Phospholipases and Phagocytosis

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Introduction

Phagocytosis, the process by which particulate matter is taken up by cells, is characteristic of many cell types including the pigmented epithelium of the eye, Langerhans cells in the skin, the microglia in the brain, and Kupffer cells in the liver. Of particular interest clinically are the "professional" phagocytes: neutrophils, monocytes, and tissue macrophages. Their ability to phagocytose foreign pathogens, senescent cells, and cellular debris is essential for homeostasis. Dendritic cells should also be mentioned as they act as sentries to take up bacteria in the skin and at mucosal surfaces. However, their primary function is to present antigens and the majority of studies address this function. Thus, we will focus on macrophages and neutrophils in this review.

Phagocytosis occurs through a variety of receptors, including those for immunoglobulin G (IgG, FcyR), complement (CR1, CR3), mannose (MR), and β-glucan (dectin-1), and the scavenger receptor that facilitates uptake of oxidized lipids and advanced glycation end products. Although much is known about the structure of phagocytic receptors and their ligands, the signaling events that accompany ingestion are the subject of ongoing investigation with exciting advances being reported on a regular basis. Recent advances in molecular biology and imaging have provided novel tools for studying phospholipids and phospholipase products in phagocytosis. This review will summarize our current understanding of the role of phospholipases in phagocytosis. As much of our knowledge of this topic is derived from macrophage models of IgG-mediated uptake, the bulk of this review will draw on that data. Relevant studies involving other targets will be included where information is available.

Phagocytosis

The initiating event in phagocytosis is the binding of phagocyte cell surface receptors to ligands on the surface of the particle to be ingested. These ligands may be intrinsic to the target, i.e., the carbohydrate coat of yeast or pathogen-associated molecular patterns (PAMPs) or may be an opsonin, a molecule such as complement or IgG that coat particles, targeting them for elimination by phagocytosis. Following target binding, receptors are recruited from outside the initial region of contact and, by sequential receptor-ligand interactions, guide actin-based pseudopods around the particle (Fig. 1). Although all phagocytosis requires actin polymerization, in some cases the phagocyte extends pseudopods to engulf the particle while in others the particle appears to sink into the cell. These morphologies are exemplified by IgG and complement opsonins, respectively. The resulting phagosome has a tightly apposed membrane that conforms to the shape of the target and undergoes a series of fusion events that change its
Figure 1. Overview of phagocytosis. A) Particles bind to cell surface receptors, cross-linking them and initiating signaling networks. Ruffling results in formation of endocytic vesicles. B) Sequential receptor-ligand interactions guide actin-based pseudopods around the particle. Insertion of VAMP3 positive vesicles into the forming phagosome provide membrane for pseudopod extension. Note that pseudopods extend beyond the membrane surface for IgG-mediated phagocytosis but complement-opsonized particles sink into the cell. C) Pseudopods fuse at the apex of the particle, enclosing the target in a phagosome (D).

composition. It transiently acquires markers of early (Rab 5, early endosome antigen 1, transferrin receptor) and late endosomes (Rab7 and mannose 6 phosphate receptor) before maturing into a phagosomes characterized by low pH (4.5-5.5) and the presence of lysosomal enzymes. It should be noted that some bacteria subvert this generic sequence to survive and grow within the macrophage. Indeed, much of our information on phagosomal maturation has been derived from studies of macrophage pathogens.

The type of receptor engaged will dictate subsequent signaling events. For example, IgG-mediated phagocytosis stimulates an inflammatory response, including activation of the respiratory burst for microbicidal killing and upregulation of inflammatory genes, including TNF-α, IL-1, and IL-12. In contrast, complement-mediated uptake is relatively silent, eliciting no burst nor activating pro-inflammatory genes. Some pathogenic bacteria, most notably Mycobacterium tuberculosis, exploit this pathway, entering macrophages via complement receptors and proliferating within this relatively safe environment. The ability of pathogens to disrupt the normal delivery of phagocytosed material to lysosomes is a large and diverse area of research and the reader is directed to several recent reviews.

The IgG-dependent signal transduction cascade can be broadly divided into three stages, (1) target binding to the phagocyte cell surface with subsequent FcγR cross-linking, (2) proximal signaling events including tyrosine phosphorylation of the FcγR and (3) more distal events involving the activation of protein kinase C, mitogen-activated protein kinase (ERK), and transcription factors. Due to space considerations, this review focuses on the involvement of phospholipases in signaling events downstream of FcγR phosphorylation. However, excellent reviews on phagocytic signaling are available.

**Phospholipases**

Phospholipases are enzymes that hydrolyze phospholipids, generating bioactive products that act as membrane detergents, enzyme cofactors, or second messengers. Phospholipids are built up on a glycerol or sphingosine backbone (Fig. 2). In higher eukaryotes, saturated and