Published in final edited form as:

Mol Microbiol. 2018 January ; 107(1): 34–46. doi:10.1111/mmi.13871.

Patatin-like phospholipases in microbial infections with emerging roles in fatty acid metabolism and immune regulation by Apicomplexa

Sarah K. Wilson[1] and **Laura J. Knoll**[1,*]

[1]Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, WI 53706

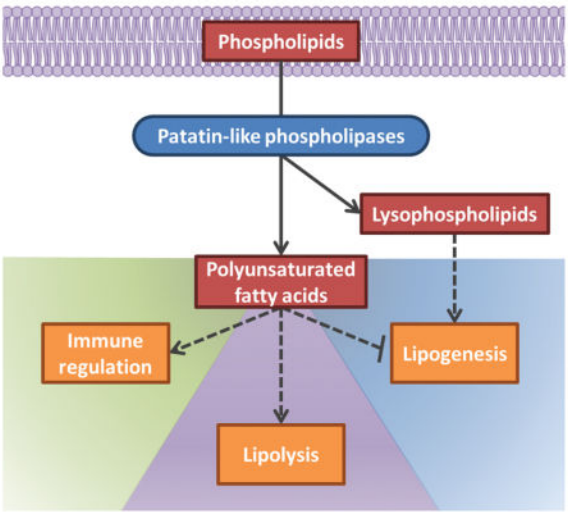
Summary

Emerging lipidomic technologies have enabled researchers to dissect the complex roles of phospholipases in lipid metabolism, cellular signaling, and immune regulation. Host phospholipase products are involved in stimulating and resolving the inflammatory response to pathogens. While many pathogen-derived phospholipases also manipulate the immune response, they have recently been shown to be involved in lipid remodeling and scavenging during replication. Animal and plant hosts as well as many pathogens contain a family of patatin-like phospholipases, which have been shown to have phospholipase A_2 activity. Proteins containing patatin-like phospholipase domains have been identified in protozoan parasites within the Apicomplexa phylum. These parasites are the causative agents of some of the most widespread human disease. Malaria, caused by *Plasmodium* spp., kills nearly half a million people worldwide each year. *Toxoplasma* and *Cryptosporidium* infect millions of people each year with lethal consequences in immunocompromised populations. Parasite-derived patatins are likely effective drug targets and progress in the tools available to the Apicomplexan field will allow for a closer look at the interplay of lipid metabolism and immune regulation during host infection.

Abbreviated Summary

Animal and plant hosts as well as many pathogens contain a family of patatin-like phospholipases, which have been shown to have phospholipase A_2 activity. Proteins containing patatin-like phospholipase domains have been identified in protozoan parasites within the Apicomplexa phylum. Parasite-derived patatins are likely effective drug targets and progress in the tools available for Apicomplexa will allow for a closer look at the interplay of lipid metabolism and immune regulation during host infection.

*To whom correspondence should be addressed: Laura J. Knoll, Department of Medical Microbiology and Immunology, University of Wisconsin - Madison, 1550 Linden Drive, Madison, WI 53706, USA Tel: 608-262-3161; Fax: 608-262-8418; ljknoll@wisc.edu.



Keywords

Apicomplexa; *Toxoplasma*; *Plasmodium*; *Cryptosporidium*; Patatin-like phospholipases

Introduction

The phospholipase A_2 (PLA_2) superfamily contains a wide range of enzymes that cleave the *sn*-2 ester bond of phospholipids to release free fatty acids and lysophospholipids (Burke & Dennis, 2009). However, there is great diversity among this family of enzymes (Six & Dennis, 2000). The most common groups of human PLA_2s are secreted PLA_2 ($sPLA_2$), calcium-independent PLA_2 ($iPLA_2$), cytosolic PLA_2 ($cPLA_2$), and patatin-like PLA_2 (PLP). The $sPLA_2$s are the most extensive group of PLA_2s and play a variety of roles in the inflammatory properties of venoms, digestion of dietary phospholipids, production of eicosanoids, and signal transduction in the inflammatory response (Lambeau & Gelb, 2008). $iPLA_2$ enzymes are important in phospholipid remodeling and homeostasis as well as signal transduction in many cell types (Winstead *et al.*, 2000). $cPLA_2$ enzymes have a high specificity for arachidonic acid (AA) at the *sn-2* position of its substrate phospholipid (Kramer & Sharp, 1997). PLP enzymes were first described as lipid acyl hydrolases involved in potato tuber storage (Senda *et al.*, 1996; Shewry, 2003). In many plants, PLPs are induced by infection to help control the spread of disease, whereas in mammals, PLPs are mostly used in lipid metabolism and turnover.

Lipoxygenases (LOX) are a family of iron-binding enzymes that catalyze the dioxygenation of polyunsaturated fatty acids (PUFA), primarily AA, released from lipids by PLA_2s. Cyclooxygenases (COX) catalyze the *bis*-dioxygenation and subsequent reduction of PUFAs (Rouzer & Marnett, 2008). In mammals, the products of LOX and COX are eicosanoids, which can help regulate immune responses via both pro-inflammatory and anti-inflammatory activities (Tam, 2013). Eicosanoids are transported from the cell via the multidrug resistance-associated protein family of ATP-dependent efflux transporters, which export compounds conjugated to anionic molecules (Gao *et al.*, 1998). Once these

eicosanoids are transported out of the cell, they can bind their specific lipoxin or leukotriene receptors to activate various signaling cascades resulting in a range of downstream effects on both the inflammatory response and cell proliferation (Wang & DuBois, 2010).

The enzyme cascades that produce eicosanoids have been increasingly studied in the context of infection. Both host and pathogens produce the enzymes involved in these pathways to regulate metabolism and the inflammatory response. Most studies of PLA_2s focus on either their role in lipid metabolism or on the downstream production of eicosanoids. In this review, we will show the connection between the two pathways (Fig. 1), with a focus on pathogen-derived enzymes during host infection. We will also discuss a family of apicomplexan-derived PLPs, which have been largely uncharacterized at the time of this review.

Patatin-like phospholipases in lipid hydrolysis

Humans have 9 PLPs, annotated in mammals as PNPLA, within the broader lipid hydrolase family. All of the PNPLAs have a catalytic serine lipase motif G-X-S-X-G, a serine-aspartate dyad, and a glycine-rich oxyanion hole. While all studied members have shown acyl-hydrolase activity, several have lacked significant phospholipase activity under the conditions tested *in vitro*. The mammalian PNPLAs have diverse and often specialized functions, but most are involved in lipid metabolism and turnover (diagram in Fig. 2). PNPLA2, or adipose triglyceride lipase (ATGL), is the rate-determining enzyme in triglyceride lipolysis (Kienesberger *et al.*, 2008; Qiao *et al.,* 2011). ATGL expression is upregulated by fasting glucocorticoids and downregulated by feeding and insulin (Villena *et al.*, 2004; Lake *et al.*, 2005; Kershaw *et al.*, 2006). However, PNPLA3 and PNPLA5 show the opposite pattern, reminiscent of genes involved in lipogenesis, such as FAS (Baulande *et al.*, 2001; Liu *et al.*, 2017). The insulin-dependent activation of PNPLA3 is mediated by the sterol regulatory element-binding protein (SREBP) transcription factors to promote triglyceride synthesis (Kim *et al.*, 2016). ATGL, PNPLA3, and PNPLA5 contain two distinct C-terminal lipid droplet targeting motifs responsible for their recruitment to lipid droplets (Murugesan *et al.*, 2013). PNPLA8 is also regulated by the SREBP family, but PNPLA8 associates with mitochondria and peroxisomes and has both PLA_1 and PLA_2 activity. PNPLA8 activation induces lipid droplet mobilization through autophagy to provide the cell with new lipids faster and with less energy consumption than uptake or *de novo* synthesis (Kim *et al.*, 2016). The fatty acids released by lipid hydrolysis can provide twice as much ATP as carbohydrates relative to their mass and are important in energy storage, especially in adipose tissue. Fatty acids are catabolized by the fatty acid oxidation, or β-oxidation, pathway (Carracedo *et al.*, 2013). The diverse cellular localizations and tissue expression patterns of the PNPLA family suggest non-redundant roles in lipid metabolism.

Patatin-like phospholipases in host cellular signaling pathways

The polyunsaturated fatty acids (PUFA) and lysophospholipids released by PLA_2 activity can act as secondary messengers for downstream cellular responses; however, in mammalian hosts these secondary messengers come from $sPLA_2$, $iPLA_2$ and $cPLA_2$ but not the PLPs. In contrast, plant PLPs play an active role in defense signaling. Arabidopsis encodes 10 PLPs and the PUFAs produced can inhibit the protein phosphatase MP2C, which is involved in the

wound-induced MAP kinase pathway (Scherer *et al.*, 2010). Arabidopsis PLP2 and PLP7 are pathogen-induced patatin-like phospholipases. PLP2 promotes a hypersensitive reaction, or programmed cell death, in infected cells that are critical in the resistance of Arabidopsis to cucumber mosaic virus but detrimental during infection with the fungus *Botrytis cinerea* and the bacteria *Pseudomonas syringae.* Higher induction of the downstream oxylipins in response to *B. cinerea* and *P. syringae* compared to the virus led to increased host damage from the induced hypersensitive reaction (Camera *et al.*, 2005; Camera *et al.*, 2009). Similarly, infection of tobacco leaves with tobacco mosaic virus strongly induced transcription and activity of three tobacco PLPs. Fatty acid conversion to 12-oxophytodienoic and jasmonic acid defense-signaling eicosanoids induced the onset of necrotic lesions to inhibit viral spread (Dhondt *et al.*, 2000, Ryu, 2004). There are also complex feedback loops involving other phospholipases. For example, phospholipase D (PLD)-derived PA can stimulate PLA_2 activity in animals, whereas PLD is inhibited by the LPE generated by PLA_2 in both plants and animals. PLD can also be activated by the PUFAs generated by PLA_2 (Ryu, 2004).

