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Phospholipid signaling in innate immune cells.

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Abstract

Phospholipids comprise a large body of lipids that define cells and organelles by forming membrane structures. Importantly, their complex metabolism represents a highly controlled cellular signaling network that is essential for mounting an effective innate immune response. Phospholipids in innate cells are subject to dynamic regulation by enzymes, whose activities are highly responsive to activation status. Along with their metabolic products, they regulate multiple aspects of innate immune cell biology, including shape change, aggregation, blood clotting, and degranulation. Phospholipid hydrolysis provides substrates for cell-cell communication, enables regulation of hemostasis, immunity, thrombosis, and vascular inflammation, and is centrally important in cardiovascular disease and associated co-morbidities. Phospholipids themselves are also recognized by innate-like T cells, which are considered essential for recognition of infection or cancer, as well as self-antigens. This review will describe the major phospholipid metabolic pathways present in innate immune cells and summarize the formation and metabolism of phospholipids as well as their emerging roles in cell biology and disease.

Main Text

Circulating innate immune cells, including neutrophils, platelets, monocytes, eosinophils, and mast cells, are considered resting basally. However, they rapidly activate during injury, such as bleeding or trauma, to trigger hemostasis and prevent bacterial invasion. Major metabolic changes to the lipid pool that accompany innate immune activation are essential in orchestrating an effective innate immune response and then initiating the process of wound healing and repair. This cellular activation is exemplified by multifaceted alterations to the phospholipid (PL) pool and includes: (i) membrane remodeling and generation of potent bioactive signaling mediators from PL-derived substrates (lysophospholipids (lysoPL), oxylipins, and platelet-activating factor (PAF) etc.); (ii) aminophospholipid (aPL) externalization, providing a negatively-charged membrane that supports clotting factor activities on the cell surface; (iii) generation of large numbers of enzymatically-oxidized PL (eoxPL), which are required for hemostasis, immediately upon innate immune cell activation; and (iv) inside the cell, PL headgroup phosphorylation that occurs within seconds, leading to formation of transient membrane anchors for kinases that regulate GPCR signaling, endocytosis, apoptosis, and cytokinesis.

Separate from changes to the PL pool, lipid-reactive T cells restricted to the CD1 family of antigen-presenting molecules, can sense the presence of bacterially-derived lipids, self-lipids during thymic selection, and self-lipids that may be perturbed during cellular stress [1, 2]. Many lipid-reactive T cells appear non-reactive to common phospholipids normally found within the cell membrane. However, T cells reactive to bacterially-derived phospholipids [3, 4], rare phospholipids [5], and lysophospholipids expressed on certain tumors [6, 7] have been described. These processes will be described in detail below.

PLs represent a diverse group of lipids also known as glycerophospholipids (GP, described using LIPID MAPS nomenclature). The five classes are subdivided based on polar headgroup and include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS). Within each class, molecular species are differentiated by fatty acid (FA) substitution at the *sn1* and *sn2* positions of the glycerol backbone (Figure 1A). Generally, *sn1* FAs are saturated or mono-unsaturated, whereas *sn2* are polyunsaturated (PUFA) with longer acyl chains. While the *sn2* FA is always an acyl substituent, the *sn1* for PE or PC can be either an alkyl-linked (ether-linked) or 1Z-alkenyl-linked (plasmalogen), with the latter particularly abundant in circulating immune cells (Figure 1B). Thus, blood cells contain literally hundreds of unique PL species, although the most quantitatively abundant contain a relatively restricted set of FAs: palmitic acid (PA, 16:0), stearic acid (SA, 18:0) or oleic acid (OA, 18:1) at *sn1*, and linoleic acid (LA, 18:2), arachidonic acid (AA, 20:4), eicosapentanoic acid (20:5, EPA) or docosahexanoic acid (22:6, DHA) at *sn2* (Figure 1 C,D). Overall, AA is present in PLs at least at 10-fold higher concentrations than other PUFAs at *sn2* in immune cells. Dietary intake of fatty acids, through ingesting high doses of fish oils, can influence relative levels of omega 3 (DHA, EPA) versus omega 6 (AA, LA) fatty acids in blood cell PLs[8, 9]. Whether this leads to changes in PL-mediated signaling in the innate immune system is unknown. However it has knock-on effects on levels of oxylipins generated in serum, with higher levels of some omega3 derived species observed [10-15].

Phospholipids are synthesized and remodeled through several interconnected enzyme pathways, including the Kennedy pathway (PC, PE synthesis), the cytidine diphosphate diacylglycerol (CDP-DAG) pathway (synthesis of PS and PI from PA), and the Lands cycle (FA removal and reattachment to PLs) (Supplementary Figure 1 A,B) [16-18]. These are common to all cell types, including circulating blood cells, and are subject to

dynamic regulation depending on metabolic state. The Lands cycle controls substrate supply for bioactive lipid signaling and is described in more detail below.

Following an acute injury, circulating cells become trapped at the wound site. The resulting clot composition is highly dependent on vessel type and co-existing inflammation [19-21]. Concurrently, trapped cells are exposed to soluble protein and non-protein mediators that activate receptor-dependent processes, including tissue factor, collagen, thrombin, ADP, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs). PAMPs include bacterial products such as lipopolysaccharide (LPS) and f-met-leu-phe (fMLP), both of which stimulate cells in the nanogram range. LPS acts via TLR complexes, while fMLP mediates signaling via a GPCR called FPR1. At the same time, a cascade of cell activation processes characterized by extracellular calcium influx and release from intracellular stores are associated with PL hydrolysis. This is mediated by several types of phospholipases, broadly categorized as PLA₁, PLA₂, PLC, PLD and named by site of PL hydrolysis. Each comprises families of multiple isoforms (Figure 2 A). Many different phospholipases are acutely switched on in activated blood cells, and their coordinated activities are responsible for initiating cell activation during the injury response. This controls much of the signaling that leads to lipid membrane remodeling and a plethora of lipid-dependent bioactivities, both intracellular and extracellular. Lipids generated during initial phospholipase activity are either released as secondary paracrine mediators (e.g. oxylipins), or retained in the cells to act locally, either physically changing membrane structure (e.g. eoxPL) or signaling at intracellular nuclear receptors (e.g. FA activation of PPARs). The following sections describe PL metabolism in innate immune cells in more detail.

1. PLA₂-mediated phospholipid hydrolysis forms lysoPL and FAs.

Pathophysiological activation of innate cells triggers immediate cell membrane hydrolysis by phospholipases. The best studied are PLA₂ isoforms, which comprise ~16 groups and several subgroups, with six main PLA₂ subtypes [22]. PLA₂ removes fatty acyl groups from the *sn*2 position of PL, leading to lysoPL and FA generation and thereby supporting the first step in bioactive oxylipin formation (Figure 2 A). Several lysoPLs are generated during platelet activation, including lyso-PE, -PC, -PI and -PS [23]. Leukocytes and platelets express at least three classes of PLA₂: secretory (sPLA₂, Groups II and X), calcium-dependent (cPLA₂, Group IV), and calcium-independent (iPLA₂, Group VI), whilst several others are known to exist in other cells. Another PLA₂ isoform, termed PAF-acetyl hydrolase (PAF-AH), is found in the circulation [24].

Regulation of innate immune cell PLA₂ is multifaceted and complex. For example, in monocytes, both MAPK-catalyzed phosphorylation and PKC activate cPLA₂ α [25]. For many years, cPLA₂ α was considered the primary source of AA for eicosanoid generation, but recent studies show a major role for iPLA₂ in several circulating cell types. In this regard, platelet cPLA₂ α can catalyze AA release from innate immune PL pools in response to collagen, enabling generation of thromboxane A₂ and 12-HETE [26]. However, iPLA₂ γ is required to mobilize AA from plasmalogen PEs and PGs for ADP-induced thromboxane B₂ generation [27]. Thus, different PLA₂ isoforms couple to distinct receptor pathways, though both can facilitate platelet eicosanoid generation. Similarly, in leukocytes, cPLA₂ α and iPLA₂ β act together to provide different signaling lipids at separate intracellular sites, with both required for migration in response to MCP-1 (also known as CCL2) [28].

Importantly, PLA₂ provide the essential link between PL metabolism and cyclooxygenase-mediated (COX-mediated) pathways in immune cells, e.g. generation of thromboxane in platelets, and prostaglandin E₂ (PGE₂) in inflammation, both of which require AA. COX-1 and COX-2 are the well-known targets of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), and more recently-developed COX-2-selective NSAIDs, underscoring their clinical importance in vascular and chronic inflammatory disease.

Circulating blood cells express several sPLA₂ isoforms, including Group IIA (platelets, leukocytes), Group X (neutrophils), and Group III (mast cells). Group IIA sPLA₂ attacks bacterial membranes during host defense in innate immunity, while Group III targets stromal fibroblasts. PG and PE are the primary targets of Group IIA, with host cells being relatively protected since PC comprises most of the external membrane leaflet of healthy cells. In contrast, in mast cells, Group III sPLA₂ attacks stromal cells via AA release and its metabolism to prostaglandin D₂, promoting mast cell maturation and ultimately anaphylaxis [29].

Hydrolysis of the *sn*1 fatty acyl group can also result in lysoPL and FA release, in this case due to PLA₁ (Figure 2 A). Little is known about PLA₁ in immune cells, although a fast release of saturated FAs occurs upon platelet activation [30]. A platelet PLA₁ isoform was purified in 2011 and proposed as a source of circulating lysoPA generated during clotting, acting in concert with autotaxin to mediate sequential hydrolysis of cellular PC [31].

Regulation of PLA₂ isoforms in innate immune cells is complex, given the large number of gene products, varied cell expression, intracellular localization, activation, control, etc. For further information on this topic, the reader is directed towards several recent reviews [25, 29, 32].

Phospholipases and their products play multiple and complex roles in disease, with symptoms noted well beyond the innate immune system. Deficiency of cPLA₂ α in humans leads to major gastrointestinal complications including bleeding, as well as increased susceptibility to infection, and acute respiratory distress syndrome. A lack of platelet thromboxane B₂ is associated with platelet defects[25]. On the other hand, mice lacking cPLA₂ α are protected from hepatic liver deposition and fibrosis[25]. Deficiency of iPLA₂ β is linked with several diseases including neurological, cancer, vascular and immune[32]. In terms of inflammation, iPLA₂ β is required for macrophage spreading/adhesion[32]. Inhibitor studies have shown that cPLA₂ and iPLA₂ isoforms are critical for development of autoimmune disease, such as experimental autoimmune encephalomyelitis[32].

