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REVIEW
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The Roles of Phospholipase A_2 in Phagocytes

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Phagocytic cells, such as macrophages, neutrophils, and dendritic cells, ingest particles larger than about 0.5 μM and thereby clear microbial pathogens and malignant cells from the body. These phagocytic cargoes are proteolytically degraded within the lumen of phagosomes, and peptides derived from them are presented on Major Histocompatibility Complexes (MHC) for the activation of T cells. Mammalian PLA_2 isozymes belong to a large family of enzymes that cleave phospholipids at the second position of the glycerol backbone, releasing a free fatty acid and a lysolipid moiety. In human macrophages, at least 15 different PLA_2 forms are expressed, and expression of many of these is dependent on pathogenic stimulation. Intriguing questions are why so many PLA_2 forms are expressed in macrophages, and what are the functional consequences of their altered gene expression after encountering pathogenic stimuli. In this review, we discuss the evidence of the differential roles of different forms of PLA_2 in phagocytic immune cells. These roles include: lipid signaling for immune cell activation, initial phagocytic particle uptake, microbial action for the killing and degradation of ingested microbes, and the repair of membranes induced by oxygen radicals. We also discuss the roles of PLA_2 in the subsequent digestion of ingested phagocytic cargoes for antigen presentation to T cells.

Keywords: phagocytosis, PLA_2, pathogens, macrophages, trafficking

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INTRODUCTION

A role for phospholipase A_2 (PLA_2) in phagocytic immune cells has been suggested since the 1980s (Waite et al., 1979; Scott et al., 1980). These phagocytic cells, such as macrophages, neutrophils, and dendritic cells, ingest particles larger than about 0.5 μM and thereby clear microbial pathogens and malignant cells from the body (Uribe-Querol and Rosales, 2020). Particularly macrophages and dendritic cells also present peptides derived from these phagocytosed antigens on major histocompatibility complex (MHC) types I and II to activate cytolytic and helper T cells, respectively, while lipids can be presented on CD1 (Burgdorf and Kurts, 2008). The process of phagocytosis and the subsequent processing of the ingested antigen are essential processes that contribute to both innate and adaptive immunity. As we will discuss in this review, evidence suggests that PLA_2 is involved in all stages of pathogen encounter, from the initial signaling, to the uptake, degradation and presentation of the antigen.

The nomenclature of PLA_2 is very complex and disordered as it follows a chronology reflecting their time-line of discovery (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). Mammalian PLA_2 isozymes belong to a large family, which have been assigned into groups I to

XVI based on their primary amino acid sequence (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). Based on their subcellular localization and Ca^{2+} requirement, these groups can be further categorized into six types called sPLA$_2$, iPLA$_2$, cPLA$_2$, LPLA$_2$, aiPLA$_2$, and PAF-AH, and all these types are further categorized into subtypes (**Table 1**) (i) sPLA$_2$ are Ca^{2+}-dependent secretory PLA$_2$ forms found in secretions, such as a tears, plasma and pancreatic juice (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). These enzymes belong to groups I, II, III, V, IX, X, XI, XII, XIII, and XIV (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). Within the sPLA$_2$ subgroup, a capital English letter indicates the subtype of enzymes. For example, sPLA$_2$-IIA is present in synovial fluid, while sPLA$_2$-IID is present in pancreas and spleen (Dennis et al., 2011). (ii) cPLA$_2$ are Ca^{2+}-dependent cytosolic enzymes which belong to group IV, and its subtypes are cPLA$_2$α, -β, -γ, -δ, -ε, and -ζ (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). (iii) iPLA$_2$ are Ca^{2+}-independent cytosolic forms which belong to group VI, and its subtypes are iPLA$_2$-β, -γ, -δ, -ε, -ζ, and -η (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). Within the cPLA$_2$ and iPLA$_2$ types, the different subtypes are mostly indicated by Greek letters (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). However, this nomenclature is not always consistently used and for example cPLA$_2$-IVα and iPLA$_2$-VIβ are also known as cPLA$_2$-IVA and iPLA$_2$-VIA. (iv) LPLA$_2$ is a lysosomal PLA$_2$ which belongs to group XV (Shayman et al., 2011; Shayman and Tesmer, 2019). (v) aiPLA$_2$ are acidic Ca^{2+}-independent PLA$_2$ forms which belong to group XVI, and are better known as peroxisomal PLA$_2$ (Sorokina et al., 2009; Fisher, 2018). (vi) platelet-activating factor hydrolases (PAFAH) belong to groups VIIA (also known as lipoprotein-associated PLA$_2$; Lp-PLA$_2$), VIIB and VIII (Stafforini, 2009; Dennis et al., 2011).

In human macrophages, 15 PLA$_2$ forms are found at the transcript level, including sPLA$_2$ (sPLA$_2$-IID, -V, and -XIIA), cPLA$_2$ (cPLA$_2$-IV α, β, and γ), iPLA$_2$ (iPLA$_2$-VI β, γ, δ, ε, ζ, and η), LPLA$_2$, LpPLA$_2$, and aiPLA$_2$ (Elstad et al., 1989; Rubio et al., 2015). Of these forms, LPLA$_2$ and LpPLA$_2$ carry a signal peptide required for cotranslational insertion into the ER and subsequent transport through the Golgi where they undergo *N*-glycosylation, while others are either cytosolic or membrane bound proteins (Dennis et al., 2011; Murakami et al., 2015; Ramanadham et al., 2015). Analysis of published gene expression data of blood monocyte-derived dendritic cells from 38 healthy individuals (Lee et al., 2014) revealed that the expression of some of these PLA$_2$ forms is dependent on pathogenic stimulation (**Figure 1**). For example, the expression of cPLA$_2$-IVα and -IVγ are upregulated, while sPLA$_2$-XIIA and PAFAHII are downregulated upon stimulation of dendritic cells with lipopolysaccharide (LPS) or influenza virus. This information is important, because it shows that many forms of PLA$_2$ undergo substantial up- or down-regulation upon pathogenic stimulation, suggesting that they have roles in the immune function of the phagocytes.

Indeed, although the roles of most PLA$_2$ forms in macrophages and dendritic cells are largely unknown, literature shows that various PLA$_2$ forms function during all the events that occur following the encounter of a pathogen: (i) the immune signaling by lipids carried out by cPLA$_2$α, (ii) the focal exocytosis to support phagosome formation by iPLA$_2$β, (iii) the killing of the microbe by sPLA$_2$-V, and LPLA$_2$ forms, and (iv) the repair of damaged phagosomal membranes inflicted by reactive oxygen species by LPLA$_2$ and aiPLA$_2$. In this review, we aim is to provide an overview of these functions of PLA$_2$ forms.