Patatin-like phospholipases in microbes

In addition to their mammalian and plant hosts, PLPs are also found in many microorganisms. In a screen of 123 bacterial genomes, 55 strains contained at least one gene with a PLP domain including *Bacillus* sp., *Brucella* sp., *Rickettsia* sp., *Staphylococcus aureus*, and *Yersinia pestis*. Genomes of pathogens and symbionts had significantly higher numbers of PLP-containing genes than the genomes of free-living bacteria, suggesting a role in host-pathogen interaction for these PLPs (Banerji & Flieger, 2004). One of the best characterized PLP is ExoU in *Pseudomonas aeruginosa* (Phillips *et al.*, 2003; Sato & Frank, 2004). ExoU is a cytotoxic effector protein secreted through the type III secretion system upon cellular contact (Sawa *et al.*, 2016). Host ubiquitination is required to activate the PLA_2 activity of ExoU leading to destruction of infected cell membranes (Anderson *et al.*, 2011; Anderson *et al.*, 2015). *Rickettsia typhi* encodes two PLPs, one of which has similar cytotoxic activity to ExoU and also requires a eukaryotic host cofactor for activation (Rahman *et al.*, 2010). The intracellular pathogen *Legionella pneumophila* encodes four PLPs, three of which are secreted into the host cell through the type IVB secretion system (Banerji *et al.*, 2008). The best characterized of these PLPs, VipD, hydrolyzes phosphatidylcholine (PC) and phosphatidylethanolamine (PE) specifically on the mitochondrial membrane and not the plasma membrane. The release of fatty acids and lysophospholipids destabilizes the membrane and allows for the release of cytochrome c from the mitochondria. Cytochrome C activates caspase 3, a major regulator of apoptotic cell death (Zhu *et al.*, 2013). VipD also interferes with endosomal trafficking and subsequent lysosomal degradation that contributes to bacterial survival within the host cell (Banerji *et al.*, 2008; Ku *et al.*, 2012).

PLPs released by fungi during infection in a mammalian host have been implicated in nutrient acquisition, tissue invasion, and modulation of the host immune response (Köhler *et al.*, 2006). The triacylglycerol (TAG) lipases in *Saccharomyces cerevisiae* contain patatin-like domains and release TAG from lipid storage particles (Athenstaedt & Daum, 2003; Athenstaedt & Daum, 2005). *S. cerevisiae* also encodes a human PNPLA6 homolog

involved in phosphatidylcholine turnover. Pathogenic fungi like *Candida albicans* produce PLPs, but their role in pathogenesis and virulence is still unclear.

Eicosanoid production and regulation

Eicosanoids are lipid mediators made by the oxidation of PUFAs and are involved in the regulation of inflammation (diagram in Fig. 3). Eicosanoids were first discovered in fungi and are involved in sexual maturation and life cycle regulation as well as pathogenesis in virulent strains (Kock *et al.*, 1991). Eicosanoids are largely produced by cells of the innate immune system, using arachidonic acid (AA as the PUFA substrate (Harizi *et al.*, 2008). The three major enzymes involved in eicosanoid production are cytochrome P450, LOX, and COX. AA metabolized by cytochrome P450 forms epoxides and hydroxyeicosatetreanoic acids (HETE), which likely contributes to tumor initiation and progression (Nebert & Dalton, 2006). COX-mediated AA oxidation forms prostaglandins (PG) and thromboxanes (TX). COX-1 and COX-2 are the two isoforms that have been identified in humans. COX-1 is constitutively expressed in most tissues, while COX-2 is induced in response to inflammation (Botting, 2007). PGs are produced by most human cells, are associated with both pro- and anti-inflammatory activity, and are responsible for many physiological symptoms of an inflammatory response. Aspirin and other nonsteroidal anti-inflammatory drugs function by blocking the active site of COX to reduce the general malaise caused by PG and TX activity (Harizi *et al.*, 2008).

The LOX superfamily is found widely in plants, animals, fungi and bacteria. The main human LOXs are 5-, 12- and 15-LOX and the names reflect the specific carbon target for oxidation on the PUFA substrate. Soybean LOX was one of the first characterized and while it displays 15-LOX activity, it only shares 25% identity with any mammalian 15-LOX. The human 15-LOXs themselves only share 35% identity with each other, highlighting the sequence diversity of this enzymatic family (Lagocki *et al.*, 1976; Steczko *et al.*, 1992; Shureiqi & Lippman, 2001). LOX-mediated AA dioxygenation forms hydroperoxyeicosatetraenoic acids (HpETE), which are rapidly reduced to HETEs. In plants, these intermediates are converted to jasmonic acid and aldehydes, which are both involved in plant defense responses (Chehab *et al.*, 2008). In other organisms, LOX-derived HETEs can be further converted to hepoxilins (HX), leukotrienes (LT), and lipoxins (LX). HXs are associated with stimulated intracellular calcium release, including stimulation of AA, diacylglycerol and insulin secretion (Nigam *et al.*, 1990; Mrsny *et al.*, 2004). LTs are mainly produced by inflammatory cells and exhibit strong pro-inflammatory properties including T cell proliferation, chemotaxis induction and secretion of cytokines involved in the Th1 response (Samuelsson *et al.*, 1987; Wang & DuBois, 2010; Norris *et al.*, 2014). Conversely, LXs are involved in inflammation resolution through downregulation of pro-inflammatory chemokines and cytokines, reduced PMN infiltration, and increased IL-10 production (Serhan, 2014; Petri *et al.*, 2015).

Mediators like PGs and LTs are produced in response to infection, but failure to resolve inflammation can be as damaging as the initial infection, so pro-resolving eicosanoids like LXs, resolvins, protectins, and maresins are produced to protect the host tissues (Tam, 2013). Signaling networks between the eicosanoids that help mediate this balance between

pro- and anti-inflammatory responses. In fungi, inhibition of PGs leads to an increase in LOX products to compensate for the loss of eicosanoids (Noverr *et al.*, 2003). PGE_2 enhances IL-10 production in dendritic cells, which inhibits activation of the 5-LOX activating protein and results in lower LTB_4 production (Harizi *et al.*, 2003). Both PGE_2 and PGD_2 can upregulate 15-LOX, leading to increased synthesis of pro-resolving LXs (Tam, 2013). Differences in eicosanoid production by immune cells contribute to their downstream effects on the immune response. PMNs are the first leukocyte responders to an inflammatory insult. At the onset of inflammation, LTB_4, PGE_2 and PGD_2 are the most abundant eicosanoids produced by PMNs and these eicosanoids enhance further leukocyte recruitment and vascular permeability. The PGs then stimulate lipid mediator class switching and upregulation of resolving eicosanoids at the sites of inflammation (Dalli & Serhan, 2012). Secreted products produced in one cell type can also be metabolized in a subsequent cell to generate the final lipid mediator. 15-HpETE or 15-HETE produced by 15-LOX in epithelial cells or monocytes can serve as substrates for neutrophil 5-LOX to produce LXA_4 and LXB_4. Myeloid 5-LOX produces pro-inflammatory LTA_4, which is then converted by platelet 12-LOX to anti-inflammatory lipoxins (Fierro & Serhan, 2001). Although there has not yet been a direct link between microbial PLPs and eicosanoid production, a mammalian PLP has been implicated in human mast cell eicosanoid production (described below under lipid scavenging). The balance of cytokines and lipid mediators is critical for an effective host response to infection, but pathogens can take advantage of host eicosanoid production to modulate the immune response. *P. aeruginosa* secretes a 15-LOX homolog which regulates the host response through the production of anti-inflammatory 15-HETE molecules (Vance *et al.*, 2004). *Listeria monocytogenes* infection of macrophages activates host $cPLA_2$ and COX-2 activity leading to increased production of PGs. The resulting downregulation of TNFα by the PGs helps *L. monocytogenes* survive (Noor *et al.*, 2008). As these mechanisms for immune regulation are studied further, the roles of microbial PLPs in eicosanoid production will likely be elucidated.

Interplay of host and parasitic PLA_2 activity during infection

Eicosanoids produced by pathogens often play a dual role in metabolism or maturation of the organism and cross-talk with the host inflammatory response (Noverr *et al.*, 2003). In protozoan parasites specifically, the PLA_2 family of enzymes have been implicated in invasion, membrane remodeling, virulence, and disease progression. PGE_2 inhibition of IL-12 plays a critical role in disease progression of *Leishmania major* (Passero *et al.*, 2008). These intracellular parasites are cholesterol auxotrophs and salvage lipids from the host during infection. An *L. major*-derived PLA_2 is responsible for hydrolyzing platelet-activating factor and contributes to virulence *in vivo* (Pawlowic & Zhang, 2012). Ca^{2+}-dependent PLA_2 produced by *Trypanosoma brucei* releases AA and stimulates Ca^{2+} influx into the parasite, which has been associated with control of infection (Eintracht *et al.*, 1998). Similar AA-regulated Ca^{2+} influx was seen in *T. cruzi* and *L. donovani* (Catisti *et al.*, 2000). *T. brucei* also has a lysoPLA that can release AA by sequentially hydrolyzing the sn1 and sn2 acyl chains in phospholipids (Ridgley & Ruben, 2001). Activation of *T. cruzi*-infection macrophages by LTB_4 induces the production of TNFα. This pro-inflammatory cytokine drives the release of NO, which mediates parasite killing (Talvani *et al.*, 2002). A lytic factor important for the virulence of *Trichomonas vaginalis* was determined to be a PLA_2 and

contributes to tissue damage and inflammation during infection through the downstream production of PGs and LTs (Lubick & Burgess, 2004).

PLA_2s have been emerging as critical enzymes in the pathogenesis of apicomplexans in recent years as well. *T. gondii* secretes both a Ca^{2+}-dependent and -independent PLA_2 that are involved in host cell penetration by facilitating membrane fusion during invasion and synthesis of LOX and COX eicosanoid products (Saffer *et al.*, 1989; Saffer & Schwartzman, 1991; Thardin *et al.*, 1993). The Ca^{2+}-independent PLA_2 is resistant to most PLA_2 inhibitors tested unlike the host enzymes, which could be exploited for drug development (Cassaing *et al.*, 2000). The released PUFAs themselves could also aid host cell invasion by increasing the fluidity and permeability of the host cell membrane (Li *et al.*, 2008). IFNγ blocks the activities of the *T. gondii* PLA_2s, suggesting another mechanism of protection for this Th1-type cytokine against active invasion by *T. gondii* (Gomez-Marín *et al.*, 2002). While the Th1 immune response is used by the host to clear parasitic infection, the cytokines from this immune response signals *T. gondii* to shift into the chronic bradyzoite stage suggesting that both stimulation and resolution of the inflammatory response aid survival of *T. gondii* to its chronic stage. Fatty acids from *T. gondii* reduce the secretion of TNFα, which is normally induced by protozoan glycolipids, through the inhibition of NF-κβ activation in the host (Debierre-Grockiego *et al.*, 2007).

