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The ER phagosome connection in the era of membrane contact sites☆

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ABSTRACT

Phagocytosis is an essential mechanism through which innate immune cells ingest foreign material that is either destroyed or used to generate and present antigens and initiate adaptive immune responses. While a role for the ER during phagosome biogenesis has been recognized, whether fusion with ER cisternae or vesicular derivatives occurs has been the source of much contention. Membrane contact sites (MCS) are tight appositions between ER membranes and various organelles that coordinate multiple functions including localized signalling, lipid transfer and trafficking. The discovery that MCS form between the ER and phagosomes now begs the question of whether MCS play a role in connecting the ER to phagosomes under different contexts. In this review, we consider the implications of MCS between the ER and phagosomes during cross-presentation and infection with intracellular pathogens. We also discuss the similarities between these contacts and those between the ER and plasma membrane and acidic organelles such as endosomes and lysosomes. This article is part of a Special Issue entitled: Membrane Contact Sites edited by Christian Ungermann and Benoit Kornmann.

1. Introduction

Phagocytosis, defined as the engulfment of particles larger than 0.5 μm in diameter into a novel membrane-enclosed vacuole called phagosome, is a mechanisms vital for immune defence. Although phagocytosis, aptly derived from the Latin words for “cell eating”, evolved as means for single-celled organisms to acquire nutrients, and while all cells retain a vestigial capacity to phagocytose, in mammals only a subset of cells coined “professional phagocytes” perform this function to any meaningful extent. These include specialized tissue-specific cells such as brain-resident microglia, bone-resorptive osteoclasts and retinal pigment epithelia, but the best studied are immune cells of the myeloid lineage: neutrophils, macrophages and dendritic cells. Neutrophils and macrophages are primarily geared towards the destruction of the ingested material which may include bacteria, but they can also ingest particulate pollutants as well as apoptotic cells, the latter function being paramount both during organism development as well as for tissue homeostasis. In contrast, dendritic cells (and also to some extent macrophages) are specialized to retain elements of the ingested material to be used for the process of antigen presentation to T lymphocytes, which, depending on the context, will either help maintain immune tolerance to self-antigens, or initiate antigen-specific immune responses. Thus phagocytosis plays a central role in both innate and adaptive immunity.

Since the initial observations in 1876 by William Osler and in 1880 by Elie Metchnikov, a great deal has been learnt about the mechanisms that allow phagocytic cells to generate a novel organelle destined to become a degradative compartment. From receptor engagement and ensuing signalling cascades that trigger cytoskeletal rearrangements that lead to engulfment, to a maturation process involving fission and fusion events that endow phagosomes with oxidative and lytic properties that allow the breakdown of the contained material, many of the numerous molecular mechanisms have been uncovered (see [1,2] for recent reviews), yet much is still unknown. When considering the similarities that phagosome maturation bears with the endocytic pathway, it is perhaps natural that many studies have focused on fusion events with different vesicular carriers like early, late, recycling, or tubular endosomes, lysosomes, or specialized secretory granules. However, and perhaps unexpectedly, a long history of research has also suggested that the endoplasmic reticulum (ER) plays a critical role, yet in ways that are less clear. For example, several intracellular pathogens that subvert the phagocytic process and create their own protected niche inside cells engage the ER or transform their parasitic vacuole into an ER-like structure [3]. Furthermore, in dendritic cells a subtype of antigen presentation termed cross-presentation requires a cohort of ER proteins [4]. Over the past decade an emerging field in ER biology has exploded with the study of membrane contact sites (MCS): tight 10–30 nm contacts between the ER and various organelles that are mediated by intermembrane protein tethers and play diverse functions such as localized signalling events, lipid transfer as well as the control

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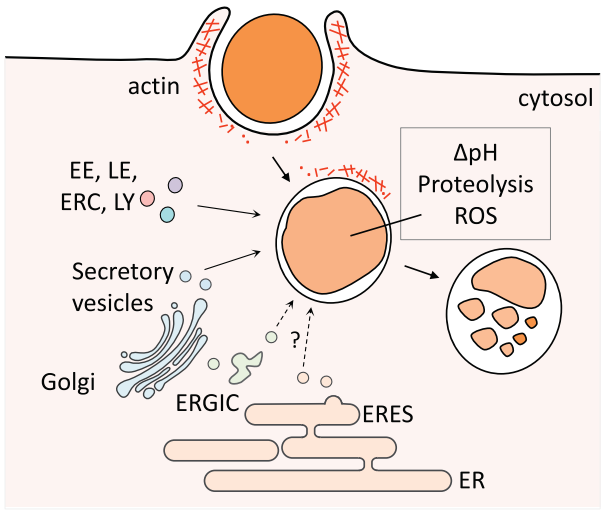
of membrane trafficking [5,6]. In the present review the ER-phagosome connection is explored through the re-examination of past research that link ER function to phagocytosis in immune cells in light of recent findings revealing the existence of ER-phagosome membrane contact sites. A summary of the recent studies demonstrating ER-phagosome MCS in the context of calcium signalling, prefaced by a short recapitulation of the major stages of phagocytosis, will be followed by an examination of the research that led to the ER-phagosome fusion hypothesis, its relevance to cross-presentation, and the recent developments that have greatly progressed the field. We will then review literature focused on two different intracellular pathogens that solicit the ER, *Toxoplasma gondii* and *Legionella pneumophilia* and discuss their implications. Finally, potential similarities and differences with ER-plasma membrane, ER-endosome and ER-lysosome contact sites will be briefly discussed, and how future studies in these fields may be mutually beneficial in the advancement of understanding of cellular physiology in health and disease.