LIPID SIGNALING BY cPLA$_2$α

Phospholipase A2 enzymes hydrolyze membrane phospholipids that are composed of a glycerol backbone esterified to two hydrophobic fatty acids tails at the *sn-* (stereospecifically numbered) 1 and 2 positions, and a hydrophilic head group at the *sn*-3 position (**Figure 2**). Phospholipids with the head groups choline, ethanolamine, serine, and inositol form the main classes of phospholipids and are called phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI), respectively. The catalytic action of PLA$_2$ releases the free fatty acid from the *sn*-2 position, such as arachidonic acid (ARA; 20:4), docosahexaenoic acid (C22:6), oleic acid (C18:1), while lysophospholipids, such as lyso-phosphatidyl-choline/-ethanolamine/-inositol (LPC/LPE/LPI), remain esterified in the membrane.

Both the lyso-phospholipids and the free fatty acids produced by PLA$_2$ can be bioactive molecules and/or form precursors for the generation of bioactive lipid hormones (Brash, 2001; Carneiro et al., 2013). PLA$_2$ activity is especially important for the generation of a class of lipid hormones called eicosanoids. Eicosanoids are a broad family of oxygenated lipid compounds that include prostaglandins, thromboxanes, and leukotrienes. Eicosanoids are generated via non-enzymatic oxidation of ARA or by the action of enzymes, such as cyclooxygenase (COX), lipooxygenase (LOX), and cytochrome P450 (CYP) (Dennis and Norris, 2015; Gil-de-Gómez et al., 2020). Eicosanoids exert immunomodulatory functions and, depending on the species of eicosanoids, can have pro- or anti-inflammatory effects (Dennis and Norris, 2015). Upon encountering a pathogen, immune phagocytes produce elevated levels of eicosanoids and an increased amount of eicosanoids in circulation is considered a hallmark of inflammation (Dennis and Norris, 2015). Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, indomethacin, and asprin, target COX and PLA$_2$ to alleviate pain, redness, and swelling associated with inflammation (Singh et al., 2005; Dennis and Norris, 2015).

Mammalian cells are rich in ARA, but this is mostly incorporated in phospholipids by esterification to the glycerol backbone at the *sn*-2 position. For example, human cultured platelets contain ∼30 μg esterified arachidonate per 10^9 cells, which approximately corresponds to 5 mM, while free ARA is only present at ∼3 pmol per 10^6 cells, corresponding to about 0.5–1 μM (Brash, 2001). However, the concentration of free ARA significantly increases upon pathogenic stimulation. For example, plasma concentrations in mice increase from ∼1 to ∼1.5 mM upon infection with *Salmonella pneumonia* (Eijkelkamp et al., 2018). Similarly, concentrations of ARA in the whole blood of sepsis patients increase significantly from ∼200 to ∼250 ng/ml

TABLE 1 | Phospholipase A_2 (PLA_2) family and role of specific forms discussed in this review.

Type	Group	Sub types	Other name	Molecular weight (kDa)	Catalytic residue	References	PLA_2 forms discussed
$cPLA_2$	IV	α (A) β (B) γ (C) δ (D) ε (E) ζ (F)		60–114	Ser/asp	Dennis et al., 2011; Leslie, 2015	$cPLA_2$-IVα in lipid signaling
$iPLA_2$	VI	β (A) γ (B) δ (C) ε (D) ζ (E) η (F)	PNPLA9 PNPLA8 PNPLA6 PNPLA3 PNPLA2 PNPLA4	27–146	Ser/Asp	Dennis et al., 2011; Ramanadham et al., 2015	$iPLA_2$-VIβ in supporting focal exocytosis at the phagocytic cup
$sPLA_2$	I II III V IX X XI XII XIII XIV	A, B A, B, C, D, E, F A, B A, B		10–19	His/Asp	Dennis et al., 2011; Murakami et al., 2015; Murakami, 2017	$sPLA_2$-IIA, V, X, XII as antimicrobial forms at the phagocytic cup and within closed phagosomes
$LPLA_2$	XV			45	Ser/His/Asp	Dennis et al., 2011; Fisher, 2018	$LPLA_2$ having bacteriocidal activity at the phagocytic cup and within phagolysosomes Also, its membrane repair mechanism
$aiPLA_2$	XVI		Peroxiredoxin 6	25	Ser/His/Asp	Fisher, 2018	$aiPLA_2$regulating NOX2 assembly, and its membrane repair mechanism
PAF-AH	VIIA VIIB VIII		Lp-PLA_2, PLA_2VII PAF-AH II PAF-AH I (PAFAH1B1 PAFAH1B2 PAFAH1B3)	26–45	Ser/His/Asp	Dennis et al., 2011; Karasawa and Inoue, 2015	Not discussed as relevant information was not found

(Bruegel et al., 2012). Phagocytosis also triggers ARA release *in vitro*, as shown for macrophages labeled with radioisotope labeled ARA (Waite et al., 1979; Gil-de-Gómez et al., 2020). This has been observed for a wide range of phagocytic cargoes, such as serum-opsonized zymosan, native zymosan, and live pathogenic bacteria (Adolph et al., 2012; Gil-de-Gómez et al., 2020). These studies indicate that pathogenic encounter results in an increased PLA_2 activity resulting in the production of free ARA. The elevated ARA levels, in turn, promote the production of eicosanoids for inflammatory signaling. As will be discussed in more detail below, the elevated ARA levels might also facilitate the phagocytic process because supplementation of ARA to macrophage-like cell lines RAW264.7 and THP-1 accelerated phagocytosis, and potentiated their anti-microbial ability against intracellular microbes (Adolph et al., 2012; Eijkelkamp et al., 2018).

The most important PLA_2 form involved in eicosanoid signaling is $cPLA_2\alpha$. $cPLA_2$ isozymes are characterized by an N-terminal CalB domain for Ca^{2+} binding, an active site Ser-Asp dyad for lipid hydrolysis, and a C-terminal phospholipid-binding domain for interacting with membranes (Leslie, 2015). $cPLA_2$ forms require mM concentrations of free Ca^{2+} for calcium-dependent membrane binding and activation (Dennis et al., 2011; Leslie, 2015). Members of this family include $cPLA_2\alpha$, -β, -γ, -δ, -ε, and -ζ, which vary in their molecular weight, tissue expression, and subcellular localization (Dennis et al., 2011; Leslie, 2015). These members have about 30% sequence homology and although they have overlapping activities, their functions are largely non-redundant. $cPLA_2\alpha$ is the only PLA_2 form that contains mitogen-activated protein kinase (MAPK) phosphorylation sites (S^{505} and S^{727}) (Murakami, 2017). Thereby, infection-induced activation of the Ras and MAPK pathways results in the phosphorylation of $cPLA_2\alpha$ (Zhou et al., 2003; Su et al., 2004). This phosphorylation results in increased activation of $cPLA_2\alpha$, which in turn leads to increased production of ARA (Dieter et al., 2002). Infection also induces $cPLA_2\alpha$ transcription via transcriptional factors, such as nuclear factor κB (NF-κB), Krüppel-like factor, hypoxia-inducible factor (Hif), specificity protein 1 (Sp1), and c-Jun, that are well known to regulate immune cell activation (Su et al., 2004; Dennis and Norris, 2015;