2. Scramblase activities leading to aPL externalization.

Normally, circulating blood cells maintain an asymmetric plasma membrane with PC the most abundant externally-facing PL and PE/PS primarily located on the cytosolic surface. PE and PC each make up about 40% of total PL in blood cells, with PS being only a few mol % [33, 34]. Maintaining asymmetry is an energy-requiring process utilizing flippase and floppase enzymes to prevent exposure of the negatively-charged aminophospholids (aPL) PS and PE on the outside of the cells. Upon activation and calcium mobilization, platelets rapidly activate scramblase, leading to externalization of aPL (Figure 2B) [35, 36]. This changes the biophysical nature of the membrane to allow coagulation factors to bind and work effectively to generate fibrin. A genetic defect in aPL externalization has been described, Scott Syndrome, that manifests as a relatively mild bleeding

phenotype[37]. This has been localized to mutations in TMEM16F, a protein proposed to play dual roles as both a channel and scramblase[38]

Coagulation factors comprise a series of serine proteases and associated co-factors that are largely inactive in the fluid phase of blood. In order to facilitate clot formation, they must self-associate on a negatively-charged surface, where they reach a high local concentration. This is classically achieved by platelets externalizing PE/PS following agonist activation [35, 36]. The headgroup of PS facilitates calcium binding to the surface, which ultimately allows binding of negatively-charged gamma-carboxyglutamic (Gla) domains of factors at the cell membrane (Figure 2B). The ability of PS to support Gla-domain-binding is enhanced by PE and eoxPL, a recent discovery that is described in more detail later [39, 40]. Platelets contain several molecular species of PE and PS. The optimum FA composition to support coagulation is around 18-20 carbons, with typical unsaturation seen in platelet PLs, though plasmalogen and acyl forms of PE are equally effective in this context [41]. Recent studies show that PE and PS associate on the surface of platelets in defined microdomains, forming “cap” structures [42]. This may further enhance hemostasis by reducing the surface area available, leading to higher local concentrations of coagulation factors on the cells.

PE/PS externalization is often measured using annexin V, which binds to negatively-charged membranes via electrostatic interactions, similarly to coagulation factors [43-45]. Often, annexin V positivity is described as PS externalization, but it can also bind PE. Another protein, lactadherin, is reportedly PS-specific [46, 47]. During inflammation, apoptotic innate immune cells can lose membrane asymmetry, also leading to PE/PS externalization [34]. This is particularly relevant in the case of neutrophils, which undergo apoptosis following infection. Neutrophil apoptosis occurs alongside elevated

coagulation during systemic infection and sepsis, suggesting a mechanistic link between neutrophil aPL exposure and associated coagulation [48].

3. Generation and action of eoxPL in innate immunity.

Oxidized PCs with truncated FAs containing reactive aldehydes at the *sn2* position have long been known to be generated non-enzymatically [49-52]. They are detected in atheroma lesions and in the circulation during a myocardial infarction caused by plaque rupture [53]. In contrast, several families of enzymatically-oxidized PL (eoxPL), comprising eicosanoids/prostaglandins attached to PLs, were recently discovered. Circulating blood cells (neutrophils, platelets, eosinophils) and resident murine peritoneal macrophages generate eoxPL in a controlled manner by as part of the innate immune response [40, 54-59]. They form via the catalytic activities of lipoxygenases (LOX) as well as COX-1 (Supplementary Figure 2A). The most abundant eoxPL are PEs, but PC forms are also found, and low levels of PI analogs were recently detected in platelets [60]. PC and PE eoxPL form as both plasmalogen and acyl species.

For many years, LOX isoforms were best known to generate monohydroperoxide derivatives of free-acid PUFAs, primarily AA (HpETEs), but also DHA (HpDOHEs) and LA (HpODEs). Cellular glutathione peroxidases (GPx) rapidly reduce lipid hydroperoxides to form hydroxyeicosatetraenoic acids (HETEs), hydroxydocosahexanoic acids (HDOHEs), and hydroxyoctadecadienoic acids (HODEs), respectively. Positional isomers of these are generated in a cell-specific manner due to LOX expression patterns, e.g. by 5-LOX (5-HETE in neutrophils, monocytes), 15-LOX (15-HETE, 17-HDOHE in human eosinophils), and 12-LOX (12-HETE, 14-HDOHE in platelets). In mice, 15-LOX is represented by a 12/15-LOX gene product, with a similar expression pattern to human cells, that generates primarily 12-HETE and 14-HDOHE. This isoform is also highly

expressed in murine peritoneal macrophages and is inducible by Th2 cytokines. Since AA is present in PL in at least 10-fold higher concentrations in immune cells than other PUFAs, its monohydroxylated products are the most abundant; for example, 12-HETE is the predominant eicosanoid generated by human platelets.

Most LOXs will only utilize free-acid PUFA due to steric hindrance at the active site; however, the 15-LOX (12/15-LOX in mice) can uniquely utilize oxidize PLs [61]. Thus, eoxPLs generated via 5- or 12-LOXs require a cycle of hydrolysis and re-esterification, involving PLA₂ and MBOAT/LPAT activities, while those from the 15- or 12/15-LOX do not (Supplementary Figure 2B) [40, 56, 58]. This is consistent with observations that neutrophil or platelet HETE-PLs are generated following agonist activation of cells to trigger PLA₂, while eosinophil/macrophage HETE-PLs are detected under basal conditions [40, 56, 58, 59]. Similarly, eoxPL containing COX-1 products, such as prostaglandin E₂-PEs, require a cycle of hydrolysis and re-esterification [54].

Circulating innate immune cells and platelets generate eoxPL via coordinated signaling pathways mediated by receptor-dependent calcium mobilization within 2-5 minutes of activation [40, 54, 56]. Unlike free-acid oxylipins, the cells retain eoxPL within the membrane compartment and do not secrete them, suggesting a different biological role to their free analogs. Up to 2016, only small numbers of eoxPL were known, typically 4-6 per cell type. However, studies using high-resolution lipidomics recently showed that human platelets generate over 100 unique molecular forms [30]. The most abundant are 12-HETE-PLs, in line with the relative abundance of platelet 12-HETE. However, multiple oxidized forms of AA are detected in platelet eoxPL, along with mono-hydroxylated forms of rarer FAs (DHA, EPA, and others) [30]. EoxPLs that contain thromboxane B₂ have never been detected, despite the abundance of this platelet lipid, suggesting that not all oxylipins are substrates for re-esterification. A recent study demonstrated that specific

eicosanoids serve as substrates for acyl-CoA synthetases, which activate eicosanoids to produce an eicosanoid-CoA species. This eicosanoid-CoA species then can be utilized by an acyltransferase (like MBOATs) to esterify the eicosanoid into PL species, indicating that cellular control of eoxPL formation may occur at the level of esterification [62]. Furthermore, correlation analysis of eoxPL from platelets shows clustering into structurally-related groups, further supporting the idea that their formation is dictated at least in part by the oxidized FA structure and resulting specificity of esterification pathways [39].

eoxPLs display multiple biological activities relevant to innate immunity and inflammation regulation. In neutrophils, HETE-PEs promote antibacterial activities, including enhancing IL-8 release and NET formation, while macrophage KETE-PEs are low affinity agonists for PPAR γ [56, 57]. Recently, HETE-PEs and -PCs were shown to be potently pro-coagulant due to their enhancement of PS binding to coagulation factors (Supplementary Figure 3A) [39]. In this, the lipids' oxidation epitope enhances the overall electronegative character of the plasma membrane, facilitating calcium interactions with PS that are required for blood clotting. Related to this, eoxPL are significantly elevated in circulating cells in patients with the thrombotic disorder antiphospholipid syndrome (aPLS), and mice lacking either platelet or eosinophil LOX isoforms display a venous bleeding defect that can be corrected by eoxPL administration [39, 63]. eoxPL also are immunogenic in vivo, with elevated eoxPL-specific IgG detected in aPLS patients [39].

4. PAF, a plasmenylcholine with potent signaling activities

PAF is a structurally unique plasmenylcholine (alkyl bond at *sn1*) with an acetyl group at the *sn2* position that signals at extremely low concentrations (around 10^{-14} M) via the PAF receptor, a GPCR, stimulating multiple signaling cascades (Supplementary Figure 3B).

The PAF receptor is expressed by numerous blood cells including neutrophils, monocytes, eosinophils, and platelets [64]. It triggers multiple biological actions, including aggregation, calcium mobilization, superoxide release, eicosanoid generation, chemotaxis, adhesion, and cytokine generation in multiple circulating cell types, including platelets, neutrophils, and eosinophils [65].

PAF is generated via both remodeling and de novo pathways [66, 67] (Supplementary Figure 3B). Remodeling is the prominent biological mechanism and involves replacing an acyl with an acetyl moiety at the *sn2* position. De novo generation involves acetylation, dephosphorylation, and PC addition to a lysoPA, with this last step thought to be the source of endogenous PAF for homeostatic functions [67].

Two enzymes are involved in dynamically modulating PAF levels in the circulation. An acetyl transferase generated by circulating neutrophils and monocytes metabolizes lyso-PAF to PAF through addition of an acetyl group at the *sn2* position. This is the prominent source of PAF during inflammation and allergies [68]. On the other hand, PAF is removed by the action of PAF acetyl hydrolase (PAF-AH), a circulating enzyme generated by multiple cell types, including innate immune blood cells. Platelets are also a source of PAF that is generated during aggregation.

Several studies have evaluated the potential for circulating PAF to act as a biomarker/predictor of cardiovascular disease or events. Despite its association with higher disease burden, PAF-AH itself may represent a target for cardiovascular prevention, since PAF metabolism can lead to generation of pro-inflammatory lipids, such as lysoPC and lysoPAF. This concept led to extensive evaluation of reversible PAF-AH inhibitors, such as darapladib, in trials; however, these yielded mixed results, lacking sufficient affect on primary endpoints [24].