Phospholipases in lipid remodeling and scavenging in Apicomplexa

Apicomplexans are auxotrophs for many lipid species such as cholesterol and phospholipids (Gupta *et al.*, 2005; Milovanović *et al.*, 2017). Phospholipids are important components of membranes and are also involved in signaling, protein turnover and attachment to membranes, and membrane fluidity (Ramakrishnan *et al.*, 2013). Apicomplexans exploit the host lipidome through a variety of mechanisms (Rub *et al.*, 2013). *T. gondii* can acquire lipid precursors from its environment and use them to synthesize all of its major glycerophospholipids (Charron & Sibley, 2002). Previous studies suggest that the parasite is capable of synthesizing only 50% of the phosphatidylserine (PS) and less than 10% of the phosphatidylcholine (PC) necessary to maintain the rate of replication (Gupta *et al.*, 2005). Accumulating enough phospholipids for replication of the parasitic membranes requires both internal synthesis and scavenging from the host and is likely a rate-limiting step in replication. The high abundance of PC in the *T. gondii* membrane, about 75%, increases membrane fluidity, which may help the parasite accommodate a wide range of hosts and environments (Gupta *et al.*, 2005; Seeber *et al.*, 2008). All of Apicomplexa have much higher levels of PC than other eukaryotic cells, which typically have 30–40% of their phospholipids comprised of PC (Mitschler *et al.*, 1994).

A mechanism for hijacking host resources involves accumulation of lipid droplets in host cells during infection and the concomitant downregulation of host genes involved in lipid metabolism, such as the mammalian patatin-like phospholipase ATGL (Hu *et al.*, 2017). In human mast cells, silencing ATGL caused an increase in neutral lipids in lipid droplets as well as a decrease in eicosanoid production (Dichlberger *et al.*, 2014). LOX and COX enzymes have been shown to localize to lipid droplets and are considered to represent a site of prostaglandin and leukotriene synthesis, so ATGL release of arachidonic acid from lipid

droplet-associated triacylglycerol could be a crucial step for eicosanoid production in mast cells (Schreiber & Zechner, 2014). During Apicomplexa infection, host lipid droplets localize near and within the parasitophorous vacuole but are too large for direct endocytosis, so host- and parasite-derived enzymes, such as PLPs, are likely involved in releasing lipids from the droplets (Nolan *et al.*, 2017). In mammalian cells, phospholipids can move freely between membranes without vesicular trafficking using mitochondrial-associated membranes. The PVM of *T. gondii* parasites can associate with the ER and mitochondria of the host, which could allow for easier lipid exchange between the host and parasite (Coppens, 2006).

The most abundant phospholipid in *Plasmodium* membranes is also PC at 40–50% of the total phospholipids (Dechamps *et al.*, 2010) *Plasmodium* cannot synthesize fatty acids or cholesterol de novo (Labaied, 2011; Fish, 1995). They have multiple pathways to overcome this by either scavenging lipid precursors such as choline and ethanolamine to be metabolized further by the parasite or actively taking up FAs and phospholipids from the erythrocyte membranes and human serum (Mitamura & Palacpac, 2003; Krishnegowda & Gowda, 2003) *Plasmodium* parasites induce a 6-fold increase in the overall phospholipid content in the membranes of their host cells, particularly infected erythrocytes (Dechamps *et al.*, 2010). The membranes become highly susceptible to phospholipase activity due to a rearrangement of those phospholipids to the outer leaflet of the host cell membrane bilayer (Joshi & Gupta, 1988). Of note, lipid metabolism is effectively absent in uninfected mature erythrocytes, so the parasite must rely on its own phospholipid synthetic machinery to meet its requirements for replication (Hsiao *et al.*, 1991) *P. berghei* PLA_2 contributes to several life cycle transitions. PLA_2 activity is involved in oocyst rupture and release of sporozoites, epithelial cell membrane penetration for migration to the liver, and finally PVM rupture in the infected hepatocyte (Burda *et al.*, 2015) and egress of merozoites into the blood stream (Bhanot *et al.*, 2005). Regulation of the immune response is also critical in the pathogenesis of cerebral malaria (CM). Patients who died or developed long-term side effects from CM had higher plasma PLA_2 levels than those who recovered. This increase in PLA_2 was also associated with excessive production of pro-inflammatory cytokines, likely leading to the severe pathogenesis (Gupta *et al.*, 2017). The PUFAs docosahexaenoic acid, eicosapentaenoic acid, AA, and linoleic acid were able to inhibit *P. falciparum* growth, but oleic acid and docosanoic acid did not inhibit growth. Addition of the oxidized derivatives of the PUFAs led to direct killing of the parasites (Kumaratilake *et al.*, 1992). PUFAs, but not their hydroperoxy derivatives, markedly increased neutrophil-mediated killing of parasites (Kumaratilake *et al.*, 1997).

A PLA_2 secreted by *Cryptosporidium parvum* is critical for host cell invasion, but the specific gene has not yet been identified (Pollok *et al.*, 2003). In *C. parvum*, PC was 65% of the total phospholipids (Mitschler *et al.*, 1994). These high levels of PC in the parasitophorous vacuole may help avoid fusion with the lysosome, as PC is more stable as a bilayer than other phospholipids. While *T. gondii* has similarly high levels of PC, *Plasmodium* may not require such high levels during the intraerythrocytic stages as red blood cells lack most fusogenic organelles. *Eimeria neischulzi* has 75–85% PC in both sporulated and unsporulated oocysts and the aqueous insolubility and relative inertness may contribute to their resistant nature (Mitschler, 1994).

Apicomplexa-derived patatin-like phospholipases

A *T. gondii* PLP, named TgPL1, was discovered during a mutagenesis screen to identify genes important for the inhibition of NO production from activated macrophages (Mordue *et al.*, 2007). This mutant had a defect in its ability to survive in active macrophages and increased cytokines with fewer inflammatory lesions in late chronic infection compared to wild type. TgPL1 localizes within the parasite during the tachyzoite stage but moves out to the PV and cyst wall during the bradyzoite stage, further supporting its role in late chronic infection (Tobin *et al.*, 2014). TgPL1 lacks the predicted catalytic serine essential for PLA_2 activity, so its effects are likely not due to PLA_2 activity (Tobin & Knoll, 2012). This led to a screen of the *T. gondii* genome where six potential PLP enzymes were found, four of which had retained the catalytic serine (Fig. 4). Recently TgPL2 was shown to be critical in maintaining membrane integrity of the apicoplast (Lévêque *et al.*, 2017). Lipid analysis revealed that TgPL2 is important for maintaining levels of apicoplast-generated fatty acids and regulating PC and LPC levels in the parasite. A third PLP, TgPL3, was previously identified in a screen for *T. gondii* virulence *in vivo* and is currently under investigation (Frankel *et al.*, 2007). In *P. falciparum*, the localization of a putative PLP, Pf3D7_0209100, was shown to be cytosolic during all asexual and gametocyte stages with additional nuclear localization during the trophozoite and gametocyte stages (Pappa *et al.*, 2017). Another predicted *Plasmodium* PLP, Pf3D7_092400, was downregulated in response to choline kinase inhibition. Choline kinase is a key enzyme in the synthesis of PC, a phospholipid comprising a large portion of the parasitophorous vacuole membrane, and is critical for *Plasmodium* replication during the asexual stages. Pf3D7_092400 inhibition in response to reduced PC synthesis could indicate a role for the PLP in maintaining membrane lipid homeostasis (Ridzuan *et al.*, 2014).

A search of Apicomplexa genomes reveals that these PLP domains are present broadly and in high numbers across the phylum. In addition to the 6 putative PLPs found in the *T. gondii* genome, there are 6 in *Cryptosporidium* and *Hammondia*, 5 in *Eimeria* and *Neospora*, and 4 in *Plasmodium* and *Sarcocystis*. Most PLPs contain a conserved proline between the lipase domain and the catalytic aspartate. The proline motif in bacterial PLPs was typically ASxxxP, whereas in mammalian PLPs, the motif was AAP (Banerji & Flieger, 2004). An alignment of the *Toxoplasma, Plasmodium* and *Cryptosporidium* PLPs revealed a mixture of both motifs but the majority aligned with ASxxxP, suggesting these PLPs have a bacterial origin (Fig. 4). TgPL2 and its *Plasmodium* ortholog, Pf3D7_1358000, notably contained the AAP motif. As discussed earlier, mammalian PLPs contribute mainly to lipid metabolism and turnover, similar to the role TgPL2 plays in the apicoplast. It will be intriguing to see if this motif distinction predicts the role of the apicomplexan PLPs in either lipid metabolism or inflammation. The Apicomplexa PLPs fall roughly into 6 ortholog groups with an additional 5 ortholog groups specific for *Cryptosporidium* or *Eimeria* species and a handful of PLPs that did not fall into any of the ortholog groups (Fig. 5). Several ortholog groups contain signal peptides and/or transmembrane domains that will direct the localization and specialized activities of the PLPs. For example, TgPL1 has a signal peptide that may be responsible for the localization shift of the protein out to the cyst wall during chronic infection. PLPs in the ortholog group named OG5_129101 have signal peptides, multiple transmembrane domains, and a domain of unknown function (DUF3336). While

functionally uncharacterized, this domain has been described adjacent to the GxSxG lipase domain in Tgl3 Tgl4 and Tgl5 in *Saccharomyces cerevisiae*. All three have triacyglycerol lipase activity and localize to lipid particles (Athenstaedt & Daum, 2003; Athenstaedt & Duam, 2005). Tgl4 is a functional ortholog of mammalian ATGL and has additional steryl ester hydrolase and phospholipase activities (Rajakumari & Duam 2010). These data give insight into the potential role of this Apicomplexa PLP ortholog group and will help guide the enzymatic assays and conditions used during further experiments. The conservation of multiple PLPs across the phylum suggests these enzymes play a critical role for the parasites. Based on the diversity, these enzymes are likely to fill unique roles to address the changing needs across the life cycle of the parasites in both lipid metabolism and host immune regulation.