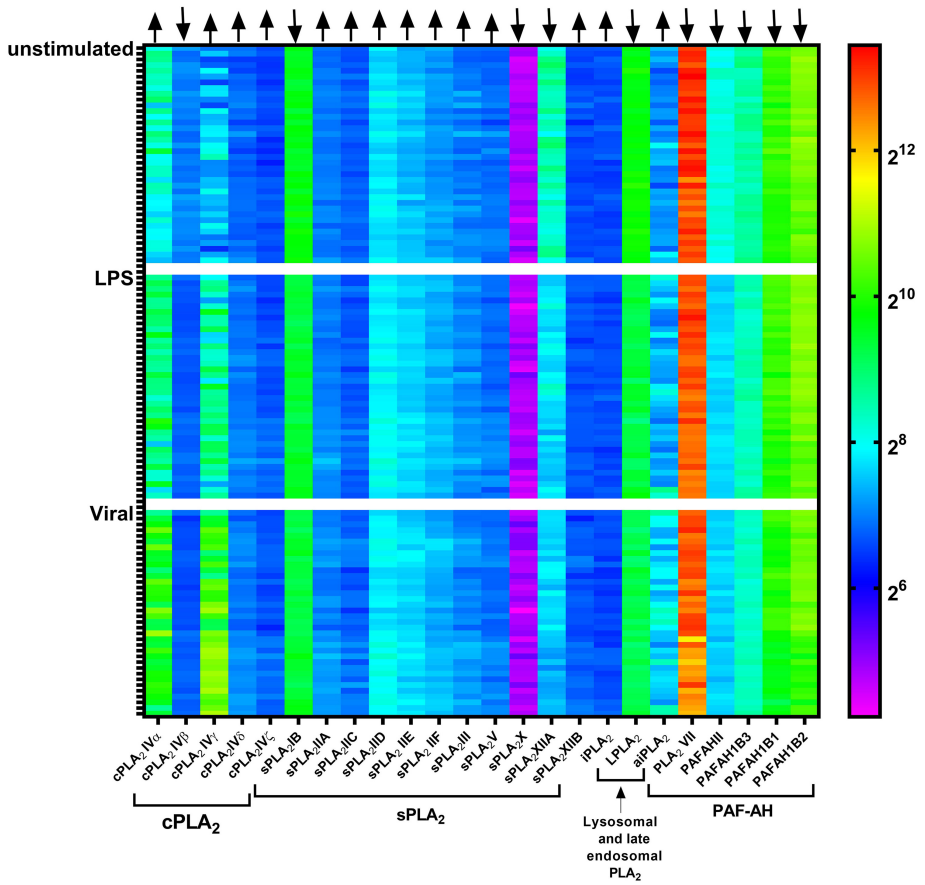

FIGURE 1 | Gene microarray heatmap of phospholipase A_2 (PLA_2) gene expression in monocyte-derived dendritic cells from a published microarray study. Each column shows a PLA_2 gene. Each row shows dendritic cells derived from an individual donor, either unstimulated or stimulated with LPS or the influenza virus (Viral). Microarray data is from the reference (Lee et al., 2014).

Lane et al., 2019). In line with this, transcriptomics analysis of human blood monocyte-derived dendritic cells revealed that both LPS and viral stimulation increase cPLA$_2$α expression (**Figure 1**).

cPLA$_2$α localizes in the cytosol in unstimulated RAW264.7 macrophage-like cells (Casas et al., 2006) and in mouse peritoneal primary macrophages (Gijón and Leslie, 1999). It translocates to a perinuclear membrane-rich area (likely the Golgi network) in response to chemically induced increases in PI (4,5)-bisphosphate [PI(4,5)P_2] (Bechler et al., 2012) and Ca^{2+} concentrations at this location (Gijón and Leslie, 1999). A similar translocation has been observed in LPS-stimulated P388D1 macrophage-like cells (Balboa et al., 2003b; Shirai et al., 2005). In mouse peritoneal macrophages, cPLA$_2$α and COX2 are both located close to the perinuclear Golgi network, which likely facilitates their functional coupling (Gijón and Leslie, 1999; Su et al., 2004). Thus, the increased expression and MAPK-mediated phosphorylation of cPLA$_2$α upon encountering a pathogen, lead to its translocation to the Golgi, resulting in increased production of ARA (Gijón and Leslie, 1999). This mechanism underlies the increased production of eicosanoids upon infection.

However, in human monocyte-derived macrophages, immunofluorescence microscopy experiments revealed that GFP–cPLA$_2$α also translocates to the phagocytic cup (Casas et al., 2010) and to zymosan containing phagosomes (Casas et al., 2009). This suggests that, next to lipid signaling, cPLA$_2$α has additional roles in the phagocytic process. These additional roles seem to be independent of its catalytic activity, because mutated cPLA$_2$α versions with an inactive catalytic domain showed no phagocytic defect in RAW264.7 macrophage-like cells, whereas mutation of the C2 domain, which is needed for membrane attachment, resulted in significantly less phagocytic ability (Zizza et al., 2012).

iPLA$_2$β SUPPORTS FOCAL EXOCYTOSIS FOR PATHOGEN UPTAKE

Unlike cPLA$_2$ forms, group-VI iPLA$_2$s do not require Ca^{2+} for their activity and membrane association (Dennis et al., 2011; Ramanadham et al., 2015). These are intracellular membrane-associated and cytoplasmic isozymes with a molecular weight ranging from 27 to 146 kDa (Dennis et al., 2011; Ramanadham et al., 2015). These isozymes are characterized by lipase (GXSXG) and nucleotide-binding (GXGXXG) consensus sequences

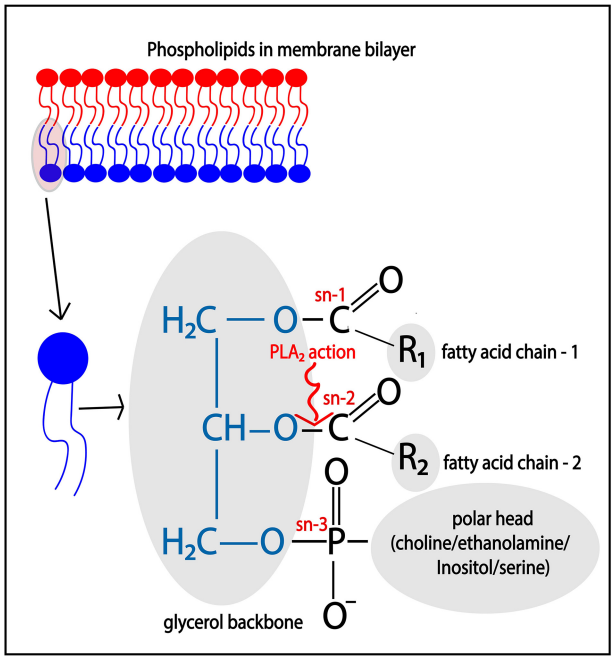

FIGURE 2 | Catalytic action of PLA$_2$. Membrane phospholipids such as phosphatidylcholine (PC)/phosphatidylethanolamine (PE)/phosphatidylinositol (PI), and phosphatidylserine (PS), are cleaved at the *sn*-2 position of the glycerol backbone by the action of PLA$_2$, thereby releasing free fatty acid such as arachidonic acid.