5. *PLC in innate immune signaling.*

PLCs are a class of enzymes that cleave PLs on the glyceride side of the phosphodiester bond, leading to diacylglycerol (DAG) formation and release of the phosphorylated PL headgroup (Figure 3A). PI-specific PLCs are the most important PL in immune cells, comprising approximately thirteen enzymes represented by six isotypes that play key roles in signal transduction [69]. PI-PLC hydrolyses PI-4,5-bisphosphate (PIP₂) to form DAG and Ins(1,4,5)P₃, which in turn can activate PKC and mobilize intracellular calcium, respectively to regulate both acute and chronic responses in immune cells. PLCs are activated by GPCRs and tyrosine kinase-linked receptors (RTKs) in platelets and leukocytes. RTK-activated PLC γ subtypes are the most important in these cells, playing key roles in supporting neutrophil respiratory burst, phagocytosis, adhesion, and cell migration. PLC γ 2 is important for hemostasis: mice lacking this isoform exhibit a bleeding defect, with glycoprotein VI- and CLEC-2-dependent platelet responses abolished [70-74]. This isoform also plays a role in platelet-dependent clot retraction [75]. Mast cells, NK cells, and murine neutrophils express both PLC γ 1 and PLC γ 2 isoforms [76-79]. In all these cells, these PLC γ isoforms modulate TLR signaling through multiple mechanisms (reviewed in [80]). In addition, several GPCR-activated PLC β isoforms have been detected in immune cells, with these playing key roles in the differentiation and activation of both innate and adaptive immune responses, with increases in free calcium and PKC activity being essential [69, 81]. Recently, PLC δ was shown to act as a negative regulator of macrophage-mediated phagocytosis [82].

6. *PLD signaling in innate immune cells.*

PLD enzymes hydrolyze PC, the most abundant external-facing PL, generating phosphatidic acid (PA) and choline (Figure 3A). They are activated in response to several GPCRs and RTKs (reviewed in: [83]). PA has a number of critical functions in innate immune signaling. With a small, negatively-charged headgroup, PA induces negative curvature thought to be important for production of vesicles and membrane fusion, thus playing a central role in membrane remodeling [84]. PA also acts as an intracellular signal, binding several cellular proteins including the GEFs DOCK2 and SOS, which activate Rac1 and Ras, whilst the PA-binding domain of cyclicAMP-PDE4A1 has been clearly defined [85-87]. Innate immune cells and platelets express the isomers PLD1 and PLD2, both known to play key roles in thrombotic disease [88-91]. *Pld1*^{-/-} mice exhibit reduced $\alpha_{IIb}\beta_3$ -dependent platelet activation, rendering them resistant to pathological hemostasis events such as strokes and pulmonary embolisms [92]. Studies in knockout mice and the use of isoform-specific inhibitors indicated that PLD1, but not PLD2 is required for neutrophil ROS production [93]. The concerted action of PLD and PLA₂ leads to formation of lysoPA, generated by thrombin-stimulated platelets, and is potentially able to stimulate aggregation itself via GPCR signaling [94] (Figure 6A); this is in addition to lysoPA generated by the action of autotaxin (lyso-PLD) upon lysoPC.

7. *Phosphoinositides :*

The seven phosphoinositides (PI(3)P, PI(4)P, PI(5)P, PI_{3,4}P₂, PI_(4,5)P₂, PI_(3,5)P₂ and PIP₃) each demonstrate signaling capabilities (Figure 3B). The phosphoinositide 3-kinase (PI3K) enzymes catalyze hydroxyl group phosphorylation at the 3-position of the inositol headgroup of PI and its phosphorylated derivatives. The eight mammalian PI3K enzymes sort into three groups: Class I (PI3K α , β , γ , δ), Class II (PI3K $C2\alpha$, β , γ) and Class

III (vps34). Class I PI3Ks phosphorylate PIP2 to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). Class II enzymes can phosphorylate both PI or PI4P to generate PI3P or PI(3,4)P2 on endosomes and the plasma membrane. Finally, Class III enzyme solely phosphorylates PI, producing PI(3)P on endosomes, which also plays a key role in autophagy [95].

Class IA PI3Ks (PI3K α and PI3K β) are ubiquitously expressed, whilst PI3K δ is restricted to myeloid and lymphoid cells (reviewed in [96]). The Class IB isoform, PI3K γ , is differentially expressed, but is most highly expressed in myeloid cells. Each Class IA PI3K α , β , and δ comprises one of five homologous regulatory subunits (p85 α , p85 β , p50 α , p55 α or p55 γ) complexed with a single p110 subunit, whereas PI3K γ is made up of p110 γ and either a p84 or p101 regulatory subunit. The soluble PI3Ks are recruited to the plasma membrane following receptor occupation. Antigen receptors, Fc receptors, integrins, cytokine receptors, and RTKs such as colony-stimulating factor (CSF) stimulate PI3K α , β , and γ activity through tyrosine phosphorylation with the SH2 domains in the regulatory domain, binding to the phosphotyrosine residues. GPCRs such as fMLP activate PI3K γ through G $\beta\gamma$ subunits binding to p84 or p101 and to p110 γ . G $\beta\gamma$ has also been shown to bind to and thus activate p110 β . Membrane association is further promoted by binding of the Ras-binding domain of the p110 subunits, associating with either Ras (p110 α , δ , γ) or Rac (p110 β).

PI3K activity generates PIP3, which binds to PH domains in target proteins and activates signaling cascades. The phosphorylation and activation of the serine kinase PKB (also known as AKT) is an ubiquitous response to PI3K activation that can activate TORC1 to promote cell growth, but also activates a metabolic shift, as in CTL activation [97]. PKB also phosphorylates and inhibits the nuclear localization of FOXO transcription factors

critical in lymphocyte differentiation. PIP3 is dephosphorylated to regenerate PI(4,5)P2 by PTEN, but can also generate PI(3,4)P2 through SHIP1/2 activity [98]. PI(4,5)P2 also binds PH domain-containing proteins and, whilst many of these also bind PIP3, there is PI(3,4)P2 selectivity notably DAPP-1 [96]. Lipidomic analysis has shown that PIP3 is not a single molecular species, with C36:1, C36:2, C36:3, C36:4, C38:2, C38:3, C38:4 and C38:5 being detected in neutrophils and macrophages [99, 100]. This variety may enable further selectivity in binding of different PH-domain proteins and thus downstream signaling [100]

The field's understanding of the distinct roles of PI3K isoforms has benefited from generation of knockout mice and selective small molecule inhibitors. These approaches have provided evidence that PI3K γ is critical for extravasation and migration of neutrophils, monocytes, and eosinophils to inflammatory sites, with additional PI3K δ involvement possibly occurring through integrin-mediated activation. PI3K β and δ regulate immune cell spreading and activation by mediating cell surface attachment to extracellular matrix or pathogens, such as fungi, and both isoforms are necessary for a maximal ROS response [101]. In addition, PI3K activity stimulates Rac through PIP3 regulation of GEFs and GAPs, leading to regulation of the actin cytoskeleton.

The development, proliferation, and differentiation of B and T cells are stringently regulated by PI3K activity, which involves PIP3-regulated signaling, including control of FOXO/FoxP3. However, PIP3 also activates BTK, which phosphorylates and stimulates PLC γ -producing DAG and calcium signaling that are important for both B cell proliferation and cytokine synthesis [102]. There has been extensive development of PI3K inhibitors, with a number approved for clinical use [103], particularly PI3K δ inhibitors to treat chronic lymphocytic leukemia [104]. The importance of the PI3K pathway in human immune

disorders is further emphasized by the presence of activating mutations in p110 δ in a number of primary immune deficiency patients, who suffer particularly from recurring respiratory tract infections [105]. In contrast, deficiency in PI3K δ signaling decreases Treg cell number and increases neutrophil numbers, which can cause an increase in endotoxic shock-related mortality [106].

8. Phospholipids as ligands for innate-like T cell populations.

Most immunologists consider T cell immunity only in the context of peptide recognition when presented by MHC molecules [107]. However, the family of MHC class I-like antigen-presenting molecules termed CD1 is ideally suited for capturing and displaying lipids for T cell surveillance [2]. This evolutionarily-conserved CD1 antigen-presenting family is sub-divided into group 1 (CD1a, CD1b, and CD1c) and group 2 (CD1d) members. CD1 group 1 is absent in mice, thus our understanding of their role in immunity is less developed than CD1d, which is present in mice and humans [108, 109]. Each CD1 isoform has distinct atomic architectures [110] and cellular trafficking properties, thereby enabling broad array of lipid classes (including PLs) that can either represent foreign or self-antigens to be bound.

Distinct populations of T cells subsequently recognize these CD1-lipid complexes. Lipid-reactive T cells play key roles in protective immunity, cancer, autoimmunity, and allergies. The most well-characterized lipid-reactive T cells are the innate-like type I Natural Killer T cells (NKT cells), which are restricted to the CD1d molecule [2]. Type I NKT cells rapidly secrete high amounts of a range of cytokines upon activation and have shown broad therapeutic promise [109].

Lipid-reactive T cells can specifically respond to a range of CD1-presented lipids, including PLs, a major lipid component of mammalian membranes. Indeed, subsets of type I NKT cells have exhibited autoreactivity towards PI, PE, and plasmalogen lysoPE (p-lysoPE) [111, 112] [113]. While the physiological role of NKT autoreactivity towards common PLs remain unclear, reactivity towards certain lysoPLs, including lysoPC, are reported to be associated with inflammatory responses [114]. Interestingly, p-lysoPE is an ether-linked PL that is generated in the peroxisome and thought to play a role in the development of NKT cells [115]. Further, infection by Hepatitis B virus (HBV) alters the levels of lipids, including lysoPLs within hepatocytes, which subsequently results in NKT cell activation, thereby playing a role in protective immunity [116].

The functional properties, T cell receptor (TCR) repertoire, and antigen reactivity of type II NKT cells are distinct from type I NKT cells [1]. Type II NKT cells are also described as responsive to bacterially-derived PLs, including PI and PG from *M. tuberculosis*, suggesting a role in protective immunity towards this devastating pathogen [4]. PG from *Listeria monocytogenes* was also shown to potently activate a subset of type II NKT cells [3].

Members of CD1 group 1 also present PLs, and T cell reactivity towards certain pollen-derived PLs has been associated with allergies [117, 118]. Further, CD1a, which is expressed predominantly in the skin and naturally present skin-derived oils [119], is associated with contact hypersensitivities [120] as well as bee sting allergies due to phospholipase activity derived from bee venom-generating LPLs that cause an inflammatory skin response [121, 122]. Moreover, aberrant reactivity towards CD1a-presented PLs is also implicated in allergies towards house dust mites [123]. CD1b-mediated autoreactivity towards rare mammalian PLs, including PG, have also recently been described and implicated in some autoimmune conditions [124] [125].

