitimate question. Professionals are faster eaters than amateurs and the pace of ingestion is likely to be crucial *in vivo*. It would not be that surprising if further investigation highlight a direct correlation between swiftness of ingestion and the identity of the upstream receptor. It is known that the triggering receptor can dictate the mechanics of ingestion in the case of phagocytosis of opsonised preys. Distinct morphological features follow in fact the engagement of either Fc receptors or complement receptors (CR3/CR4).^{50,51} If we extrapolate this to engulfment and also consider a differential usage of receptors by professionals and amateurs, we may envision multiple facets of the engulfment as a phagocytic event. This speculation has recently found support in a comparative study of engulfment dynamics and morphology by professionals (microglia) and non-professionals

(BHK and lens epithelial cells).⁵² This work highlights major differences in the behaviour of the two classes. The highly motile professionals ingest early apoptotic cells quickly after the first contact whereas the sessile non-professionals considerably delay ingestion after recognition. In addition, the presence of a membrane signal emerging only on ripe apoptotic cells appears to be required for initiating engulfment by non-professionals. As for now however it is not known whether and how these dramatic differences are related to the nature of the recognising receptor.

The redundancy of engulfment receptors in mammals

As previously underlined, a specialised high affinity engulfment receptor does not exist; the situation is essentially the opposite, and '*in vitro*' even the combined inhibition of several receptors fails to completely prevent the clearance of apoptotic cells by phagocytes. This points out receptor redundancy as a major characteristic of the process, without providing any clue for the underlying rationale.

Redundancy of low affinity receptors may well act as a safety device; it reduces escape possibilities for the prey and multiplies the chances of an optimal clearance (Figure 1a). Translated in cell biology terms, this would mean that each engulfment receptor can trigger the ingestion of an apoptotic particle via a receptor-specific entry sites into a common effector cascade. Alternatively redundancy may

Figure 1 The redundancy of engulfment receptors and phagosome formation. (a) Each receptor is able and sufficient to trigger phagosome formation. The composition of phagosomes and the morphology of ingestion may thus vary accordingly to the triggering molecule. (b) Zippering mediated by recruitment of multiple receptors around an apoptotic corpse leads to the formation of phagosomes of similar membrane composition. (c) Engulfment starts at sites with peculiar membrane properties, where the engulfment receptors cluster, possibly with the help of ABC1. Symbols schematise the different receptor molecules. Polymerised actin is shown. The scheme does not account for morphological differences between ingestion undertaken by professionals or amateur phagocytes. We may however speculate that a situation type C or A, respectively, occurs

be necessary to reinforce tethering around the prey via the local recruitment of multiple low potency molecules (Figure 1b). In this modified version of the classical zipper mechanism⁵³ reinforcement does not come from the multiplication of the same receptor/ligand interaction but rather from the concerted action of several distinct receptor/ligand pairs, all concomitantly activating a common signalling cascade. Finally, redundancy could barely reflect topological constraints (Figure 1c). Indeed preferential start sites for engulfment may exist on the cell surface as specialised membrane domains. It is here relevant to underline that most if not all the engulfment receptors are enriched in detergent insoluble membrane domains or rafts.^{54–57} Considering the recent developments indicating the existence of a multiplicity of rafts with diverse biochemical features,^{58–60} it would be of interest to establish whether the plethora of engulfment receptors is clustered in domains of the same size and floating characteristics on a macrophage which lacks canonical caveolae. This view brings us back to the fluidity of the membrane, or more generally, to its biophysical properties as determinants for the efficiency of engulfment. Local lipid homeostasis is the critical determinant of the biophysical characteristics of a membrane domain and, intriguingly, lipid metabolism or cellular cholesterol trafficking are key words frequently associated to engulfment. Class A and B scavenger receptor mediate cholesterol influx from high density lipoproteins,^{61–64} whereas ABC1 function controls the Apolipoprotein A-I mediated extraction from cell membranes of phospholipids and cholesterol.⁶⁵ Similarly depletion/repletion of plasma membrane cholesterol is known to affect the lateral diffusion parameters of lipid species^{66–69} and is thus likely to exert a dramatic impact on events requiring impressive membrane turnover and plasticity like engulfment.^{70,71} Disclosing the details of the relationship between lipid dynamics and the safe elimination of dying cells may well become the challenge of the new millennium.

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