(Dennis et al., 2011; Ramanadham et al., 2015). They also carry ankyrin repeats that mediate protein-protein interactions and this enables them to form homo-oligomers which is essential for their activity (Dennis et al., 2011; Ramanadham et al., 2015). Members of this family include iPLA$_2$-β, -γ, -δ, -ε, -ζ, and -η. Notably, transcripts of iPLA$_2$-β, -γ, -δ, -ε, -ζ, and -η have been detected in human monocyte derived macrophages (Rubio et al., 2015), iPLA$_2$-β in human primary blood monocytes (Mishra et al., 2008) and the human monocyte cell line U937 (Tay and Melendez, 2004). Of these, only iPLA$_2$-β (a cytoplasmic form), and -γ (membrane-associated form) isozymes are widely characterized (Dennis et al., 2011; Ramanadham et al., 2015).

iPLA$_2$-β localizes to the ER–Golgi intermediate compartment (ERGIC), likely by associating to the cytosolic leaflet of the ERGIC membrane (Ben-Tekaya et al., 2010; Bechler et al., 2012). Studies in mammalian cell lines, such as HeLa, showed that iPLA$_2$-β mediates the formation of membrane tubules that bridge between separate ERGIC clusters and thereby regulate intra-ERGIC trafficking (Ben-Tekaya et al., 2010; Bechler et al., 2012). However, the widely described role of iPLA$_2$-β is a housekeeping function of remodeling phospholipids (Winstead et al., 2000; Ramanadham et al., 2015) that occurs in nearly all cellular membranes via two pathways: the Lands and the Kennedy (or *de novo*) pathways (Pasternak and Bergeron, 1970; Dabral and Coorssen, 2017). The Lands pathway maintains cellular homeostasis and operates in the presence of low concentrations of free ARA, which typically is the case in a resting cell, and iPLA$_2$-β is a critical enzyme for this pathway (Kennedy and Weiss, 1956; Lands, 1958). In the Lands pathway, iPLA$_2$-β generates free ARA, but most of it rapidly gets re-incorporated into phospholipids by first linking to Co-Enzyme A (CoA) by long-chain fatty acyl CoA synthetases. CoA-linked ARA then gets incorporated into PC by a CoA-dependent acyltransferase (Balsinde et al., 1997). Thus, iPLA$_2$β-produced ARA is an intermediate of connected de-acylation and re-acylation reactions of membrane phospholipids. In unstimulated P388D1 macrophages, re-acylation dominates over de-acylation, which limits the presence of free ARA (Chilton et al., 1996; Balsinde et al., 1997), thereby also limiting eicosanoid production. The Kennedy pathway is a low-affinity mechanism that incorporates free ARA in membranes as triacylglycerol (TAG) and diacylglycerol (DAG) species (Kennedy and Weiss, 1956; Astudillo et al., 2012; Gil-de-Gómez et al., 2020). Thus, the incorporation of free ARA into membranes depends upon the concentration of free ARA: at low concentrations most ARA will be incorporated in phospholipids via the Lands pathway, while at higher concentrations it will be incorporated in TAG and DAG species via the Kennedy pathway. Since pathogenic stimulation results in increased activities of cPLA$_2$ as described above, the rate of ARA hydrolysis exceeds that of its reincorporation into membrane phospholipids, leading to activation of the Kennedy pathway to esterify bulk ARA back in the membrane.

However, evidence suggests that iPLA$_2$-β has a second role in supporting the phagocytic uptake. During phagocytosis, iPLA$_2$-β translocates to the plasma membrane within 5 mins after particle engagement (Tay and Melendez, 2004). The catalytic activity of iPLA$_2$-β is essential for particle uptake, as its inhibition using bromoenolactone (BEL)–a selective iPLA$_2$-β and -γ inhibitor (Ramanadham et al., 2015)–leads to the polarized accumulation of electronlucent structures that appear as intracellular vesicles in the cytosol polarized toward the target attachment site in primary human monocytes (Lennartz et al., 1997). Moreover, enrichment of BEL-sensitive iPLA$_2$ to F-actin rich pseudopods occurs in murine monocytes that are stimulated with monocyte chemoattractant protein-1 (MCP-1) (Mishra et al., 2008), a chemokine known to induce increased ARA release in human monocytes (Gschwandtner et al., 2019). Together, these findings indicate that vesicles carrying BEL-sensitive iPLA$_2$ translocate to the plasma membrane at the site of the nascent phagosome, presumably to support membrane extension by promoting the local fusion of these vesicles with the plasma membrane (Bajno et al., 2000). These iPLA$_2$ carrying vesicles might be of endosomal nature, as iPLA$_2$ has been reported on endosomes (Karli et al., 1990; Mayorga et al., 1993, 1994). Alternatively or additionally, these might be secretory lysosomes, as BEL-sensitive iPLA$_2$ is required for lysozyme secretion from these compartments (Balboa et al., 2003a).

ANTI-MICROBIAL ROLES OF sPLA$_2$

Bactericidal Roles of sPLA$_2$ Forms

Members of the sPLA$_2$ group were first discovered in snake and bee venom, and in bovine pancreatic juice (Dennis et al.,

2011). Humans express 10 catalytically active sPLA$_2$ forms (IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XIIA), and one inactive form (XIIB) (Murakami et al., 2015). Several of these forms are expressed in phagocytic immune cells. For instance, human primary macrophages express sPLA$_2$-IIA, -IID, -V, -XIIA, and -XIIB (Anthonsen et al., 2000; Rubio et al., 2015), the macrophage-like cell line U937 expresses sPLA$_2$-IID, -V, and -XIIA (Dennis et al., 2011), human monocyte-derived dendritic cells express sPLA$_2$-IIA, -IIC, -IID, -IIE, -IIF, -III, -V, -X, -XIIA, and -XIIB (Anthonsen et al., 2000; Lee et al., 2014), and human monocytes express sPLA$_2$-IIA, and -V (Anthonsen et al., 2000). sPLA$_2$ forms are Ca^{2+}-dependent and low molecular weight proteins (<10–19 kDa) which are secreted into the extracellular environment. They carry a His-Asp catalytic dyad, have a 6–8 intramolecular disulfide bonds, and contain a highly conserved Ca^{2+} binding loop (Dennis et al., 2011; Murakami et al., 2015). Intracellular sPLA$_2$ is believed to be inactive and reside within the lumen of secretory vesicles and it becomes active after its secretion (Murakami et al., 2015).