Future perspectives

As seen in the PLP sequence alignments, Apicomplexa PLPs more closely resemble bacterial PLPs than eukaryotic PLPs. Additionally, compounds identified as potent inhibitors of ExoU PLA_2 activity did not inhibit the activity of mammalian-derived PLA_2s (Lee *et al.*, 2007). These parasite PLPs will likely be excellent drug targets as TgPL2 has already been characterized as a critical gene to parasite growth and replication (Lévêque *et al.*, 2017). Treatment of *T. gondii* with atglistatin, an inhibitor of ATGL, led to a decrease in parasite replication proportional to drug concentration and disorganization of the parasites within the PV. There was also accumulation of lipids in the lumen of the PV and between the parasites, indicative of dysfunctional lipid metabolism (Nolan *et al.*, 2017). This paper suggested the effects of the drug were based solely on the mammalian ATGL, however the drug could also be acting on one or more of the six parasite-derived PLPs. Anti-bacterial drugs gentamicin and amikacin have modest effects on Plasmodium development from the ring stage to mature trophozoites and schizonts. While the ring stage matures in erythrocytes, it ingests host cell cytoplasm through endocytosis. The endocytic vesicle fuses with the parasite food vacuole and phospholipases contribute to the digestion of its contents. Drug treatment resulted in reduced host cytosol digestion concurrent with reduced PLA_2 activity in the parasite cytosol, suggesting these drugs may function through the inhibition of a parasite-derived PLA_2 (Krugliak *et al.*, 1987). Anti-malarials chloroquine, quinine and arteether also produce minor inhibition of PLA_2 activity associated with *P. falciparum* infection of erythrocytes with IC_{50}s of 1.3, 1.0 and 1.8 mM, respectively (Zidovetzki *et al.*, 1993). Arteether and quinine have been shown to disrupt the structure of lipid membranes, which could alter the ability of PLA_2s to access their substrates leading to reduced activity. In chloroquine-sensitive parasites, the drug enters the parasite in a neutral form that can move freely across the food vacuole membrane, but in this acidic environment, it becomes charged and can no longer diffuse out of the vacuole (Saliba *et al.*, 1998). The accumulation of chloroquine to higher concentrations within the food vacuole compared to the rest of the parasite may give some therapeutic potential for the current IC_{50} of this drug on PLA_2 activity. However, these drugs are likely more useful as a starting point in designing more specific and potent drugs against the parasitic PLA_2 enzymes. A mammalian PLA_2 inhibitor, AACOCF3, had an inhibitory anti-malarial effect at low μM concentrations, but whether this AA analogue is targeting host or parasite-derived PLA_2 is not yet known (Pappa *et al.*, 2017). An alternative route to fight the spread of malaria is to target the mosquito vector

where oocyst formation occurs. When active or inactive PLA_2 from snake and bee venoms was fed to mosquitoes, the protein associated with the midgut surface and prevented ookinete attachment and invasion of the midgut epithelium, critical for oocyst formation (Zieler *et al.*, 2001). Further, mosquitoes were genetically manipulated to express an inactive form of PLA_2 under the control of a midgut-specific promoter and showed similar inhibition of oocyst development (Rodrigues *et al.*, 2008).

There is crosstalk among the many pathways discussed here and inhibition of one enzyme can stimulate activity in another pathway to maintain a balance (Buczynski *et al.*, 2009; Quehenberger & Dennis, 2011; Norris & Dennis, 2012). A systemic approach is needed to fully understand the entire lipidomic response to host and pathogen interactions. The emerging field of lipidomics is largely driven by the improving mass spectrometry methods integrated with better detection and characterization of the enzymes involved in lipid anabolism and catabolism. In addition to the lipidomics advancements, Apicomplexa have become more genetically manipulatable through new methods such as CRISPR/Cas9 (Sidik *et al.*, 2014; Brown *et al.*, 2014). Combining drug development with lipidomics and targeted parasite genetic manipulation will allow for a better understanding of the interwoven roles these PLPs play in parasite development and survival in the host.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

Anderson DM, Schmalzer KM, Sato H, Casey M, Terhune SS, Haas AL, Feix JB, Frank DW. Ubiquitin and ubiquitin-modified proteins activate the *Pseudomonas aeruginosa* T3SS cytotoxin, ExoU. Mol Microbiol. 2011; 82:1454–1467. [PubMed: 22040088]

Anderson DM, Sato H, Dirck AT, Feix JB, Frank DW. Ubiquitin activates patatin-like phospholipases from multiple bacterial species. J Bacteriol. 2015; 197:529–541. [PubMed: 25404699]

Athenstaedt K, Daum G. YMR313c/TGL3 encodes a novel triacylglycerol lipase located in lipid particles of *Saccharomyces cerevisiae*. J Biol Chem. 2003; 278:23317–23323. [PubMed: 12682047]

Athenstaedt K, Daum G. Tgl4p and Tgl5p, two triacylglycerol lipases of the yeast *Saccharomyces cerevisiae* are localized to lipid particles. J Biol Chem. 2005; 280:37301–37309. [PubMed: 16135509]

Banerji S, Flieger A. Patatin-like proteins: A new family of lipolytic enzymes present in bacteria? Microbiology. 2004; 150:522–525. [PubMed: 14993300]

Banerji S, Aurass P, Flieger A. The manifold phospholipases A of *Legionella pneumophila* - Identification, export, regulation, and their link to bacterial virulence. Int J Med Microbiol. 2008; 298:169–181. [PubMed: 18178130]

Baulande S, Lasnier F, Lucas M, Pairault J. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. J Biol Chem. 2001; 276:33336–33344. [PubMed: 11431482]

Bhanot P, Schauer K, Coppens I, Nussenzweig V. A surface phospholipase is involved in the migration of *Plasmodium sporozoites* through cells. J Biol Chem. 2005; 280:6752–6760. [PubMed: 15590623]

Botting RM. Cyclooxygenases in Biology and Disease. Encycl Pain. 2007; 12:511–515.

Brown KM, Lee TD, Sibley LD. Efficient gene disruption in diverse strains of *Toxoplasma gondii* using CRISPR / CAS9 Efficient Gene Disruption in Diverse. MBio. 2014; 5:1–11.

Buczynski M, Dumlao D, Dennis EA. An integrated omics analysis of eicosanoid biology. J Lipid Res. 2009; 50:1015–1038. [PubMed: 19244215]

Burda PC, et al. A *Plasmodium* phospholipase is involved in disruption of the liver stage parasitophorous vacuole membrane. PLoS Pathog. 2015; 11:e1004760. [PubMed: 25786000]

Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. Journal of lipid research. 2009; 50:S237–S242. [PubMed: 19011112]

Camera SL, et al. A pathogen-inducible patatin-like lipid acyl hydrolase facilitates fungal and bacterial host colonization in *Arabidopsis*. Plant J. 2005; 44:810–825. [PubMed: 16297072]

Camera SL, et al. The *Arabidopsis* Patatin-Like Protein 2 (PLP2) Plays an Essential Role in Cell Death Execution and Differentially Affects Biosynthesis of Oxylipins and Resistance to Pathogens. Mol Plant-Microbe Interact. 2009; 22:469–481. [PubMed: 19271961]

Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: fatty acid oxidation in the limelight. Nat Rev Cancer. 2013; 13:227–32. [PubMed: 23446547]

Cassaing S, et al. *Toxoplasma gondii* secretes a calcium-independent phospholipase A2. Int J Parasitol. 2000; 30:1137–1142. [PubMed: 11027777]

Catisti R, Uyemura SA, Docampo R, Vercesi AE. Calcium mobilization by arachidonic acid in trypanosomatids. Mol Biochem Parasitol. 2000; 105:261–271. [PubMed: 10693748]

Charron AJ, Sibley D. Host cells: mobilizable lipid resources for the intracellular parasite *Toxoplasma gondii*. J cell Sci. 2002; 115:3049–3059. [PubMed: 12118061]

Chehab EW, et al. Distinct roles of jasmonates and aldehydes in plant-defense responses. PLoS One. 2008; 3:1–10.

Coppens I. Contribution of host lipids to *Toxoplasma* pathogenesis. Cell Microbiol. 2006; 8:1–9. [PubMed: 16367861]

Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: Microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood. 2012; 120:60–72.

Debierre-Grockiego F, et al. Fatty acids isolated from *Toxoplasma gondii* reduce glycosylphosphatidylinositol-induced tumor necrosis factor alpha production through inhibition of the NF-kappaB signaling pathway. Infect Immun. 2007; 75:2886–93. [PubMed: 17387164]

Dechamps S, Shastri S, Wengelnik K, Vial HJ. Glycerophospholipid acquisition in Plasmodium – A puzzling assembly of biosynthetic pathways. Int J Parasitol. 2010; 40:1347–1365. [PubMed: 20600072]

Dhondt S, Geoffroy P, Stelmach BA, Legrand M, Heitz T. Soluble phospholipase A2 activity is induced before oxylipin accumulation in tobacco mosaic virus-infected tobacco leaves and is contributed by patatin-like enzymes. Plant J. 2000; 23:431–440. [PubMed: 10972869]

Dichlberger A, Schlager S, Maaninka K, Schneider WJ, Kovanen PT. Adipose triglyceride lipase regulates eicosanoid production in activated human mast cells. J Lip Res. 2014; 55:2471–2478.

Eintracht J, Maatha R, Mellors A, Ruben L. Calcium entry in *Trypanosoma brucei* is regulated by phospholipase A2 and arachidonic acid. Biochem J. 1998; 336:659–666. [PubMed: 9841878]

Fierro IM, Serhan CN. Mechanisms in anti-inflammation and resolution: The role of lipoxins and aspirin-triggered lipoxins. Brazilian J Med Biol Res. 2001; 34:555–566.

Fish WR. Lipid and Membrane Metabolism of the Malaria Parasite and the African Trypanosome. Mol Biochem Parasitol. 1995; 8:133–145.