The conundrum of how lipid-reactive T cells distinguish between rare and common PLs was recently determined to arise from the ideal shape and charge complementarity between the TCR and CD1b-PG complex, whereas the polar headgroups of more common PLs are sterically-disfavored from binding to the TCR [5]. Further, T cell targeting of specific PLs, including methyl-lysophosphatidic acid (mLPA), when presented by CD1c+ leukemic cells is considered to play a role in anti-tumor immunity and hence may represent a likely candidate for novel immunotherapeutic approaches [6]. Collectively, T cell reactivity towards CD1-restricted PLs is emerging as a component of protective and aberrant immunity, although the breadth of reactivity towards distinct PLs and their role in physiology and pathophysiology remains germinal.

9. Summary

Herein, we summarized the major PL-dependent signaling pathways in circulating innate immune cells and their recognition by specialized populations of innate T cells during challenge. The importance of PLs in the acute response to injury extends far beyond their structural role, as they provide a major repository for activation-dependent signaling molecules such as PGs, oxylipins, and PAF during acute injury and inflammation and through T cell recognition, representing a way for the body to sense danger and appropriately respond during challenge. Importantly, PL-dependent signaling is not only mediated through long-known soluble paracrine mediators (oxylipins such as prostaglandins), but also through changing membrane biophysics that alter protein binding and activation both on the outside and inside of cells (eoxPL, phosphoinositides). Many questions remain in the emerging study of PL signaling, particularly relating to how PLs dynamically change, the role(s) of CoA-dependent versus –independent recycling pathways in their metabolism, how tissues recognize endogenous changes and respond,

and ultimately how to harness these processes to improve detection, diagnosis, and treatment of human disease associated with aberrant PL generation and signaling.

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Figure Legends

Figure 1. Structures of the major PL classes. Panel A. The five phospholipid classes are shown: PS: phosphatidylserine, PI: phosphatidylinositol, PG: phosphatidylglycerol, PE: phosphatidylethanolamine, PC: phosphatidylcholine. Panel B. Ether and plasmalogen PLs. Panels C,D. Structures of the common fatty acids in PLs.

Figure 2. PL cleavage and PL asymmetry in innate immune cells. Panel A. Phospholipases hydrolyze PLs and are named by the site of hydrolysis, as shown. Panel

B. Platelets externalize PE and PS on activation, providing a negatively charged surface that supports coagulation factor binding via calcium-dependent mechanisms.

Figure 3. PLC, PLD and Phosphoinositide pathways. Panel A. PLC and PLD generate lipid intermediates that promote innate immunity. Panel B. PI is phosphorylated/dephosphorylated by a series of known enzymes, generating a complex array of transient lipid molecular species which display potent bioactivity.

References

1. Rossjohn, J., et al., *Recognition of CD1d-restricted antigens by natural killer T cells*. Nat Rev Immunol, 2012. **12**(12): p. 845-857.
2. Van Rhijn, I., et al., *Lipid and small-molecule display by CD1 and MR1*. Nat Rev Immunol, 2015. **15**(10): p. 643-654.
3. Wolf, B.J., et al., *Identification of a Potent Microbial Lipid Antigen for Diverse NKT Cells*. The Journal of Immunology, 2015. **195**(6): p. 2540-2551.
4. Tatituri, R.V., et al., *Recognition of microbial and mammalian phospholipid antigens by NKT cells with diverse TCRs*. Proc Natl Acad Sci U S A, 2013. **110**(5): p. 1827-32.
5. Shahine, A., et al., *A molecular basis of human T cell receptor autoreactivity toward self-phospholipids*. Sci Immunol, 2017. **2**(16).
6. Lepore, M., et al., *Targeting leukemia by CD1c-restricted T cells specific for a novel lipid antigen*. Oncoimmunology, 2015. **4**(3): p. e970463.
7. Lepore, M., et al., *A novel self-lipid antigen targets human T cells against CD1c(+) leukemias*. J Exp Med, 2014. **211**(7): p. 1363-77.
8. Haban, P., E. Zidekova, and J. Klvanova, *Supplementation with long-chain n-3 fatty acids in non-insulin-dependent diabetes mellitus (NIDDM) patients leads to*

- the lowering of oleic acid content in serum phospholipids.* Eur J Nutr, 2000. **39**(5): p. 201-6.
9. Siener, R., et al., *Change in the fatty acid pattern of erythrocyte membrane phospholipids after oral supplementation of specific fatty acids in patients with gastrointestinal diseases.* Eur J Clin Nutr, 2010. **64**(4): p. 410-8.
 10. Colas, R.A., et al., *Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue.* Am J Physiol Cell Physiol, 2014. **307**(1): p. C39-54.
 11. Kalish, B.T., et al., *Intravenous fish oil lipid emulsion promotes a shift toward anti-inflammatory proresolving lipid mediators.* Am J Physiol Gastrointest Liver Physiol, 2013. **305**(11): p. G818-28.
 12. Lundstrom, S.L., et al., *Lipid mediator serum profiles in asthmatics significantly shift following dietary supplementation with omega-3 fatty acids.* Mol Nutr Food Res, 2013. **57**(8): p. 1378-89.
 13. Mas, E., et al., *Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation.* Clin Chem, 2012. **58**(10): p. 1476-84.
 14. Psychogios, N., et al., *The human serum metabolome.* PLoS One, 2011. **6**(2): p. e16957.
 15. Skarke, C., et al., *Bioactive products formed in humans from fish oils.* J Lipid Res, 2015. **56**(9): p. 1808-20.
 16. Kennedy, E.P., *Biosynthesis of complex lipids.* Fed Proc, 1961. **20**: p. 934-40.
 17. Kennedy, E.P. and S.B. Weiss, *The function of cytidine coenzymes in the biosynthesis of phospholipides.* J Biol Chem, 1956. **222**(1): p. 193-214.
 18. Lands, W.E., *Metabolism of glycerolipides; a comparison of lecithin and triglyceride synthesis.* J Biol Chem, 1958. **231**(2): p. 883-8.

19. Marder, V.J., et al., *Analysis of thrombi retrieved from cerebral arteries of patients with acute ischemic stroke*. *Stroke*, 2006. **37**(8): p. 2086-93.
20. Niesten, J.M., et al., *Histopathologic composition of cerebral thrombi of acute stroke patients is correlated with stroke subtype and thrombus attenuation*. *PLoS One*, 2014. **9**(2): p. e88882.
21. Yunoki, K., et al., *Relationship of thrombus characteristics to the incidence of angiographically visible distal embolization in patients with ST-segment elevation myocardial infarction treated with thrombus aspiration*. *JACC Cardiovasc Interv*, 2013. **6**(4): p. 377-85.
22. Dennis, E.A., et al., *Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention*. *Chem Rev*, 2011. **111**(10): p. 6130-85.
23. Thomas, L.M. and B.J. Holub, *Eicosanoid-dependent and -independent formation of individual [¹⁴C]stearoyl-labelled lysophospholipids in collagen-stimulated human platelets*. *Biochim Biophys Acta*, 1991. **1081**(1): p. 92-8.
24. Stafforini, D.M. and G.A. Zimmerman, *Unraveling the PAF-AH/Lp-PLA2 controversy*. *J Lipid Res*, 2014. **55**(9): p. 1811-4.
25. Leslie, C.C., *Cytosolic phospholipase A(2): physiological function and role in disease*. *J Lipid Res*, 2015. **56**(8): p. 1386-402.
26. Wong, D.A., et al., *Discrete role for cytosolic phospholipase A(2)alpha in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A(2)*. *J Exp Med*, 2002. **196**(3): p. 349-57.
27. Yoda, E., et al., *Group VIB calcium-independent phospholipase A2 (iPLA2gamma) regulates platelet activation, hemostasis and thrombosis in mice*. *PLoS One*, 2014. **9**(10): p. e109409.
28. Cathcart, M.K., *Signal-activated phospholipase regulation of leukocyte chemotaxis*. *J Lipid Res*, 2009. **50 Suppl**: p. S231-6.

29. Murakami, M., et al., *A new era of secreted phospholipase A(2)*. J Lipid Res, 2015. **56**(7): p. 1248-61.
30. Slatter, D.A., et al., *Mapping the Human Platelet Lipidome Reveals Cytosolic Phospholipase A2 as a Regulator of Mitochondrial Bioenergetics during Activation*. Cell Metab, 2016. **23**(5): p. 930-44.
31. Bolen, A.L., et al., *The phospholipase A1 activity of lysophospholipase A-I links platelet activation to LPA production during blood coagulation*. J Lipid Res, 2011. **52**(5): p. 958-70.
32. Ramanadham, S., et al., *Calcium-independent phospholipases A2 and their roles in biological processes and diseases*. J Lipid Res, 2015. **56**(9): p. 1643-68.
33. Daleke, D.L., *Phospholipid flippases*. J Biol Chem, 2007. **282**(2): p. 821-5.
34. Ravichandran, K.S., *Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums*. J Exp Med, 2010. **207**(9): p. 1807-17.
35. Heemskerk, J.W., E.M. Bevers, and T. Lindhout, *Platelet activation and blood coagulation*. Thromb Haemost, 2002. **88**(2): p. 186-93.
36. Zwaal, R.F., P. Comfurius, and E.M. Bevers, *Surface exposure of phosphatidylserine in pathological cells*. Cell Mol Life Sci, 2005. **62**(9): p. 971-88.
37. Weiss, H.J., *Scott syndrome: a disorder of platelet coagulant activity*. Semin Hematol, 1994. **31**(4): p. 312-9.
38. Picollo, A., M. Malvezzi, and A. Accardi, *TMEM16 proteins: unknown structure and confusing functions*. J Mol Biol, 2015. **427**(1): p. 94-105.
39. Lauder, S.N., et al., *Networks of enzymatically oxidized membrane lipids support calcium-dependent coagulation factor binding to maintain hemostasis*. Sci Signal, 2017. **10**(507).
40. Morgan, L.T., et al., *Thrombin-activated human platelets acutely generate oxidized docosahexaenoic-acid-containing phospholipids via 12-lipoxygenase*. Biochem J, 2010. **431**(1): p. 141-8.