Because its activity is largely confined to the extracellular environment, sPLA$_2$ in principle can only cleave phospholipids that are exposed to the outside of the cell. Thus, sPLA$_2$ enzymes can mainly hydrolyze lipids in the exoplasmic leaflet of the plasma membrane, and might hence act on the same cell that produces it in an autocrine manner, or paracrine on other cells. In addition, sPLA$_2$ might act on other membranous extracellular structures such as extracellular vesicles, mitochondria, lipoproteins, and microbes (Dennis et al., 2011; Murakami et al., 2015). Both sPLA$_2$-IIA and -V have specificity for phospholipids head-groups and cleave phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) more efficiently than phosphatidylcholine (PC) (Dennis et al., 2011; Murakami et al., 2015). Because the outer monolayer of the mammalian plasma membrane is rich in PC, whereas bacterial membranes are mainly composed of PG and PE, sPLA$_2$-IIA and -V are considered so-called bactericidal or inflammatory sPLA$_2$s that primarily hydrolyze phospholipids of invading bacteria (Murakami et al., 2015).

Pathogenic stimulation promotes the secretion and expression of sPLA$_2$-IIA and -V (Lee et al., 2014). In healthy human individuals, circulating sPLA$_2$-IIA levels are low (∼3 ng/ml), and likely insufficient to kill bacteria (Grönroos et al., 2002; Movert et al., 2013). However, upon microbial infection, sPLA$_2$-IIA production increases resulting in increased levels (250–500 ng/ml) in human and mouse serum, which are sufficient to kill microbe as shown by *in vitro* dose-response curves (Grönroos et al., 2002; Bruegel et al., 2012; Movert et al., 2013; Murakami et al., 2015). Unstimulated mouse peritoneal macrophages express sPLA$_2$-V and an intracellular pool is present at the Golgi network and recycling endosomes (Balestrieri et al., 2006). Activation of macrophages results in the release of this sPLA$_2$-V pool, as stimulating P388D1 macrophage-like cells with LPS for 6 h resulted in an increased localization of sPLA$_2$-V in caveolin-rich vesicles near the perinuclear area (Balboa et al., 2003b). The pathogenic stimulation also increases the expression of sPLA$_2$-V, as has been reported for LPS-stimulated primary human monocyte-derived macrophages (Rubio et al., 2015) and rat liver macrophages (Dieter et al., 2002). In line with this, transcriptomics analysis (Lee et al., 2014) also showed an upregulation in the expression of sPLA$_2$-V in LPS and viral stimulated human monocyte-derived dendritic cells (**Figure 1**). However, sPLA$_2$-XIIA seems down regulated upon pathogenic stimulation of monocyte-derived dendritic cells (**Figure 1**), but the functional significance of this is unknown.

The release of sPLA$_2$-V might occur in a polarized fashion at the phagocytic cup, as it translocates from the Golgi and recycling endosomes to the forming phagosome in zymosan-stimulated mouse peritoneal macrophages (Balestrieri et al., 2006, 2009). This translocation of sPLA$_2$-V from its juxtanuclear resting position to the forming phagosome was also observed within 5 mins after phagocytic cup formation in zymosan-stimulated peritoneal mouse macrophages (Balestrieri et al., 2006). Therefore, sPLA$_2$-V at the phagocytic cup could potentially damage the attached microbe even before its internalization, especially since its activity will be promoted by the high extracellular calcium concentration (mM) (Mitsuishi et al., 2006; Balestrieri et al., 2009). Evidence supporting such a role in the killing of pathogenic microbes at the phagocytoc cup and/or later in closed phagosomes, comes from the finding that mouse peritoneal macrophages lacking sPLA$_2$-V show delayed phagocytosis, delayed phagosomal maturation, and impaired *Candida albicans* killing (Lennartz et al., 1997). Similarly, human recombinant sPLA$_2$-IIA, V, X, and XII substantially inhibited growth of *Staphylococcus aureus,* and *Listeria monocytogenes* in colony forming unit (CFU) assays (Koduri et al., 2002). However, the contribution of these sPLA$_2$ forms in the killing of infectious pathogens *in vivo* is yet unknown.

Bacterial Killing and Immune Modulation by LPLA$_2$

Lysosomal PLA$_2$ (LPLA$_2$; group XV) is a glycoprotein and is highly mannosylated. The glycosylated form has a molecular weight of about 45 kDa (Shayman et al., 2011; Shayman and Tesmer, 2019). It carries a signal peptide for targeting to the ER, and is characterized by the conserved lipase motif AXSXG, containing an active site serine which is essential for its hydrolytic action (Shayman et al., 2011; Shayman and Tesmer, 2019). Its catalytic activity is calcium-independent, and it primarily localizes to the lumen of acidic lysosomes and late endosomes (Shayman et al., 2011; Shayman and Tesmer, 2019). Besides the canonical PLA$_2$-mediated hydrolysis which releases a free *sn*-2 fatty acid from a phospholipid, LPLA$_2$ is capable of transferring the fatty acid to the OH group at the C1 position of a short-chain ceramide in the so-called transacylase reaction. LPLA$_2$ has an acidic pH optimum and is expressed in murine alveolar macrophages, RAW264.7 macrophage-like cells, and peritoneal mouse macrophages (Shayman et al., 2011; Shayman and Tesmer, 2019). The physiological roles of LPLA$_2$ are still incompletely understood, and several functions have been proposed.

First, LPLA$_2$ might regulate phospholipid metabolism, because phospholipids (i.e., PC, PE, and plasminogen-PE) were found to be accumulated in the alveolar lavage fluid of LPLA$_2$ knock-out mice (Shayman and Tesmer, 2019).

Second, LPLA$_2$ might mediate pathogen killing similar to sPLA$_2$ described above, because LPLA$_2$ is also released in the extracellular space of zymosan-stimulated mouse peritoneal and alveolar macrophages (Wightman et al., 1981; Abe et al., 2008). Also similar to sPLA$_2$, LPLA$_2$ translocates to forming phagosomes in RAW264.7 macrophage-like cells within 4 mins after particle engagement, and the fusion of lysosomes with the plasma membrane results in the exocytosis of LPLA$_2$ (Sun et al., 2020). Because the V-ATPase at the plasma membrane pumps H^+ ions across the membrane to the extracellular side, potentially lowering the local extracellular pH at the phagocytic cup, this might provide the required acidic microenvironment for secreted LPLA$_2$ to attack the microbial cargo.