Frankel MB, Mordue DG, Knoll LJ. Discovery of parasite virulence genes reveals a unique regulator of chromosome condensation 1 ortholog critical for efficient nuclear trafficking. Proc Natl Acad Sci USA. 2007; 104:10181–10186. [PubMed: 17535896]

Gao M, Yamazaki M, Loe DW, Westlake CJ, Grant CE, Cole SP, Deeley RG. Multidrug Resistance Protein Identification of Regions Required for Active Transport of Leukotriene C4. Journal of Biological Chemistry. 1998; 273:10733–10740. [PubMed: 9553138]

Gomez-Marín JE, et al. Involvement of secretory and cytosolic phospholipases a2 during infection of THP1 human monocytic cells with *Toxoplasma gondii*. Effect of interferon γ. Parasitol Res. 2002; 88:208–216. [PubMed: 11954905]

Gupta N, Zahn MM, Coppens I, Joiner KA, Voelker DR. Selective disruption of phosphatidylcholine metabolism of the intracellular parasite *Toxoplasma gondii* arrests its growth. J Biol Chem. 2005; 280:16345–16353. [PubMed: 15708856]

Gupta S, et al. Extensive alterations of blood metabolites in pediatric cerebral malaria. PLoS One. 2017; 12:1–13.

Harizi H, Corcuff JB, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. Trends Mol Med. 2008; 14:461–469. [PubMed: 18774339]

Harizi H, Juzan M, Moreau JF, Gualde N. Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B4 production from dendritic cells via an IL-10-dependent mechanism. J Immunol. 2003; 170:139–146. [PubMed: 12496393]

Hsiao LL, Howard RJ, Aikawa M, Taraschi TF. Modification of host cell membrane lipid composition by the intra-erythrocytic human malaria parasite *Plasmodium falciparum*. Biochem J. 1991; 274:121–132. [PubMed: 2001227]

Hu X, Binns D, Reese ML. The coccidian parasites *Toxoplasma* and *Neospora* dysregulate mammalian lipid droplet biogenesis. J Biol Chem. 2017; 292:11009–11020. [PubMed: 28487365]

Hurley BP, Siccardi D, Mrsny RJ, McCormick BA. Polymorphonuclear Cell Transmigration Induced by *Pseudomonas aeruginosa* Requires the Eicosanoid Hepoxilin A3. J Immunol. 2004; 173:5712–5720. [PubMed: 15494523]

Joshi P, Gupta CM. Abnormal membrane phospholipid organization in *Plasmodium falciparum*-infected human erythrocytes. Br J Haematol. 1988; 68:255–259. [PubMed: 3280006]

Kershaw EE, Hamm JK, Verhagen LA, Peroni O, Katic M, Flier JS. Adipose triglyceride lipase: function, regulation by insulin, and comparison with adiponutrin. Diabetes. 2006; 55:148–157. [PubMed: 16380488]

Kienesberger PC, Oberer M, Lass A, Zechner R. Mammalian patatin domain containing proteins: a family with diverse lipolytic activities involved in multiple biological functions. J Lipid Res. 2008; 50:S63–S68. [PubMed: 19029121]

Kim KY, et al. SREBP-2/PNPLA8 axis improves non-alcoholic fatty liver disease through activation of autophagy. Sci Rep. 2016; 6:35732. [PubMed: 27767079]

Kock J, Coetzee D, Van Dyk M, Truscott M, Cloete P, Van Wyk V, Augustyn O. Evidence for pharmacologically active prostaglandins in yeasts. S Afr J Sci. 1991; 87:73–76.

Köhler GA, et al. Phospholipase A2 and Phospholipase B activities in fungi. Biochim Biophys Acta - Mol Cell Biol Lipids. 2006; 1761:1391–1399.

Kramer RM, Sharp JD. Structure, function and regulation of Ca^{2+}-sensitive cytosolic phospholipase A2 (cPLA2). FEBS letters. 1997; 410:49–53. [PubMed: 9247121]

Krishnegowda G, Gowda DC. Intraerythrocytic *Plasmodium falciparum* incorporates extraneous fatty acids to its lipids without any structural modification. Mol Biochem Parasitol. 2003; 132:55–58. [PubMed: 14563537]

Krugliak M, Waldman Z, Ginsburg H. Gentamicin and amikacin repress the growth of *Plasmodium falciparum* in culture, probably by inhibiting a parasite acid phospolipase. Life Sci. 1987; 40:1253–1257. [PubMed: 3550344]

Ku B, et al. VipD of *Legionella pneumophila* targets activated Rab5 and Rab22 to interfere with endosomal trafficking in macrophages. PLoS Pathog. 2012; 8:e1003082. [PubMed: 23271971]

Kumaratilake LM, Ferrante A, Robinson BS, Jaeger T, Poulos A. Enhancement of neutrophil-mediated killing of *Plasmodium falciparum* asexual blood forms by fatty acids: Importance of fatty acid structure. Infect Immun. 1997; 65:4152–4157. [PubMed: 9317021]

Kumaratilake LM, Robinson BS, Ferrante A, Poulos A. Antimalarial Properties of n-3 and n-6 polyunsaturated fatty acids: In vitro effects on *Plasmodium falciparum* and in vivo effects on *P. berghei*. J Clin Invest. 1992; 89:961–967. [PubMed: 1541684]

Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, Coppens I. *Plasmodium* salvages cholesterol internalized by LDL and synthesized *de novo* in the liver. Cell Microbiol. 2011; 13:569–586. [PubMed: 21105984]

Lagocki JW, Emken EA, Law JH, Kezdy FJ. Kinetic Analysis of the Action of Soybean Lipoxygenase Linoleic Acid. J Biol Chem. 1976; 251:6001–6006. [PubMed: 823156]

Lake AC, Sun Y, Li JL, Kim JE, Johnson JW, Li D, et al. Expression, regulation, and triglyceride hydrolase activity of Adiponutrin family members. J Lipid Res. 2005; 46:2477–2487. [PubMed: 16150821]

Lambeau G, Gelb MH. Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem. 2008; 77:495–520. [PubMed: 18405237]

Lee VT, et al. Pseudolipasin A is a specific inhibitor for phospholipase A2 activity of *Pseudomonas aeruginosa* cytotoxin ExoU. Infect Immun. 2007; 75:1089–1098. [PubMed: 17178785]

Lévêque MF, et al. TgPL2, a patatin-like phospholipase domain-containing protein, is involved in the maintenance of apicoplast lipids homeostasis in *Toxoplasma*. Mol Microbiol. 2017; 0:1–17.

Li L, Li X, Yan J. Alterations of concentrations of calcium and arachidonic acid and agglutinations of microfilaments in host cells during *Toxoplasma gondii* invasion. Vet Parasitol. 2008; 157:21–33. [PubMed: 18706765]

Liu Y, et al. PNPLA5-knockout rats induced by CRISPR/Cas9 exhibit abnormal bleeding and lipid level. J Integr Agric. 2017; 16:169–180.

Lubick KJ, Burgess DE. Purification and Analysis of a Phospholipase A_2-Like Lytic Factor of *Trichomonas vaginalis*. Infect Immun. 2004; 72:1284–1290. [PubMed: 14977929]

Milovanović I, Busarčević M, Trbovich A, Ivović V, Uzelac A, Djurković-Djaković O. Evidence for host genetic regulation of altered lipid metabolism in experimental toxoplasmosis supported with gene data mining results. PLoS One. 2017; 12:e0176700. [PubMed: 28459857]

Mitamura T, Palacpac NM. Lipid metabolism in *Plasmodium falciparum*-infected erythrocytes: possible new targets for malaria chemotherapy. Microbes Infect. 2003; 5:545–552. [PubMed: 12758284]

Mitschler, RR. Doctoral thesis. Kansas State University; Kansas, USA: 1994. Lipid biology of *Cryptosporidium parvum* and *Eimeria nieschulzi* (Apicomplexa).

Mitschler RR, Welti R, Upton SJ. A Comparative Study of Lipid Compositions of *Crypfosporidium parvum* (Apicomplexa) and Madin-Darby Bovine Kidney Cells. J Euk Microbiol. 1994; 41:8–12. [PubMed: 8124271]

Moltke J, Trinidad NJ, Moayeri M, Kintzer AF, Wang SB, Rooijen N, Brown CR, Krantz BA, Leppla SH, Gronert K, Vance RE. Rapid induction of inflammatory lipid mediators by the inflammasome *in vivo*. Nature. 2012; 490:107–111. [PubMed: 22902502]

Mordue DG, Scott-Weathers CF, Tobin CM, Knoll LJ. A patatin-like protein protects *Toxoplasma gondii* from degradation in activated macrophages. Mol Microbiol. 2007; 63:482–496. [PubMed: 17166175]

Mrsny RJ, Gewirtz AT, Siccardi D, Savidge T, Hurley BP, Madara JL, McCormick BA. Identification of Hepoxilin A3 in inflammatory events: a required role in neutrophil migration across intestinal epithelia. Proc Natl Acad Sci USA. 2004; 101:7421–7426. [PubMed: 15123795]

Murugesan S, Goldberg EB, Dou E, Brown WJ. Identification of Diverse Lipid Droplet Targeting Motifs in the PNPLA Family of Triglyceride Lipases. PLoS One. 2013; 8:e64950. [PubMed: 23741432]

Nebert DW, Dalton TP. The role of cytochrome P450 enzymes in endogenous signaling pathways and environmental carcinogenesis. Nat Rev Cancer. 2006; 6:947–960. [PubMed: 17128211]

Nigam S, Nodes J, Cichon G, Corey EJ, Pace-Asciak CR. Receptor-mediated action of hepoxilin A3 releases diacylglycerol and arachidonic acid from human neutrophils. Biochem Biophys Res Commun. 1990; 171:944–948. [PubMed: 2171511]

Nolan SJ, Romano JD, Coppens I. Host lipid droplets: An important source of lipids salvaged by the intracellular parasite *Toxoplasma gondii*. PLoS Pathog. 2017; 13:e1006362. [PubMed: 28570716]

Noor S, et al. Activation of cytosolic phospholipase A2α in resident peritoneal macrophages by *Listeria monocytogenes* involves listeriolysin O and TLR2. J Biol Chem. 2008; 283:4744–4755. [PubMed: 18083708]

Norris PC, Gosselin D, Reichart D, Glass CK, Dennis EA. Phospholipase A2 regulates eicosanoid class switching during inflammasome activation. Proc Natl Acad Sci. 2014; 111:12746–12751. [PubMed: 25139986]

Norris PC, Dennis EA. Omega-3 fatty acids cause dramatic changes in TLR4 and purinergic eicosanoid signaling. Proc Natl Acad Sci USA. 2012; 109:8517–8522. [PubMed: 22586114]

Noverr MC, Erb-Downward JR, Huffnagle GB. Production of eicosanoids and other oxylipins by pathogenic eukaryotic microbes. Clin Microbiol Rev. 2003; 16:517–533. [PubMed: 12857780]

Pappa V, Mony V, Fajardo JE, Guo P, Kieken F, Ankarklev J, Noy T, Almo SC, Fiser A, Daily JP. *Plasmodium falciparum* PLA2: a potential novel anti-malarial target. Mol & Biochem Parasitology. 2017 In press.