41. Clark, S.R., et al., *Characterization of platelet aminophospholipid externalization reveals fatty acids as molecular determinants that regulate coagulation*. Proc Natl Acad Sci U S A, 2013. **110**(15): p. 5875-80.
42. Whyte, C.S., et al., *Plasminogen associates with phosphatidylserine-exposing platelets and contributes to thrombus lysis under flow*. Blood, 2015. **125**(16): p. 2568-78.
43. Andree, H.A., et al., *Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers*. J Biol Chem, 1990. **265**(9): p. 4923-8.
44. Koopman, G., et al., *Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis*. Blood, 1994. **84**(5): p. 1415-20.
45. Meers, P. and T. Mealy, *Phospholipid determinants for annexin V binding sites and the role of tryptophan 187*. Biochemistry, 1994. **33**(19): p. 5829-37.
46. Otzen, D.E., et al., *Lactadherin binds to phosphatidylserine-containing vesicles in a two-step mechanism sensitive to vesicle size and composition*. Biochim Biophys Acta, 2012. **1818**(4): p. 1019-27.
47. Shi, J. and G.E. Gilbert, *Lactadherin inhibits enzyme complexes of blood coagulation by competing for phospholipid-binding sites*. Blood, 2003. **101**(7): p. 2628-36.
48. Iba, T., et al., *Neutrophil cell death in response to infection and its relation to coagulation*. J Intensive Care, 2013. **1**(1): p. 13.
49. Cole, A.L., et al., *Oxidized phospholipid-induced endothelial cell/monocyte interaction is mediated by a cAMP-dependent R-Ras/PI3-kinase pathway*. Arterioscler Thromb Vasc Biol, 2003. **23**(8): p. 1384-90.
50. Lee, H., et al., *Role for peroxisome proliferator-activated receptor alpha in oxidized phospholipid-induced synthesis of monocyte chemotactic protein-1 and interleukin-8 by endothelial cells*. Circ Res, 2000. **87**(6): p. 516-21.

51. Loidl, A., et al., *Oxidized phospholipids in minimally modified low density lipoprotein induce apoptotic signaling via activation of acid sphingomyelinase in arterial smooth muscle cells*. J Biol Chem, 2003. **278**(35): p. 32921-8.
52. O'Donnell, V.B. and R.C. Murphy, *New families of bioactive oxidized phospholipids generated by immune cells: identification and signaling actions*. Blood, 2012. **120**(10): p. 1985-92.
53. DeFilippis, A.P., et al., *Circulating levels of plasminogen and oxidized phospholipids bound to plasminogen distinguish between atherothrombotic and non-atherothrombotic myocardial infarction*. J Thromb Thrombolysis, 2016. **42**(1): p. 61-76.
54. Aldrovandi, M., et al., *Human platelets generate phospholipid-esterified prostaglandins via cyclooxygenase-1 that are inhibited by low dose aspirin supplementation*. J Lipid Res, 2013. **54**(11): p. 3085-97.
55. Aldrovandi, M., et al., *DioxolaneA3-phosphatidylethanolamines are generated by human platelets and stimulate neutrophil integrin expression*. Redox Biol, 2017. **11**: p. 663-672.
56. Clark, S.R., et al., *Esterified eicosanoids are acutely generated by 5-lipoxygenase in primary human neutrophils and in human and murine infection*. Blood, 2011. **117**(6): p. 2033-43.
57. Hammond, V.J., et al., *Novel keto-phospholipids are generated by monocytes and macrophages, detected in cystic fibrosis, and activate peroxisome proliferator-activated receptor-gamma*. J Biol Chem, 2012. **287**(50): p. 41651-66.
58. Maskrey, B.H., et al., *Activated platelets and monocytes generate four hydroxyphosphatidylethanolamines via lipoxygenase*. J Biol Chem, 2007. **282**(28): p. 20151-63.

59. Morgan, A.H., et al., *Phosphatidylethanolamine-esterified eicosanoids in the mouse: tissue localization and inflammation-dependent formation in Th-2 disease*. J Biol Chem, 2009. **284**(32): p. 21185-91.
60. O'Connor, A., et al., *LipidFinder: A computational workflow for discovery of lipids identifies eicosanoid-phosphoinositides in platelets*. JCI Insight, 2017. **2**(7): p. e91634.
61. Kuhn, H. and V.B. O'Donnell, *Inflammation and immune regulation by 12/15-lipoxygenases*. Prog Lipid Res, 2006. **45**(4): p. 334-56.
62. Klett, E.L., et al., *Long-chain acyl-CoA synthetase isoforms differ in preferences for eicosanoid species and long-chain fatty acids*. J Lipid Res, 2017. **58**(5): p. 884-894.
63. Uderhardt, S., et al., *Enzymatic lipid oxidation by eosinophils propagates coagulation, hemostasis, and thrombotic disease*. J Exp Med, 2017. **214**(7): p. 2121-2138.
64. Montrucchio, G., G. Alloatti, and G. Camussi, *Role of platelet-activating factor in cardiovascular pathophysiology*. Physiol Rev, 2000. **80**(4): p. 1669-99.
65. Barnes, P.J., K.F. Chung, and C.P. Page, *Platelet-activating factor as a mediator of allergic disease*. J Allergy Clin Immunol, 1988. **81**(5 Pt 1): p. 919-34.
66. Snyder, F., *Chemical and biochemical aspects of platelet activating factor: a novel class of acetylated ether-linked choline-phospholipids*. Med Res Rev, 1985. **5**(1): p. 107-40.
67. Snyder, F., *Platelet-activating factor and related acetylated lipids as potent biologically active cellular mediators*. Am J Physiol, 1990. **259**(5 Pt 1): p. C697-708.
68. Palur Ramakrishnan, A.V., et al., *Platelet activating factor: A potential biomarker in acute coronary syndrome?* Cardiovasc Ther, 2017. **35**(1): p. 64-70.

69. Cocco, L., et al., *Phosphoinositide-specific phospholipase C in health and disease*. J Lipid Res, 2015. **56**(10): p. 1853-60.
70. Zheng, Y., et al., *Restoration of responsiveness of phospholipase Cgamma2-deficient platelets by enforced expression of phospholipase Cgamma1*. PLoS One, 2015. **10**(3): p. e0119739.
71. Mangin, P., et al., *A PLC gamma 2-independent platelet collagen aggregation requiring functional association of GPVI and integrin alpha2beta1*. FEBS Lett, 2003. **542**(1-3): p. 53-9.
72. Munnix, I.C., et al., *The glycoprotein VI-phospholipase Cgamma2 signaling pathway controls thrombus formation induced by collagen and tissue factor in vitro and in vivo*. Arterioscler Thromb Vasc Biol, 2005. **25**(12): p. 2673-8.
73. Suzuki-Inoue, K., et al., *A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2*. Blood, 2006. **107**(2): p. 542-9.
74. Suzuki-Inoue, K., et al., *Murine GPVI stimulates weak integrin activation in PLCgamma2-/- platelets: involvement of PLCgamma1 and PI3-kinase*. Blood, 2003. **102**(4): p. 1367-73.
75. Suzuki-Inoue, K., et al., *Involvement of Src kinases and PLCgamma2 in clot retraction*. Thromb Res, 2007. **120**(2): p. 251-8.
76. Caraux, A., et al., *Phospholipase C-gamma2 is essential for NK cell cytotoxicity and innate immunity to malignant and virally infected cells*. Blood, 2006. **107**(3): p. 994-1002.
77. Jakus, Z., et al., *Critical role of phospholipase Cgamma2 in integrin and Fc receptor-mediated neutrophil functions and the effector phase of autoimmune arthritis*. J Exp Med, 2009. **206**(3): p. 577-93.
78. Ting, A.T., et al., *Fc gamma receptor activation induces the tyrosine phosphorylation of both phospholipase C (PLC)-gamma 1 and PLC-gamma 2 in natural killer cells*. J Exp Med, 1992. **176**(6): p. 1751-5.

79. Wen, R., et al., *Phospholipase C gamma 2 is essential for specific functions of Fc epsilon R and Fc gamma R*. J Immunol, 2002. **169**(12): p. 6743-52.
80. Bae, Y.S., et al., *Phospholipase Cgamma in Toll-like receptor-mediated inflammation and innate immunity*. Adv Biol Regul, 2017. **63**: p. 92-97.
81. Kawakami, T. and W. Xiao, *Phospholipase C-beta in immune cells*. Adv Biol Regul, 2013. **53**(3): p. 249-57.
82. Kudo, K., et al., *Phospholipase C delta1 in macrophages negatively regulates TLR4-induced proinflammatory cytokine production and Fcgamma receptor-mediated phagocytosis*. Adv Biol Regul, 2016. **61**: p. 68-79.
83. Selvy, P.E., et al., *Phospholipase D: enzymology, functionality, and chemical modulation*. Chem Rev, 2011. **111**(10): p. 6064-119.
84. Ammar, M.R., et al., *Lipids in Regulated Exocytosis: What are They Doing?* Front Endocrinol (Lausanne), 2013. **4**: p. 125.
85. Baillie, G.S., et al., *TAPAS-1, a novel microdomain within the unique N-terminal region of the PDE4A1 cAMP-specific phosphodiesterase that allows rapid, Ca²⁺-triggered membrane association with selectivity for interaction with phosphatidic acid*. J Biol Chem, 2002. **277**(31): p. 28298-309.
86. Nishikimi, A., et al., *Sequential regulation of DOCK2 dynamics by two phospholipids during neutrophil chemotaxis*. Science, 2009. **324**(5925): p. 384-7.
87. Zhao, C., et al., *Phospholipase D2-generated phosphatidic acid couples EGFR stimulation to Ras activation by Sos*. Nat Cell Biol, 2007. **9**(6): p. 706-12.
88. Elvers, M., et al., *Impaired alpha(IIb)beta(3) integrin activation and shear-dependent thrombus formation in mice lacking phospholipase D1*. Sci Signal, 2010. **3**(103): p. ra1.
89. Hong, K.W., et al., *Non-synonymous single-nucleotide polymorphisms associated with blood pressure and hypertension*. J Hum Hypertens, 2010. **24**(11): p. 763-74.