Third, LPLA$_2$ might play a role in antigen presentation of lipids to T cells in CD1, because LPLA$_2$ knock-out mice displayed lower T cell activation and recruitment to infected lungs (Shayman and Tesmer, 2019). Furthermore, *Mycobacterium* infected LPLA$_2$ knock-out mice show an enhanced bacterial burden and a low recruitment of CD1-expressing cells to the infection site (Schneider et al., 2014). This role in lipid presentation is supported by the finding that LPLA$_2$ can cleave bacterial cardiolipin, and the resulting lipid species are incorporated into the membranes of phagosomes and other organelles in *Mycobacterium bovis* infected murine macrophages (Fischer et al., 2001). Similarly, LPLA$_2$ can cleave mycobacterial tetra-acylated glycolipid antigens (phosphatidyl-*myo*-inositol mannosides) into diacylated forms in THP-1 monocyte-like cells (Balboa et al., 2003a). Because cardiolipin carries four fatty acid chains, just as mycobacterial tetra-acylated glycolipids, LPLA$_2$ appears to preferentially cleave tetra-acylated lipids (Fischer et al., 2001; Gilleron et al., 2016). Thus, current evidence suggests that LPLA$_2$ processes microbial tetra-acylated lipids within phagolysosomes, and the resulting diacylated forms might be presented on CD1 molecules to stimulate T cells.

Fourth, LPLA$_2$ could play a role in membrane repair, as explained in the next section.

MEMBRANE REPAIR BY LPLA$_2$ AND aiPLA$_2$

Transacylase Activity of LPLA$_2$ for Repair of Membrane Damage

In addition to its antimicrobial roles described in the previous section, LPLA$_2$ also has a potential role in membrane repair. It can remove oxidized fatty acids at the *sn*-2 position of phospholipids and transfer these to ceramides in the transacylase reaction (Shayman and Tesmer, 2019). This ability of LPLA$_2$ might be important, because in neutrophils, macrophages, and dendritic cells, the killing of ingested pathogens is a radical-mediated mechanism where the NADPH oxidase NOX2 generates large amounts of reactive oxygen species (ROS) in the lumen of phagolysosomes (Vulcano et al., 2004). These radicals not only damage the ingested pathogen, but also oxidize membranes of the host cell (Dingjan et al., 2016). Therefore, the host cell could need a mechanism to repair damage to the phagosomal membrane, particularly because polyunsaturated fatty acids, such as ARA, are highly susceptible to lipid peroxidation (Yin et al., 2011). LPLA$_2$ might thus potentially limit or repair the damaging effects of ROS, by removing oxidized ARA from phospholipids and transfer it to ceramides. Supporting this transfer, ceramides containing long acyl chains such as ARA are high on mature phagosomes while ceramide synthase 2 expression and activity are high on early phagosomes in RAW264.7 macrophage-like cells (Pathak et al., 2018).

Regulation of Oxidative Damage by aiPLA$_2$

Acidic Ca^{2+}-independent PLA$_2$ (aiPLA$_2$) has a molecular weight of ∼25 kDa and is better known as peroxiredoxin 6 (Fisher, 2011, 2018). It is expressed in mouse alveolar macrophages (Chatterjee et al., 2011) and mainly localizes to the cytosol where it has only low PLA$_2$ activity due to its acidic optimum of pH 4.0 (Fisher, 2018). However, a fraction of aiPLA$_2$ is also targeted to the lumen of lysosomes and late endosomes, in a manner depending on direct interactions with the chaperone 14-3-3ε (Sorokina et al., 2009; Fisher, 2018). Besides possessing PLA$_2$ activity, aiPLA$_2$ is a multifunctional enzyme that also possesses lyso-phosphatidyl acyltransferase and glutathione peroxidase activities (Fisher, 2011).

Because aiPLA$_2$ binds to liposomes carrying oxidized lipids, and translocates to the plasma membrane in A549 cells following treatment with an oxidization agent (Manevich et al., 2009), membrane oxidation might promote association of aiPLA$_2$ with the membrane. Hence, oxidized *sn-2* fatty acids might be replaced by the lyso-phosphatidyl acyltransferase activity of aiPLA$_2$ (Fisher, 2018; Fisher et al., 2018). Additionally, aiPLA$_2$ can reduce peroxidized phospholipids to their corresponding alcohol due to its glutathione peroxidase activity (Chatterjee et al., 2011; Fisher, 2018; Fisher et al., 2018). Therefore, similar to LPLA$_2$, aiPLA$_2$ can protect against oxidative damage due to its ability to (i) hydrolyze the *sn*-2 position peroxidized fatty acids to generate lyso-phospholipids, (ii) reacylate these lyso-phospholipids to form a new phospholipid, and (iii) convert oxidized phospholipids to their corresponding alcohol (Fisher et al., 2018). The physiological roles of aiPLA$_2$ therefore include the repair of oxidized membranes (Chatterjee et al., 2011).

However, aiPLA$_2$ also promotes ROS production by promoting the assembly of the NOX2 complex in alveolar macrophages (Chatterjee et al., 2011). As mentioned above, NOX2 generates ROS within the lumen of phagolysosomes, and also at the plasma membrane, in order to kill and degrade microbial pathogens (Burgdorf and Kurts, 2008; Uribe-Querol and Rosales, 2020). The assembly and activation of NOX2 occurs when MAPK phosphorylates cytosolic aiPLA$_2$ at T^{177} which results in the translocation of aiPLA$_2$ to the plasma membrane (Fisher et al., 2018). At the plasma membrane, aiPLA$_2$ generates lyso-PC which in turn converts to lyso-phospatidic acid (LPA) by lysophospholipase D (Vázquez-Medina et al., 2016; Fisher, 2018). Binding of LPA to the LPA receptor-1 activates the small-GTPase Rac, which is a component required for the activation of the NOX2 complex (Fisher, 2018). In stark contrast, another