Passero LFD, Laurenti MD, Tomokane TY, Corbett CEP, Toyama MH. The effect of phospholipase A2 from *Crotalus durissus collilineatus* on *Leishmania (Leishmania) amazonensis* infection. Parasitol Res. 2008; 102:1025–1033. [PubMed: 18180953]

Pawlowic MC, Zhang K. *Leishmania* parasites possess a platelet-activating factor acetylhydrolase important for virulence. Mol Biochem Parasitol. 2012; 186:11–20. [PubMed: 22954769]

Petri MH, Thul S, Ovchinnikova O, Back M. Differential regulation of monocytic expression of leukotriene and lipoxin receptors. Prostaglandins Other Lipid Mediat. 2015; 121:138–143. [PubMed: 26248046]

Phillips RM, Six DA, Dennis EA, Ghosh P. In Vivo Phospholipase Activity of the *Pseudomonas aeruginosa* Cytotoxin ExoU and Protection of Mammalian Cells with Phospholipase A2 Inhibitors. J Biol Chem. 2003; 278:41326–41332. [PubMed: 12915403]

Pollok RCG, McDonald V, Kelly P, Farthing MJG. The role of *Cryptosporidium parvum*-derived phospholipase in intestinal epithelial cell invasion. Parasitol Res. 2003; 90:181–6. [PubMed: 12783305]

Qiao A, et al. Mouse patatin-like phospholipase domain-containing 3 influences systemic lipid and glucose homeostasis. Hepatology. 2011; 54:509–521. [PubMed: 21547936]

Quehenberger O, Dennis EA. The human plasma lipidome. N Engl J Med. 2011; 365:1812–1823. [PubMed: 22070478]

Rahman MS, Ammerman NC, Sears KT, Ceraul SM, Azad AF. Functional characterization of a phospholipase A2 homolog from *Rickettsia typhi*. J Bacteriol. 2010; 192:3294–3303. [PubMed: 20435729]

Rajakumari S, Duam G. Multiple Functions as Lipase, Steryl Ester Hydrolase, Phospholipase, and Acyltransferase of Tgl4p from the Yeast *Saccharomyces cerevisiae*. J Biol Chem. 2010; 285:5769–15776.

Ramakrishnana S, Serricchio M, Striepen B, Bütikofer P. Lipid Synthesis in Protozoan Parasites: a Comparison Between Kinetoplastids and Apicomplexans. Prog Lipid Res. 2013; 52:488–512. [PubMed: 23827884]

Ridgley EL, Ruben L. Phospholipase from *Trypanosoma brucei* releases arachidonic acid by sequential *sn*-1, *sn*-2 deacylation of phospholipids. Mol Biochem Parasitol. 2001; 114:29–40. [PubMed: 11356511]

Ridzuan M, et al. Effect of choloine kinase inhibitor hexadecyltrimethylammonium bromide on *Plasmodium falciparum* gene expression. Southeast Asian J Trop Med Public Health. 2014; 45:259–266. [PubMed: 24968665]

Rodrigues FG, et al. Expression of a mutated phospholipase A 2 in transgenic *Aedes fluviatilis* mosquitoes impacts *Plasmodium gallinaceum* development. Insect Mol Biol. 2008; 17:175–183. [PubMed: 18353106]

Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. J Lipid Res. 2008; 50:S29–S34. [PubMed: 18952571]

Rub A, Arish M, Husain SA, Ahmed N, Akhter Y. Host-lipidome as a potential target of protozoan parasites. Microbes and Infect. 2013; 15:649–660. [PubMed: 23811020]

Ryu SB. Phospholipid-derived signaling mediated by phospholipase A in plants. Trends Plant Sci. 2004; 9:229–235. [PubMed: 15130548]

Saffer LD, Schwartzman JD. A soluble phospholipase of *Toxoplasma gondii* associated with host cell penetration. J Protozool. 1991; 38:454–460. [PubMed: 1920144]

Saffer LD, Long Krug SA, Schwartzman JD. The role of phospholipase in host cell penetration by *Toxoplasma gondii*. Am J Trop Med Hyg. 1989; 40:145–149. [PubMed: 2919724]

Saliba KJ, Folb PI, Smith PJ. Role for the *Plasmodium falciparum* digestive vacuole in chloroquine resistance. Biochem Pharmacol. 1998; 56:313–320. [PubMed: 9744568]

Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science. 1987; 237:1171–1176. [PubMed: 2820055]

Sato H, Frank DW. ExoU is a potent intracellular phospholipase. Mol Microbiol. 2004; 53:1279–1290. [PubMed: 15387809]

Sawa T, et al. *Pseudomonas aeruginosa* Type III secretory toxin ExoU and its predicted homologs. Toxins. 2016; 8:1–15.

Scherer GFE, Ryu SB, Wang X, Matos AR, Heitz T. Patatin-related phospholipase A: Nomenclature, subfamilies and functions in plants. Trends Plant Sci. 2010; 15:693–700. [PubMed: 20961799]

Schreiber R, Zechner R. Lipolysis meets inflammation–arachidonic acid mobilization from fat. J Lip Res. 2014; 55:C055673.

Seeber F, Limenitakis J, Soldati-Favre D. Apicomplexan mitochondrial metabolism: a story of gains, losses and retentions. Trends Parasitol. 2008; 24:468–478. [PubMed: 18775675]

Senda K, Yoshioka H, Doke N, Kawakita K. A cytosolic phospholipase A2 from potato tissues appears to be patatin. Plant Cell Physiol. 1996; 37:347–353. [PubMed: 8673343]

Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014; 510:92–101. [PubMed: 24899309]

Shewry PR. Tuber storage proteins. Annals of botany. 2003; 91:755–769. [PubMed: 12730067]

Shureiqi I, Lippman SM. Lipoxygenase modulation to reverse carcinogenesis. Cancer Res. 2001; 61:6307–6312. [PubMed: 11522616]

Sidik SM, Hackett CG, Tran F, Westwood NJ, Lourido S. Efficient genome engineering of *Toxoplasma gondii* using CRISPR/Cas9. PLoS One. 2014; 9:e100450. [PubMed: 24971596]

Six DA, Dennis EA. The expanding superfamily of phospholipase A 2 enzymes: classification and characterization. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2000; 1488:1–19. [PubMed: 11080672]

Steczko J, Donoho GP, Clemens JC, Dixon JE, Axelrod B. Conserved histidine residues in soybean lipoxygenase: functional consequences of their replacement. Biochemistry. 1992; 31:4053–4057. [PubMed: 1567851]

Talvani A, et al. Leukotriene B4 Induces Nitric Oxide Synthesis in *Trypanosoma cruzi* -Infected Murine Macrophages and Mediates Resistance to Infection. Infect Immun. 2002; 70:4247–4253. [PubMed: 12117933]

Tam VC. Lipidomic profiling of bioactive lipids by mass spectrometry during microbial infections. Semin Immunol. 2013; 25:240–248. [PubMed: 24084369]

Thardin JF, et al. Eicosanoid production by mouse peritoneal macrophages during *Toxoplasma gondii* penetration: Role of parasite and host cell phospholipases. Infect Immun. 1993; 61:1432–1441. [PubMed: 8454347]

Tobin CM, Knoll LJ. A patatin-like protein protects *Toxoplasma gondii* from degradation in a nitric oxide-dependent manner. Infect Immun. 2012; 80:55–61. [PubMed: 22006568]

Tobin CM, Pittman KJ, Moser LA, Boldon KM, Knoll LJ. A *Toxoplasma* patatin-like protein changes localization and alters the cytokine response during Toxoplasmic encephalitis. Infect Immun. 2014; 82:618–625. [PubMed: 24478077]

Vance RE, Hong S, Gronert K, Serhan CN, Mekalanos JJ. The opportunistic pathogen *Pseudomonas aeruginosa* carries a secretable arachidonate 15-lipoxygenase. Proc Natl Acad Sci USA. 2004; 101:2135–2139. [PubMed: 14766977]

Villena JA, Roy S, Sarkadi-Nagy E, Kim KH, Sul HS. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. J Biol Chem. 2004; 279:47066–47075. [PubMed: 15337759]

Wang D, DuBois RN. Eicosanoids and cancer. Nature Reviews Cancer. 2010; 10:181–193. [PubMed: 20168319]

Winstead MV, Balsinde J, Dennis EA. Calcium-independent phospholipase A2: structure and function. Biochimica et Biophysica Acta. 2000; 1488:28–39. [PubMed: 11080674]

Zhu W, Hammad LA, Hsu F, Mao Y, Luo ZQ. Induction of caspase 3 activation by multiple *Legionella pneumophila* Dot/Icm substrates. Cell Microbiol. 2013; 15:1783–1795. [PubMed: 23773455]

Zidovetzki R, Sherman IW, O'Brien L. Inhibition of *Plasmodium falciparum* Phospholipase A2 by Chloroquine, Quinine, and Arteether. J Parasitol. 1993; 79:565–570. [PubMed: 8331477]

Zieler H, Keister DB, Dvorak JA, Ribeiro JM. A snake venom phospholipase A2 blocks malaria parasite development in the mosquito midgut by inhibiting ookinete association with the midgut surface. J Exp Biol. 2001; 204:4157–4167. [PubMed: 11809789]