90. Schonberger, T., et al., *Pivotal role of phospholipase D1 in tumor necrosis factor- α -mediated inflammation and scar formation after myocardial ischemia and reperfusion in mice*. *Am J Pathol*, 2014. **184**(9): p. 2450-64.
91. Stegner, D., et al., *Pharmacological inhibition of phospholipase D protects mice from occlusive thrombus formation and ischemic stroke--brief report*. *Arterioscler Thromb Vasc Biol*, 2013. **33**(9): p. 2212-7.
92. Elvers, M., et al., *Impaired $\alpha_{IIb}\beta_3$ Integrin Activation and Shear-Dependent Thrombus Formation in Mice Lacking Phospholipase D1*. *Science Signaling*, 2010. **3**(103): p. 1-10.
93. Norton, L.J., et al., *PLD1 rather than PLD2 regulates phorbol-ester-, adhesion-dependent and Fc $\{\gamma\}$ -receptor-stimulated ROS production in neutrophils*. *J Cell Sci*, 2011. **124**(Pt 12): p. 1973-83.
94. Eichholtz, T., et al., *The bioactive phospholipid lysophosphatidic acid is released from activated platelets*. *Biochem J*, 1993. **291** (Pt 3): p. 677-80.
95. Axe, E.L., et al., *Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum*. *The Journal of Cell Biology*, 2008. **182**(4): p. 685-701.
96. Hawkins, P.T. and L.R. Stephens, *PI3K signalling in inflammation*. *Biochimica et Biophysica Acta*, 2015. **1851**: p. 882-897.
97. Engelmann, J.A., J. Luo, and L.C. Cantley, *The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism*. *Nature Reviews Genetics*, 2006. **7**: p. 606-619.
98. Rudge, S.A. and M.J. Wakelam, *Phosphatidylinositolphosphate phosphatase activities and cancer*. *J Lipid Res*, 2016. **57**(2): p. 176-92.
99. Milne, S.B., et al., *A targeted mass spectrometric analysis of phosphatidylinositol phosphate species*. *Journal of Lipid Research*, 2005. **46**: p. 1796-1802.

100. Wakelam, M.J.O., *The uses and limitations of the analysis of cellular phosphoinositides by lipidomic and imaging methodologies*. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 2014. **1841**(8): p. 1102-1107.
101. Kulkarni, S., et al., *PI3Kbeta plays a critical role in neutrophil activation by immune complexes*. *Sci Signal*, 2011. **4**(168): p. ra23.
102. Fruman, D.A. and G. Bismuth, *Fine tuning the immune response with PI3K*. *Immunol Rev*, 2009. **228**(1): p. 253-72.
103. Fruman, D.A., et al., *The PI3K Pathway in Human Disease*. *Cell*, 2017. **170**(4): p. 605-635.
104. Pongas, G. and B.D. Cheson, *PI3K signaling pathway in normal B cells and indolent B-cell malignancies*. *Semin Oncol*, 2016. **43**(6): p. 647-654.
105. Lucas, C.L., et al., *PI3Kdelta and primary immunodeficiencies*. *Nat Rev Immunol*, 2016. **16**(11): p. 702-714.
106. Okeke, E.B., et al., *Deficiency of Phosphatidylinositol 3-Kinase delta Signaling Leads to Diminished Numbers of Regulatory T Cells and Increased Neutrophil Activity Resulting in Mortality Due to Endotoxic Shock*. *J Immunol*, 2017. **199**(3): p. 1086-1095.
107. Rossjohn, J., et al., *T cell antigen receptor recognition of antigen-presenting molecules*. *Annu Rev Immunol*, 2015. **33**: p. 169-200.
108. Godfrey, D.I., et al., *Antigen recognition by CD1d-restricted NKT T cell receptors*. *Semin Immunol*, 2010. **22**(2): p. 61-67.
109. Godfrey, D.I., et al., *The burgeoning family of unconventional T cells*. *Nat Immunol*, 2015. **16**(11): p. 1114-1123.
110. Adams, E.J. and A.M. Luoma, *The Adaptable Major Histocompatibility Complex (MHC) Fold: Structure and Function of Nonclassical and MHC Class I-Like Molecules*. *Annual Review of Immunology*, 2013. **31**(1): p. 529-561.

111. Gapin, L., D.I. Godfrey, and J. Rossjohn, *Natural Killer T cell obsession with self-antigens*. Current Opinion in Immunology, 2013. **25**(2): p. 168-173.
112. Mallewaey, T., et al., *A Molecular Basis for NKT Cell Recognition of CD1d-Self-Antigen*. Immunity, 2011. **34**(3): p. 315-326.
113. Gumperz, J.E., et al., *Murine CD1d-Restricted T Cell Recognition of Cellular Lipids*. Immunity, 2000. **12**(2): p. 211-221.
114. Fox, L.M., et al., *Recognition of Lyso-Phospholipids by Human Natural Killer T Lymphocytes*. PLoS Biol, 2009. **7**(10): p. e1000228.
115. Facciotti, F., et al., *Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus*. Nature Immunology, 2012. **13**(5): p. 474-480.
116. Zeissig, S., et al., *Hepatitis B virus-induced lipid alterations contribute to natural killer T cell-dependent protective immunity*. Nature Medicine, 2012. **18**(7): p. 1060-1068.
117. Agea, E., et al., *Human CD1-restricted T cell recognition of lipids from pollens*. The Journal of Experimental Medicine, 2005. **202**(2): p. 295-308.
118. Russano, A.M., et al., *Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted gammadelta T cells*. J Allergy Clin Immunol, 2006. **117**(5): p. 1178-84.
119. de Jong, A., et al., *CD1a-autoreactive T cells recognize natural skin oils that function as headless antigens*. Nat Immunol, 2014. **15**(2): p. 177-85.
120. Kim, J.H., et al., *CD1a on Langerhans cells controls inflammatory skin disease*. Nat Immunol, 2016. **17**(10): p. 1159-1166.
121. Subramaniam, S., et al., *Elevated and cross-responsive CD1a-reactive T cells in bee and wasp venom allergic individuals*. European Journal of Immunology, 2016. **46**(1): p. 242-252.

122. Bourgeois, E.A., et al., *Bee venom processes human skin lipids for presentation by CD1a*. J Exp Med, 2015.
123. Jarrett, R., et al., *Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite–derived phospholipase*. Science Translational Medicine, 2016. **8**(325): p. 325ra18-325ra18.
124. Van Rhijn, I., et al., *Human autoreactive T cells recognize CD1b and phospholipids*. Proceedings of the National Academy of Sciences, 2016. **113**(2): p. 380-385.
125. Bagchi, S., et al., *CD1b-autoreactive T cells contribute to hyperlipidemia-induced skin inflammation in mice*. The Journal of Clinical Investigation, 2017. **127**(6).

Figure 1

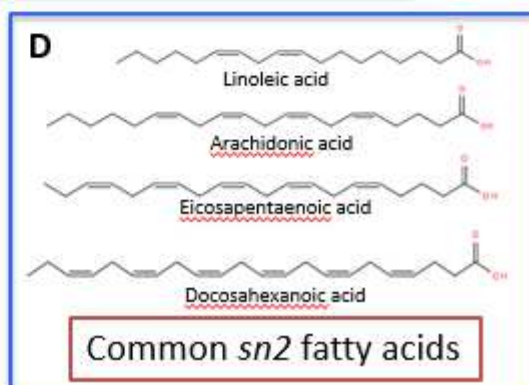
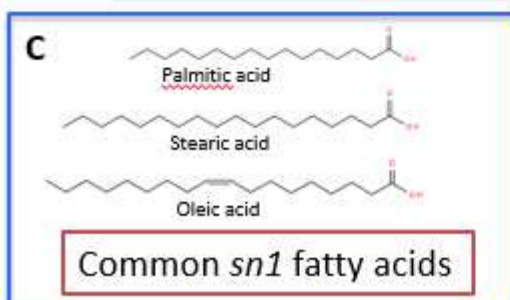
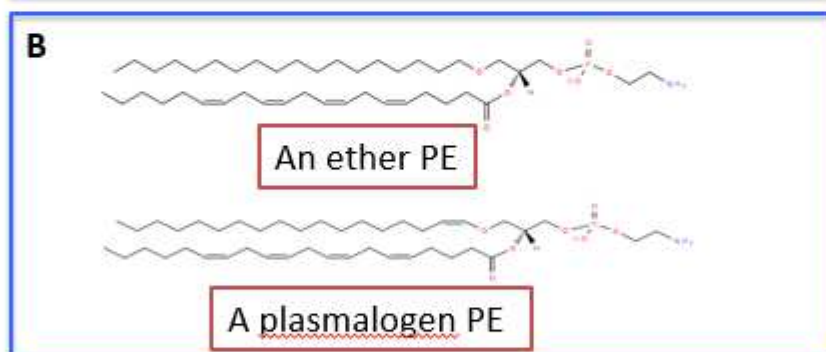
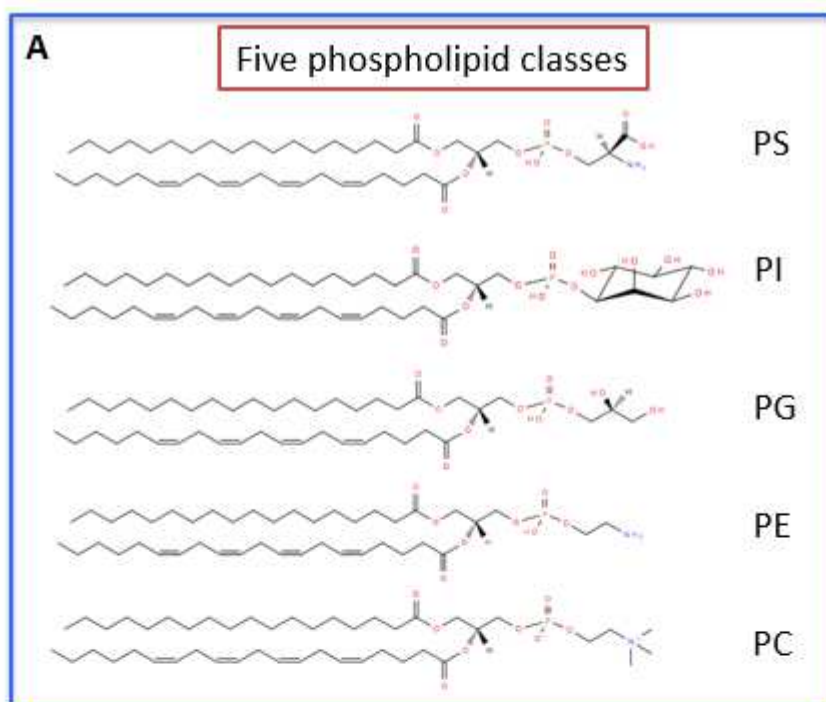