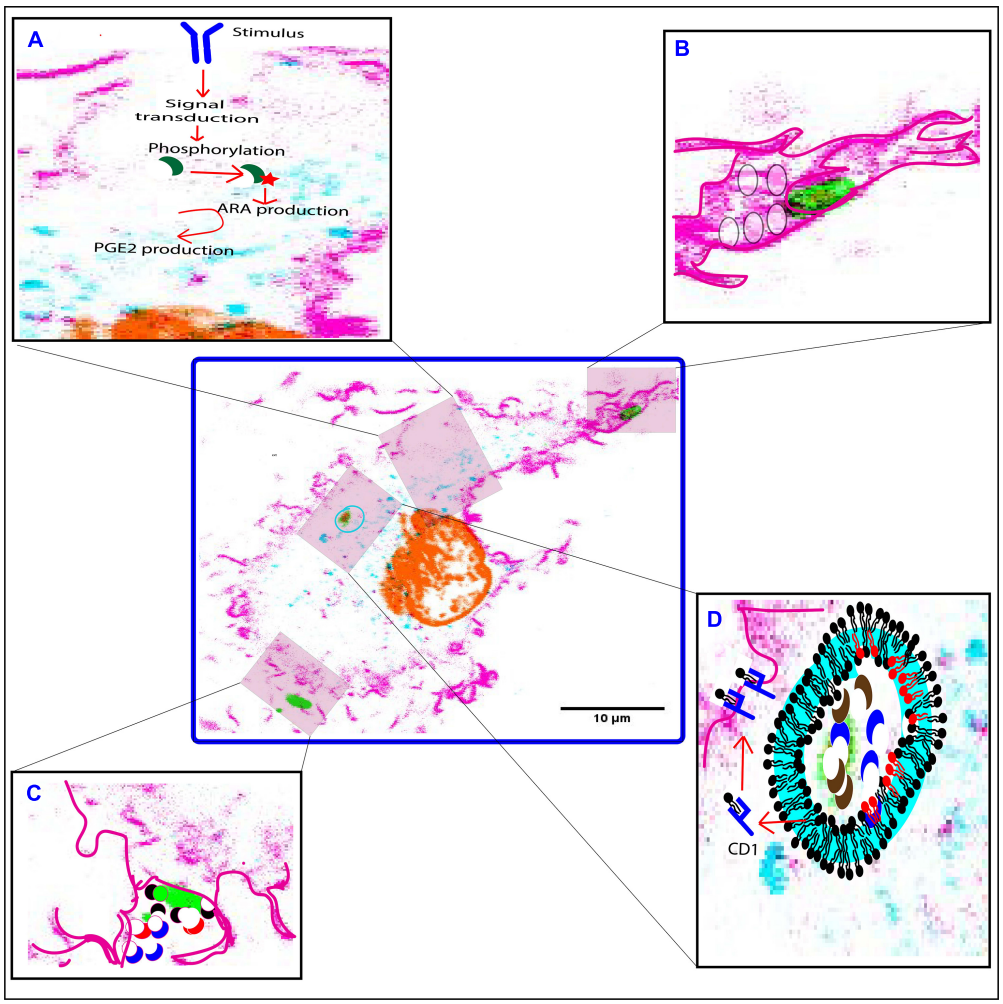

FIGURE 3 | Functions of PLA$_2$ forms at different stages of phagocytosis. **(A)** cPLA$_2\alpha$ (green crescent) is phosphorylated in response to an extracellular pathogenic stimulus. This leads to translocation of cPLA$_2\alpha$ to the perinuclear area, where it hydrolyzes membrane phospholipids to generate arachidonic acid (ARA) which in-turn gets metabolized to eicosanoids by the action of the COX-2 enzyme. **(B)** iPLA$_2$ (not shown) translocates to the phagocytic cup, and facilitates fusion of secretory vesicles (translucent circles) to provide additional membrane required for the extension of the pseudopodia. **(C)** sPLA$_2$-II/V (red and black crescent) and LPLA$_2$ (blue crescent) are released at the phagocytic cup to degrade invading microbes (green). **(D)** LPLA$_2$ (blue crescent) and aiPLA$_2$ (brown crescent) work together to degrade and process microbes within the phagolysosome. The processed lipids are then loaded onto a CD1 molecule (blue structure) to be presented on the cell surface to activate T cells. Both LPLA$_2$ and aiPLA$_2$ repair peroxidized lipids of the phagosomal membrane that might occur due to increased NOX2 activity. The repaired phospholipids are shown in red. F-actin is pink, DNA is orange, LAMP1 is turquoise and LPS is green. Highlighted pink boundary in insets **(B–D)** shows pseudopodia, phagocytic cup, and plasma membrane, respectively. Highlighted turquoise boundary at the phagosome membrane is to show recruited LAMP1.

component of the NOX2, p67^{phox}, can bind to phosphorylated form of aiPLA$_2$, and this inhibits its PLA$_2$ activity (Fisher, 2018).

Thus, aiPLA$_2$ has a dual function: it both repairs oxidation-induced membrane damage and promotes NOX2-mediated ROS formation. Perhaps at initial stages of pathogen recognition, the membrane damage by NOX2-produced ROS is still low. At this stage, unassembled p67^{phox} (i.e., not in the NOX2 complex) might negatively regulate aiPLA$_2$ activity, as the need to repair damaged membranes is low. However, as more NOX2 is assembled and ROS production is increased, most p67^{phox} might be assembled in the NOX2 complex and no longer be available to inhibit aiPLA$_2$. At this stage, aiPLA$_2$ would be free to repair peroxidized phospholipids in the membrane.

aiPLA$_2$ might also mediate the repair of membranes damaged by intracellular pathogens. The expression of aiPLA$_2$ shows a biphasic response in *Brucella suis* infected RAW267.4 macrophages: aiPLA$_2$ expression initially decreases until 10 h post-infection, after which it increases until 50 h post-infection (Wang et al., 2019). During the first phase, *B. suis* is non-replicative within phagosomes (Celli, 2019), whereas it becomes replicative in the second phase and this eventually leads to rupture of the phagosomal membrane (Celli, 2019; Wang et al., 2019). Therefore, the expression of aiPLA$_2$ might initially be low because there is limited need for repair of membrane damage, whereas later its expression increases to perhaps repair the damaged

phagosomal membrane. This mechanism is speculative and needs experimental support.

DISCUSSION AND CONCLUDING REMARKS

As discussed above, PLA$_2$ forms have different effects on phagocytosis: aiPLA$_2$, LPLA$_2$, and sPLA$_2$ forms mainly act on the luminal and/or extracellular leaflet of the (nascent) phagosomal membrane and play roles in the killing of the pathogen and the repair of the membrane from oxidative damage. In contrast, iPLA$_2$ and cPLA$_2$ forms mainly act on the cytosolic leaflets of the plasma membrane, phagosomes and other organelles, and play roles in eicosanoid signaling and regulation of organellar trafficking. These roles are summarized in **Figure 3**. In addition, since the products from PLA$_2$ hydrolysis directly alter the physicochemical properties of the membrane, PLA$_2$s might also directly affect the phagocytic process. ARA and LPC have negative and positive spontaneous curvatures, respectively, and thereby can directly stabilize or destabilize membrane assemblies (Chernomordik et al., 1995). Increased levels of ARA in the *cis* leaflets of merging bilayers (i.e., the cytoplasmic leaflet of the plasma membrane and the outer leaflet of the organellar membrane) promote fusion, while more LPC blocks fusion (Chernomordik et al., 1995). In contrast, more ARA in the *trans* leaflets (i.e., outer leaflet of the plasma membrane and luminal leaflet of the organellar membrane) of merging bilayers blocks fusion, while more LPC promotes fusion (Chernomordik et al., 1995). Therefore, membrane fusion might be both promoted and inhibited by these PLA$_2$ metabolites depending on the site of PLA$_2$ action, the PLA$_2$ substrates, and downstream metabolism of the PLA$_2$ products. Moreover, the fusion of intracellular membrane compartments at the phagocytic cup results in the delivery of more PLA$_2$s to the nascent phagosome, including cPLA$_2$, sPLA$_2$, LPLA$_2$, aiPLA$_2$, and iPLA$_2$ forms. This local delivery of more PLA$_2$ at the nascent phagosome might thus modulate the phagocytic process by either facilitating or inhibiting the membrane reshaping required for the membrane wrapping and internalization of the phagocytic cargo.