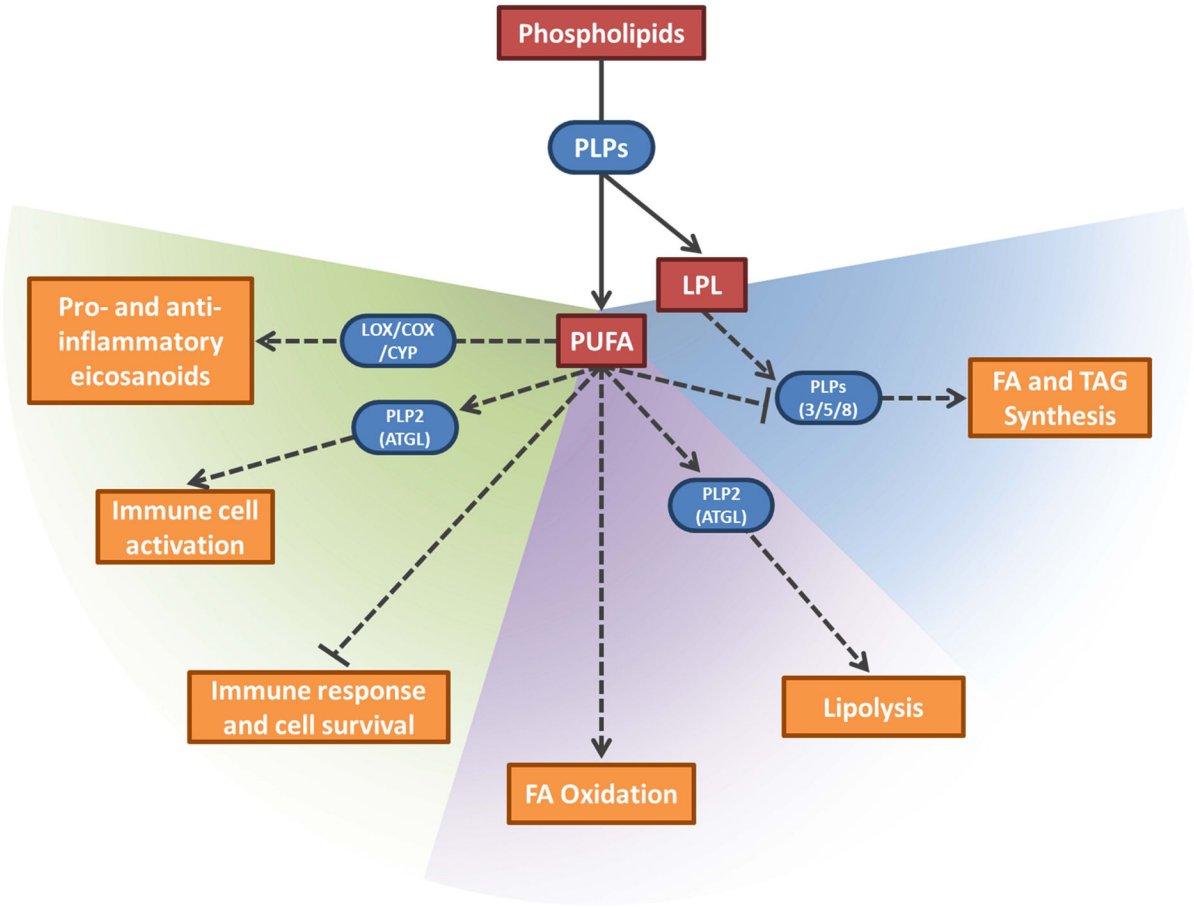


Fig. 1.

Diverse roles of patatin-like phospholipases (PLPs) and their products in immune regulation (left/green region), lipolysis (middle/purple region), and lipogenesis (right/blue region). PLPs release polyunsaturated fatty acids (PUFA) and lysophospholipids (LPL) from phospholipids. These products can directly alter membrane fluidity and can also act as signaling molecules to regulate lipid metabolism and cellular signaling pathways. PUFAs can be oxidized by LOX, COX, and CYP to form both pro- and anti-inflammatory eicosanoids (figure 3 for details). Some PLPs (e.g. PNPL2/ATGL, PNPLA3, PNPLA5, and PNPLA8 in mammals) can be regulated by PUFAs and LPLs to maintain stringent control over immune responses and lipid homeostasis (figure 2 for details). Substrates and signaling molecules are red, enzymes are blue and rounded, and altered downstream processes are orange. Activation and inhibition are indicated with arrows and bar-headed lines, respectively. Dashed lines indicate indirect pathways.

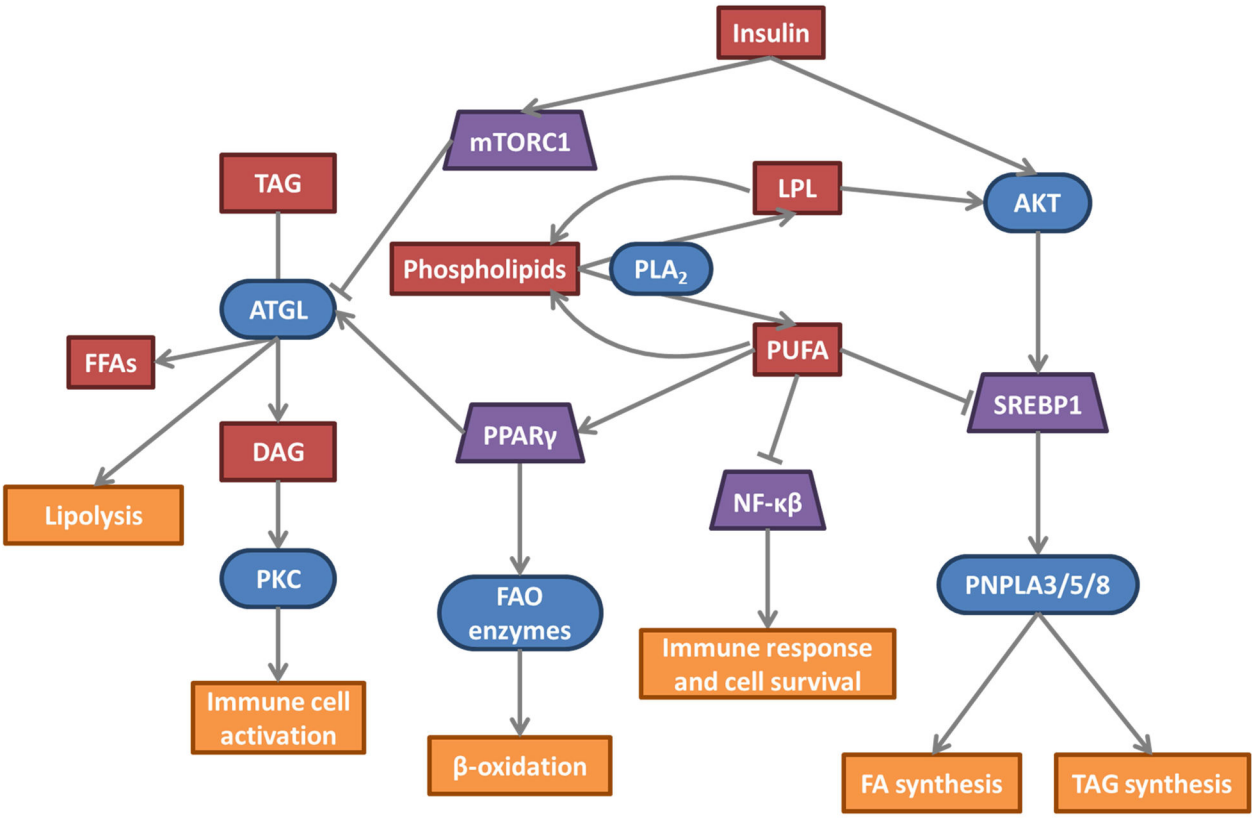
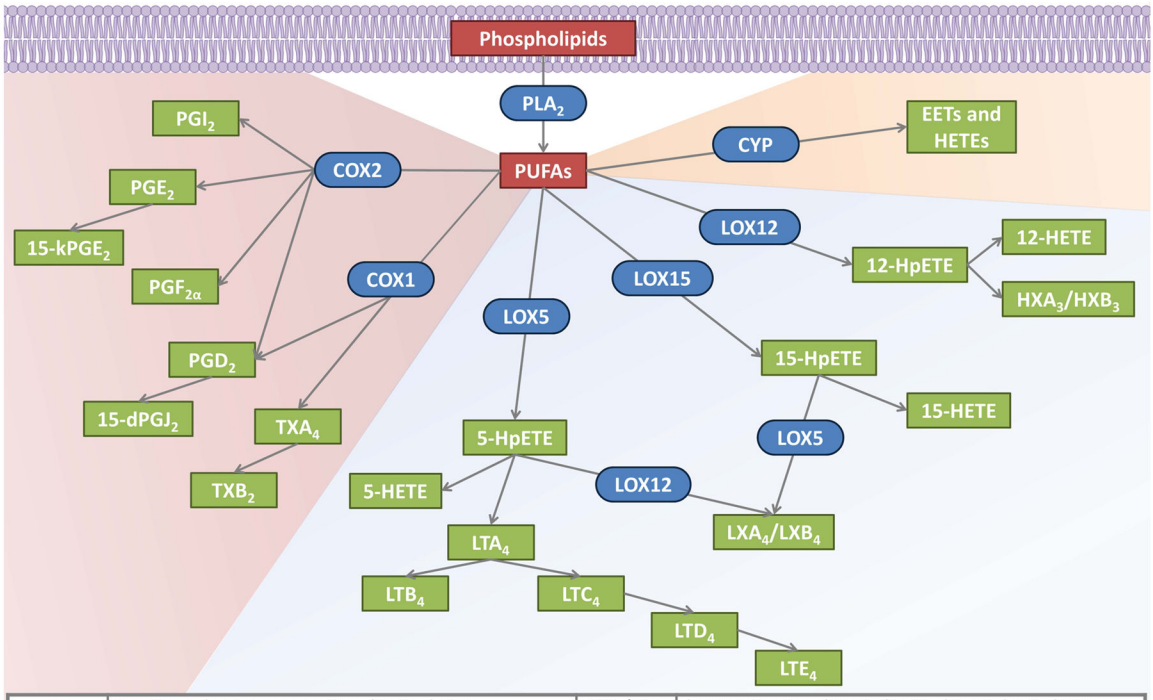


Fig. 2.