Figure 2

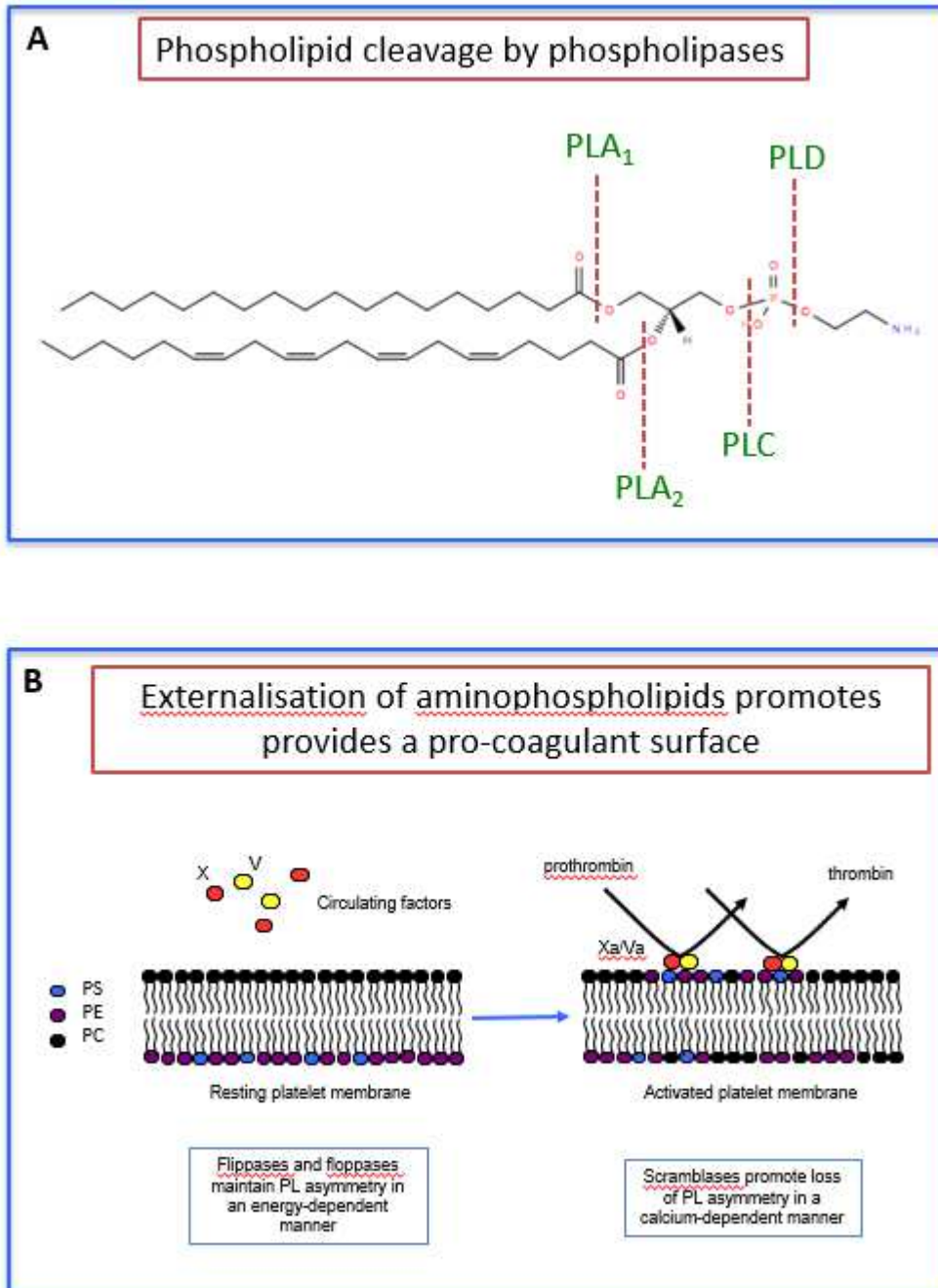
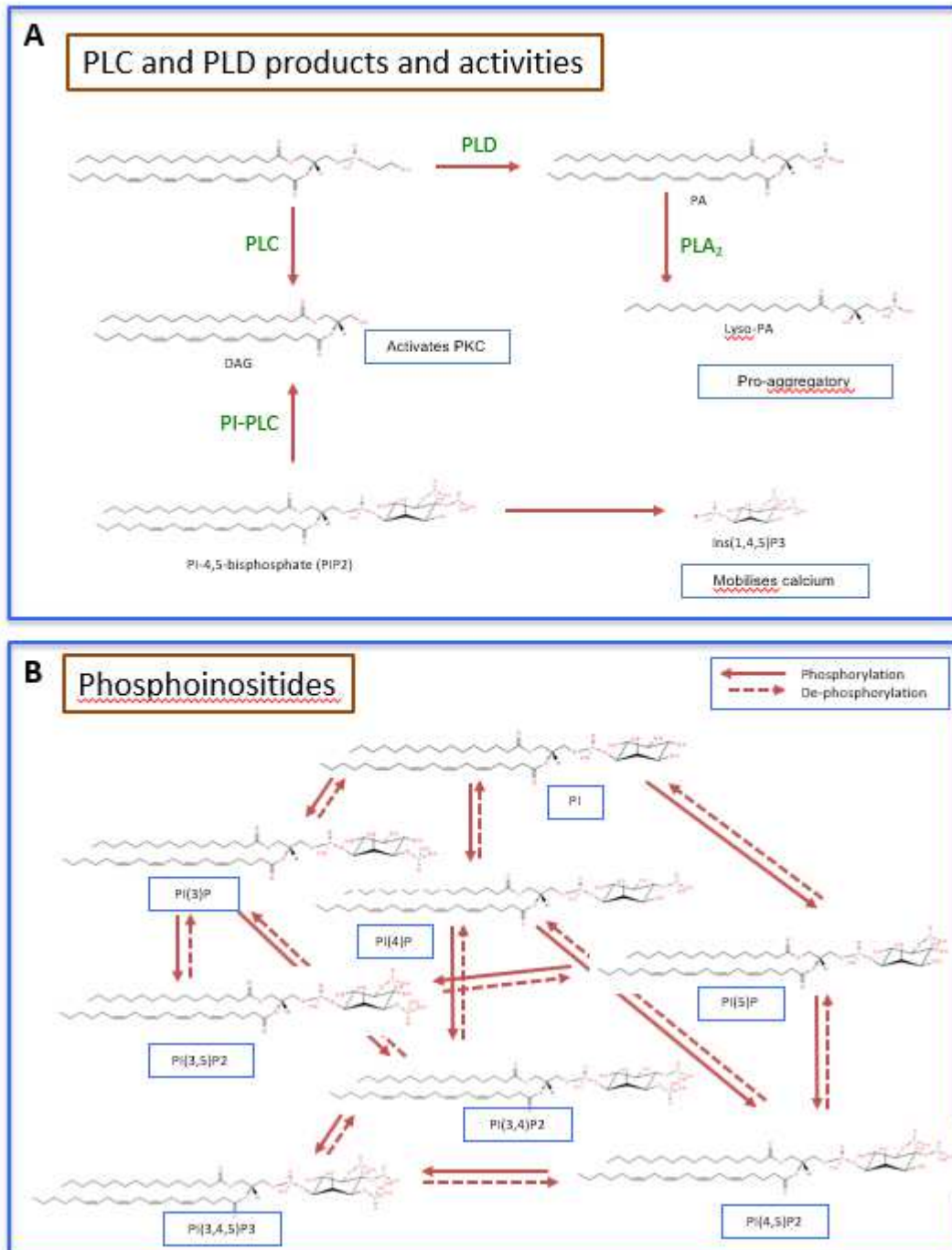
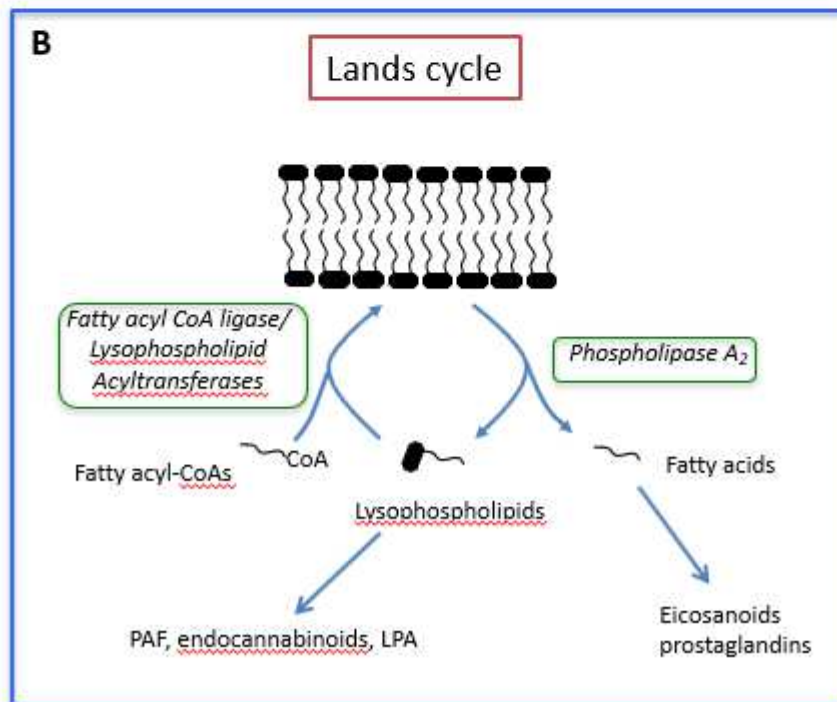
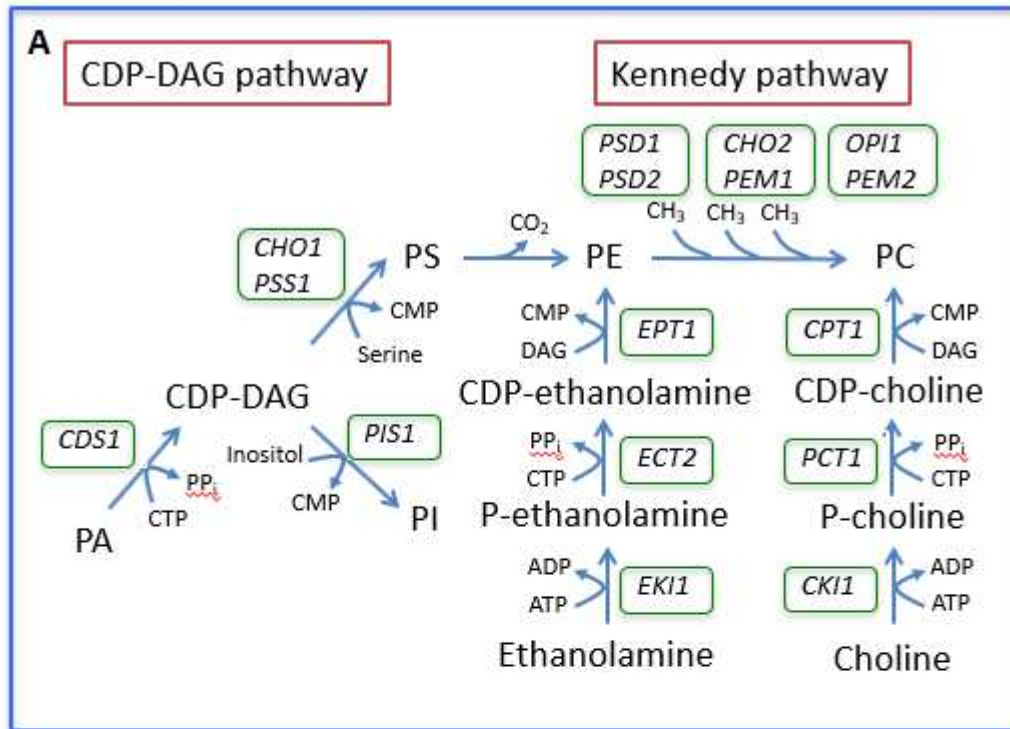