In addition to these spontaneous effects on membrane fusion by the physicochemical properties of PLA$_2$ products, PLA$_2$ might potentially also affect phagocytosis by affecting soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE)-mediated membrane fusion. In animal cells, all organellar membrane fusion (except mitochondrial fusion) is mediated by members of the SNARE protein family (Hong, 2005). Cognate SNARE proteins in both the vesicular and target membranes, called v- and t-SNAREs, engage and "zipper" from their N-terminal toward their C-terminal termini, thereby forming a tight alpha-helical coiled-coil bundle that overcomes the energy barrier of membrane fusion (Dingjan et al., 2018). In mouse chromaffin cells, the transmembrane domain of the v-SNAREs VAMP2 is affected by the PLA$_2$ metabolites LPC and oleic acid in a leaflet specific manner that either promotes or inhibits fusion pore formation and expansion, hence affecting neurotransmitter release (Dhara et al., 2020). In line with this, the administration of LPC and oleic acid to the cell culture medium, which increases their concentrations in the outer leaflet of the plasma membrane, accelerated and deaccelerated nurotransmitter release, respectively (Dhara et al., 2020). In contrast, the intracellular administration of LPC and oleic acid by microinjection, which increased their concentration in the inner leaflet of the plasma membrane, blocked and promoted membrane fusion, respectively (Dhara et al., 2020). Similar results were also obtained in homotypic fusion of cortical vesicles isolated from eggs of the sea urchin, in which increasing the LPC concentrations in the outer leaflets of these vesicles by external LPC blocked fusion (Dabral and Coorssen, 2019). However, the PLA$_2$ products might not only affect the membrane fusion, but also upstream events as the external application of ARA also affected the SNARE dependent docking or priming in homotypic fusion of cortical vesicles (Dabral and Coorssen, 2019). Because many SNARE proteins are both functionally and structurally homologous, and since many SNARE proteins are involved in endosomal trafficking and phagocytosis (Dingjan et al., 2018), similar interactions with PLA$_2$ metabolites could potentially inhibit or promote membrane fusion in phagocytosis as well.

Whereas the roles of the different PLA$_2$ types in phagocytes are fairly well established, as discussed in this review, a main open question is why immune cells express so many different members of each PLA$_2$ group. For example, human monocyte derived macrophages express three forms of sPLA$_2$ (sPLA$_2$-IID, -V, and -XIIA), three forms of cPLA$_2$ (cPLA$_2$IV-α, -β, and -γ) and six forms of iPLA$_2$ (iPLA$_2$VI-β, -γ, -δ, -ε, -ζ, and -η) (Rubio et al., 2015). While these different types have non-overlapping functions in eicosanoid signaling, membrane repair, organellar trafficking and pathogen killing, it is largely unknown why multiple members of each different type are expressed. PLA$_2$ members from each type perhaps are essential for carrying out the same critical processes. Hence, multiple genes coding for these essential enzymes might ensure the functional redundancy in the absence of one of the forms, thereby increasing the fidelity of the immune response. It might also be that these different members have different specificities for headgroups and acyl chains, and/or they might have different subcellular localizations. Thereby, the various PLA$_2$ subgroup members might have non-overlapping roles, and could for instance act during different stages of the phagocytic process: antigen recognition, formation of the phagocytic cup, particle internalization, and maturation of the phagosome into a phagolysosome. Supporting such non-overlapping roles, is the finding that expression of different subtype members of cPLA$_2$ is differently regulated upon pathogenic stimulation: transcript levels of cPLA$_2$-IVα and -IVγ are upregulated, whereas cPLA$_2$-IVβ is downregulated (**Figure 1**) (Lee et al., 2014).

Another open question is whether microbial PLA$_2$ forms also modulate phagocytic process. Several bacterial pathogens express PLA$_2$ enzymes as virulence factors. For example, PLA$_2$ activity of *Helicobacter pylori* is responsible for the degradation of the gut mucosal barrier (Baj et al., 2020). The putative PLA$_2$ protein RV3091 of *Mycobacterium tuberculosis* is involved in the phagosomal escape of this pathogen (Cui et al., 2020) and a secreted form of PLA$_2$ by *Toxoplama gondii* contributes to its

replicative cycle (Cassaing et al., 2000). These bacterial PLA$_2$s are structurally similar to mammalian sPLA$_2$ forms and thus are evolutionary conserved (Murakami et al., 2015). Hence, we presume that within the lumen of a phagosome or at the nascent phagocytic cup, both host and microbial PLA$_2$ likely engage in a contest to cleave the lipids of the bacterial and host membranes, respectively, but the functional impact of such engagement in phagocytic cells is yet unexplored.

Experimentally, it is difficult to discriminate the actions of the different PLA$_2$ forms, both from host and microbial origin, because many PLA$_2$ inhibitors are not entirely specific for one specific species of PLA$_2$. Moreover, all PLA$_2$ forms act on membrane phospholipids, and lipidomics approaches therefore do not readily allow the assignment of the results to a single species of PLA$_2$. The potential functional redundancy of PLA$_2$s also make them difficult to study, as knockout or knockdown might not always result in a clear phenotype. Nevertheless, we expect that the side-by-side comparison of mammalian cell lines with specific knockouts of one or more PLA$_2$ forms, for instance using CRISPR technology, coupled with MS based organellar lipidomics and high resolution microscopy, will enable to address this functional redundancy and better delineate the unique and overlapping roles of the different PLA$_2$ forms.

As summarized in **Figure 3**, PLA$_2$ is important for the clearance of pathogens, because it (i) triggers eicosanoid signaling shaping the downstream immune response, (ii) stimulates or inhibits the phagocytic process, (iii) directly supports the killing of pathogenic microbes, and (iv) repairs oxidation induced phagosomal membrane damage. Therefore, the understanding of the expression, membrane association, substrate specificity and the associated immunomodulatory actions of PLA$_2$ forms in regulating the immune response in phagocytic cells is critical, as this might also lead to new therapeutic approaches to combat microbial infections.

AUTHOR CONTRIBUTIONS

DD conceptualize and drafted the original draft. GB reviewed and provided critical feedback. Both authors equally contributed to the final preparation of the manuscript.

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