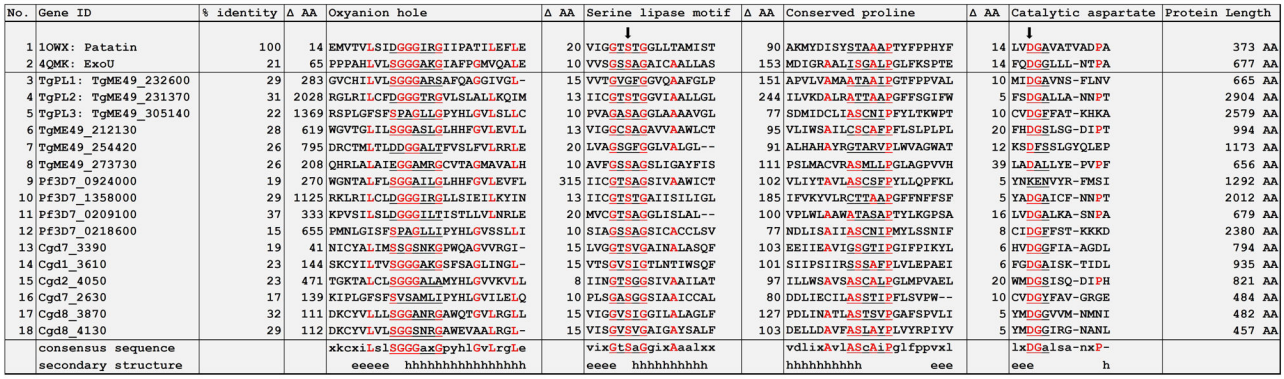
PLA_2 enzymes in lipid metabolism and cellular signaling. PUFAs and lysophospholipids (LPLs) are cleaved from phospholipids by PLA_2. These products can be recycled back into the lipid membrane or serve as signaling molecules to regulate lipolysis, lipogenesis, and the immune responses. Downstream patatin-like PLA_2s (ATGL, PNPLA3, PNPLA5, and PNPLA8) respond to insulin levels, adding another layer of regulation on these lipid metabolism and cellular signaling pathways. Substrates and signaling molecules are red, enzymes are blue and rounded, transcription factors are purple trapezoids, and altered downstream processes are orange. Activation and inhibition are indicated with arrows and bar-headed lines, respectively. mTORC1 (mammalian target of rapamycin complex 1), Akt (Protein kinase B), PKC (Protein kinase C), DAG (diacylglycerol), PPARγ (Peroxisome proliferator-activated receptor gamma)



PGI_2	Vasodilator, ↓platelet aggregation, ↑IL-10, ↓TNFα	LXA_4/LXB_4	↓PMN infiltration, ↑TGFβ, ↑IL-10, ↓IL-1β, ↓IL-8, ↓TNFα
PGE_2	Vasodilator, ↓DC antigen presentation, ↑IL-10, ↓TNFα, ↓IL-12, ↑PMN eicosanoid class switching	LTB_4	↑vascular leakage, ↑chemotaxis of leukocytes, ↑T-cell proliferation, ↑IFNγ, ↑IL-1, ↑IL-2
$PGF_{2\alpha}$	Vasoconstrictor	$LTC_4/D_4/E_4$	↑vascular leakage, ↑neutrophil recruitment
PGD_2	Vasodilator, ↑allergic response, ↑eosinophil recruitment	HXA_3/HXB_3	↑neutrophil recruitment, ↑intracellular Ca^{2+}
$15\text{-}dPG_{j2}$	↓NF-κβ signaling, activates PPARγ	HpETE/HETE	Vasodilators, ↓COX2
TXA_4/TXB_4	Vasoconstrictor, ↑platelet aggregation, ↓T-cell activation	EETs	Vasodilators, ↓COX2, tumor initiation and progression

Fig. 3.

Eicosanoid production and inflammatory roles. PUFAs, released from phospholipids by PLA_2, are oxidized by three main pathways to form both pro- and anti-inflammatory eicosanoids. COX pathways to produce PGs and TXs are shown in the red-shaded region. LOX production of HETEs, HXs, LTs, and LXs are shown in the blue-shaded region. Cytochrome P450 (CYP) products are shown in the orange-shaded region. Substrates are red, products are green, and enzymes are blue and rounded. The table summarizes some of the discussed downstream pro- and anti-inflammatory effects of the eicosanoids.



No.	Gene ID	% identity	Δ AA	Oxyanion hole	Δ AA	Serine lipase motif	Δ AA	Conserved proline	Δ AA	Catalytic aspartate	Protein Length
1	1OWX: Patatin	100	14	EMVTVLSIDGGGIRGIIPATILEFLE	20	VIGGTSTGGLLTAMIST	90	AKMYDISYSTAAAPTYFPPHYF	14	LVDGAVATVADPA	373 AA
2	4QMK: ExoU	21	65	PPPAHLVLSGGGGAKGIAFPGMVQALE	10	VVSGSSAGAICAALLAS	153	MDIGRAALISGALPGLFKSPTE	14	FQDGGLLL-NTPA	677 AA
3	TgPL1: TgME49_232600	29	283	GVCHILVLSGGGARSAFQAGGIVGL-	15	VVTGVGFGGVQAAFGLP	151	APVLVAMAATAAIPGTFPPVAL	10	MIDGAVNS-FLNV	665 AA
4	TgPL2: TgME49_231370	31	2028	RGLRILCFDGGGTRGVLSLALLKQIM	13	IICGTSTGGVIAALLGL	244	ILVKDALRATTAAPGFFSGIFW	5	FSDGALLA-NNPT	2904 AA
5	TgPL3: TgME49_305140	22	1369	RSPLGFSFSPAGLLGPYHLGVLSLLC	10	PVAGASAGGLAAAAVGL	77	SDMIDCLIASCNIPFYLTKWPT	10	CVDGFFAT-KHKA	2579 AA
6	TgME49_212130	28	619	WGVTGLILSGGASLGLHHFGVLEVLL	13	VIGGCSAGAVVAAWLCT	95	VLIWSAILCSCAFPFLSLPLPL	20	FHDGSLSG-DIPT	994 AA
7	TgME49_254420	26	795	DRCTMLTLDDGGALTFVSLFVLRRLE	20	LVAGSGFGGLVALGL--	91	ALHAHAYRGTARVPLWVAGWAT	12	KSDFSSLGYQLEP	1173 AA
8	TgME49_273730	26	208	QHRLALAIEGGAMRGCVTAGMAVALH	10	AVFGSSAGSLIGAYFIS	111	PSLMACVRASMLLPGLAGPVVH	39	LADALLYE-PVPF	656 AA
9	Pf3D7_0924000	19	270	WGNTALFLSGGAILGLHHFGVLEVFL	315	IICGTSAGSIVAAWICT	102	VLIYTAVLASCSFPYLLQPFKL	5	YNKENVYR-FMSI	1292 AA
10	Pf3D7_1358000	29	1125	RKLRILCLDGGGIRGLLSIEILKYIN	13	IICGTSTGAIISILIGL	185	IFVKYVLRCTTAAPGFFNFFSF	5	YADGAICF-NNPT	2012 AA
11	Pf3D7_0209100	37	333	KPVSILSLDGGGILTISTLLVLNRLE	20	MVCGTSAGGLISLAL--	100	VPLWLAAWATASAPTYLKGPSA	16	LVDGALKA-SNPA	679 AA
12	Pf3D7_0218600	15	655	PMNLGISFSPAGLLIPYHLGVSSLLI	10	SIAGSSAGSICACCLSV	77	NDLISAIIASCNIPMYLSSNIF	8	CIDGFFST-KKKD	2380 AA
13	Cgd7_3390	19	41	NICYALIMSSGSNKGPWQAGVVRGI-	15	LVGGTSVGAINALASQF	103	EEIIEAVIGSGTIPGIFPIKYL	6	HVDGGFIA-AGDL	794 AA
14	Cgd1_3610	23	144	SKCYILTVSGGGAKGSFSAGLINGL-	15	VTSGVSIGTLNTIWSQF	101	SIIPSIIRSSSAFPLVLEPAEI	6	FGDGAISK-TIDL	935 AA
15	Cgd2_4050	23	471	TGKTALCLSGGGALAMYHLGVVKVLL	8	IINGTSGGSIVAAILAT	97	ILLWSAVSASCALPGLMPVAEL	20	WMDGSISQ-DIPH	821 AA
16	Cgd7_2630	17	139	KIPLGFSFSVSAMLIPYHLGVILELQ	10	PLSGASGGSIAAICCAL	80	DDLIECILASSTIPFLSVPW--	10	CVDGYFAV-GRGE	484 AA
17	Cgd8_3870	32	111	DKCYVLLLSGGANRGAWQTGVLRGLL	15	VIGGVSIGGILALAGLF	127	PDLINATLASTSVPGAFSPVLI	5	YMDGGVVM-NMNI	482 AA
18	Cgd8_4130	29	112	DKCYVLVLSGGSNRGAWEVAALRGL-	15	VISGVSVGAIGAYSALF	103	DELLDAVFASLAYPLVYRPIYV	5	YMDGGIRG-NANL	457 AA
	consensus sequence			xkcxiLslSGGGaxGpyhlGvLrgLe		vixGtSaGgixAaalxx		vdlixAvlAScAiPglfppvxl		lxDGalsa-nxP-	
	secondary structure			eeeee hhhhhhhhhhhhhhh		eeee hhhhhhhhh		hhhhhhhhhh eee		eee h	

Fig. 4.

Alignment of conserved patatin domains in Apicomplexa PLPs. 16 *Toxoplasma, Plasmodium,* and *Cryptosporidium* PLP genes were aligned to PDB structures of Patatin and *P. aeruginosa* ExoU using the CLUSTAL Omega and MAFFT methods in MegAlign Pro (DNASTAR). PROMALS3D and SWISS-MODEL were used to confirm the predicted secondary and tertiary structures aligned with the known PLP structures. The known conserved patatin motifs are underlined and residues conserved in more than 60% of the sequences are colored red. Arrows indicate the catalytic S/A dyad. % identity = 100*(1 – distance) where distance is the uncorrected pairwise distance between the full sequence of each gene compared to patatin. ΔAA is the number of residues before and after each motif.