2. The phagocytic process in a nutshell

Phagocytosis begins when a phagocytic cell touches its prey. While phagocytosis may be receptor independent, in immune cells phagocytosis if often a receptor-mediated event. Several different families of phagocytic receptors exist that bind molecules generated by immune system such as complement or immunoglobulins, collectively called opsonins, which are recognized by the complement (CR) and Fc receptor (FcR) families, respectively [1,7]. Alternatively, other receptors, for instance the beta-glucan receptor Dectin-1 and the mannose receptor CD206, may directly interact with microbial ligands [1]. Receptor engagement and clustering initiates intracellular signalling cascades that often include Src and Syk phosphorylation as well as downstream phospholipase-C or –D (PLC or PLD)-driven calcium signals [8]. These signals then induce cytoskeletal rearrangements that in turn drive either the protrusion of actin-rich pseudopods that closely zipper around the target, or that lead to an invagination of the plasma membrane (PM), akin to endocytosis, into what is termed the phagocytic cup [1,7]. In both cases, the connection to the plasma membrane is severed when the target becomes completely surrounded and the novel organelle is officially born. In fact, a membrane barrier at the tips of pseudopods allows the sorting of both lipids and proteins into the nascent organelle well before cup closure, and thus, while the phagosomal membrane originates from and initially shares several features with the PM, it is a distinct structure from the earliest time points [7]. Also initiating potentially just before or just after cup closure is the shedding of the actin coat and fusion events with endomembranes. What happens subsequently is largely dependent on cell type (Fig. 1).

Macrophages are by far the best studied phagocytes, and phagosomal maturation in macrophages is the most similar to its ancestral form, closely resembling that observed in phagocytic single-celled organisms such the amoeba *Dictyostelium discoideum* [9]. Shortly after cup closure, the early delivery of vacuolar-ATPase (V-ATPase) subunits from tubular endosomes incites a rapid and intense acidification that reaches a pH of < 5.0 within the first 10 min [10]. Active lipid remodelling is observed, characterized by consecutive peaks of different phospholipid species appearing and receding as the phagosome matures [11]. Concomitantly, and reminiscent of endosomal maturation, a sequential series of fusion events with early and late endosomes, followed by lysosomes, occurs with the successive acquisition of first Rab5 and then Rab7, culminating with the acquisition of hydrolytic enzymes that lead to the degradation of the ingested material. Simultaneously, fission events maintain the volume of the phagosome relatively constant [1,2]. It is then presumed that the material is decomposed into its basic constituents, and the lipids, proteins and carbohydrates are recycled by the cell or extruded into the extracellular environment to be either recycled or excreted from the body [2]. Around 15 years ago studies inspired by biochemical data showing an early enrichment of ER



B

Maturation Step	MΦ	NΦ	DCs
Ingest. /Actin shedding	+	+	+
Endomembrane Fusion	EE, LE, LY ERGIC?	1°, 2°, 3° granules EE, LE, LY	EE, LE, LY Rab27⁺ LY, ERC, ERGIC, ERES ..?
Proteolysis	+++	+++	++
ROS	+	+++	++
pH	4.0-5.0	7.0-9.0	4.0-8.0?

Fig. 1. Phagosomal maturation.A. Actin-driven remodelling of the plasma membrane leads to the phagocytic engulfment of large particles into a newly formed membrane-bound organelle called phagosome. Once ingested, phagosomes undergo maturation that involves actin shedding, fusion with a variety of endomembranes, pH changes, ROS production and the acquisition of hydrolytic properties. Cell-type dependent differences in phagosomal maturation in macrophages (MΦ), neutrophils (NΦ) and dendritic cells (DCs) are summarized in B. Calcium-dependent mechanisms are highlighted by red boxes, partial calcium-dependence is indicated by boxes with dotted lines. EE = early endosomes, LE = late endosomes, LY = lysosomes, ERGIC = ER-to-Golgi intermediate compartment, ERES = ER exit sites, Rab27$^+$ LY = Rab27-positive lysosome-like vesicles, ERC = endocytic recycling compartment, 1° = primary, 2° = secondary, 3° = tertiary.

proteins on isolated phagosomes, and electron microscopy (EM) images displaying a close association of phagosomes with the ER, led to the provocative hypothesis that fusion of macrophage phagosomes with the ER could provide growing phagosomes with an additional membrane source [12]. A few years later this hypothesis was strongly contested [13] and the controversy is discussed in further detail below.

In neutrophils, phagocytosis is marked by an early and profound release of reactive oxygen species (ROS), termed the oxidative burst, mediated by the phagocyte-specific NADPH oxidase complex containing NOX2, which is highly expressed in these cells and is activated by phosphokinase C phosphorylation as well as calcium signalling [14]. ROS production, which may last up to 30 min, has been linked to a delayed delivery of the V-ATPase in a phagosome-specific manner, and is responsible for an initial alkalinisation of neutrophil phagosomes, which acidify only at later time points [14–17]. Additionally, neutrophils harbour a set of specialized secretory granules, named primary (or azurophilic), secondary (or specific) and tertiary (or gelatinase) granules, that each contain an assortment of hydrolytic and antimicrobial enzymes, and that similar to endosomes and lysosomes, also fuse