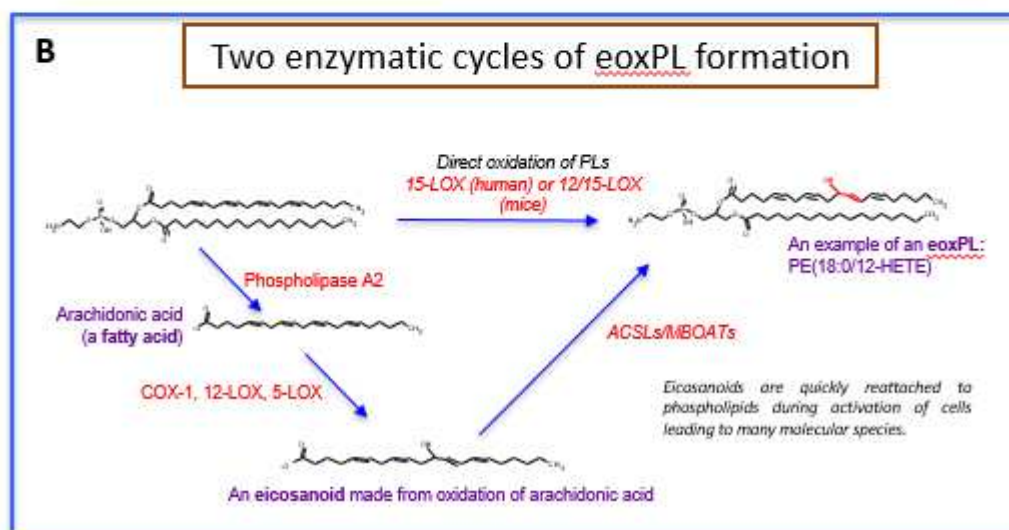
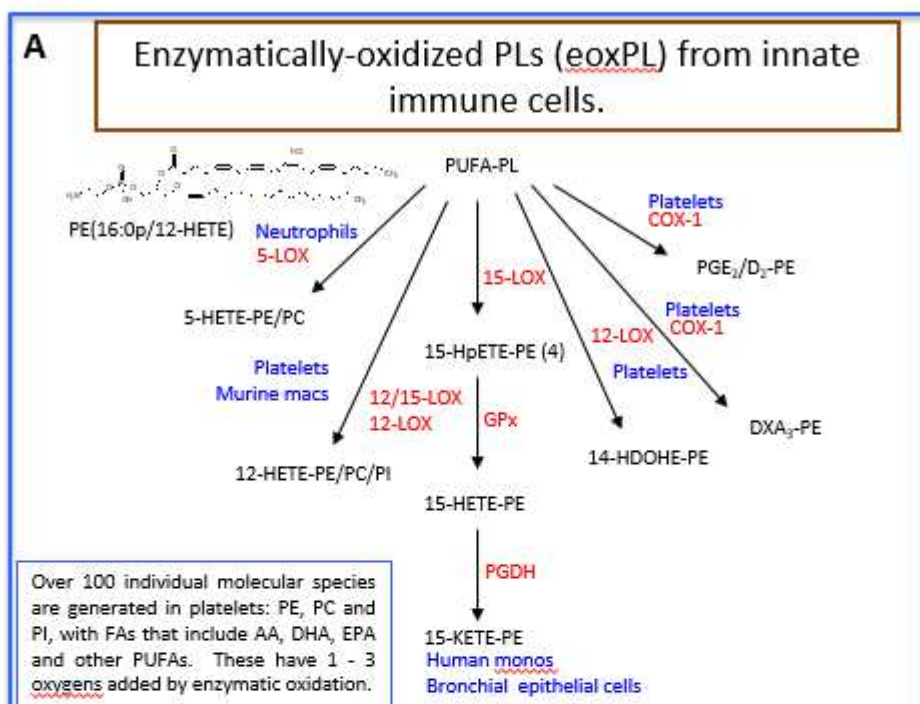
Figure 3



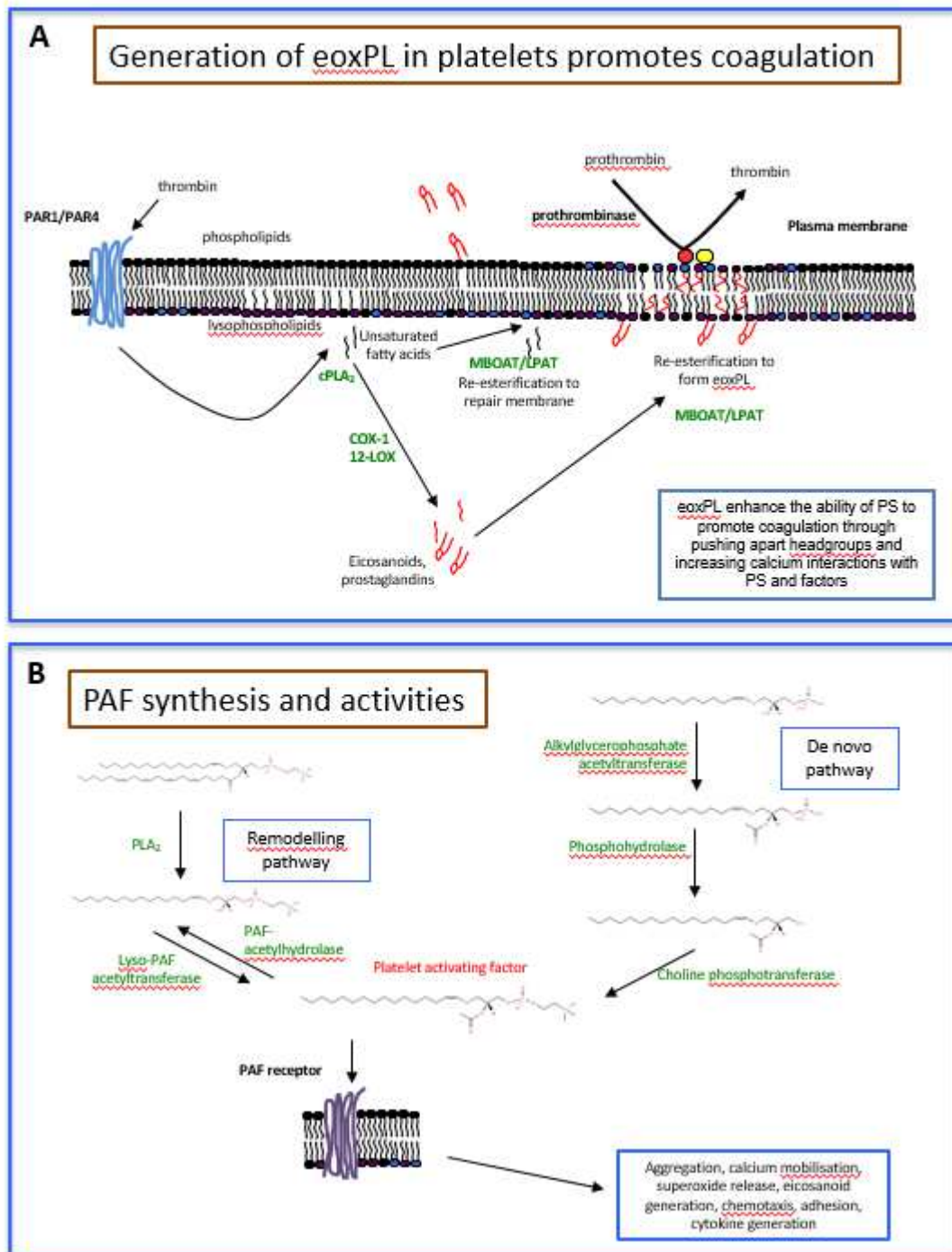
Supplementary Figure 1



Supplementary Figure 1. Pathways of PL generation and recycling. Panel A. CDP-DAG and Kennedy pathways. Gene names are indicated in italics. Panel B. Lands cycle. Hydrolysis and re-acylation of fatty acids into lysoPLs to generate phospholipids.



Supplementary Figure 2. Innate immune cells generate eoxPL via LOX or COX activities. Panel A. The diversity of eoxPL generated by immune cells. Panel B. Enzymatic cycles of eoxPL generation include direct oxidation and esterification of free acid eicosanoids into LPL, forming membrane-associated lipids.



Supplementary Figure 3. Generation of exoPL in platelets and the synthesis and activities of PAF. Panel A. exoPL are generated in platelets following thrombin-mediated activation via a co-ordinated pathway including PLA₂, COX-1/12-LOX, MBOAT/LPAT enzymes. Panel B. PAF is generated by two pathways and is potently bioactive through binding and activating the PAF receptor.