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Phospholipases A and Lysophospholipases in protozoan parasites

Perrine Hervé^{1*}, Sarah Monic¹, Frédéric Bringaud¹, Loïc Rivière^{1*}.

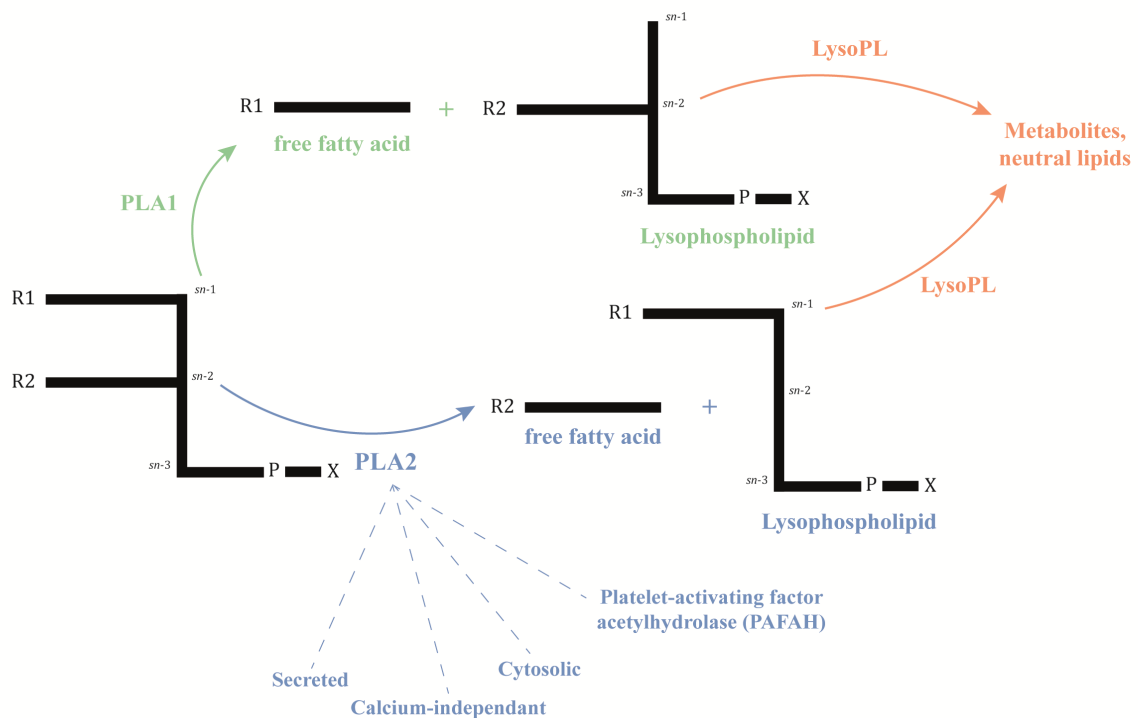
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27 Phospholipases (PLs) and lysophospholipases (LysoPLs) are a widespread and complex group of
 28 lipolytic enzymes responsible for the hydrolysis of phospholipids and lysophospholipids, respectively.
 29 They are classified as A1, A2, C and D according to their site of cleavage (Fig 1) (1). These enzymes
 30 are involved in several biological processes including modulation of the immune response, cell
 31 signaling, membrane remodeling and lipid metabolism (2,3). Some of them, mainly phospholipases A
 32 (PLAs) and LysoPLs, play important roles in the virulence and pathogenicity of several groups of
 33 pathogens such as bacteria (*Pseudomonas*, *Rickettsia*) and fungi (*Candida*) (4,5). PLAs are also major
 34 toxins of venomous species (6). For detailed information regarding PLs, please refer to Aloulou *et al.*
 35 (1).



36

37

38 **Fig 1. Simplified phospholipid structure and the site of cleavage of Phospholipases A and**
 39 **Lysophospholipases.** A phospholipid consists of a glycerol-3-phosphate esterified with nonpolar fatty
 40 acids at its *sn*-1 (R1) and *sn*-2 (R2) positions and a polar headgroup (X = choline, ethanolamine,
 41 serine...) at its phosphoryl group. Phospholipases A are named A1 or A2 depending on their site on

42 cleavage and the resulting molecules, indicated by arrows. Lysophospholipases act on
43 lysophospholipids previously hydrolyzed by a PLA (1).

44 Protozoans form a group of eukaryotic unicellular organisms that contain parasites responsible for
45 severe and even fatal diseases worldwide. Their modes of transmission are diverse and include vectors
46 (insects, gasteropods, annelids, etc.), contaminated food, water or soil, congenital transmission, blood
47 transfusion or organ transplantation. Some of the diseases they cause are especially prevalent in
48 developing countries due to lower standards of living and a favorable climate for the presence of
49 vectors. However, migration and climate change could favor the spread of these parasites globally.
50 While some parasites such as the Apicomplexan *Plasmodium* spp. and *Toxoplasma* are heavily
51 studied, others remain neglected despite the high risks they represent for susceptible populations (7,8).

52 The importance of PLAs and LysoPLs for virulence in pathogenic species and their ability to
53 manipulate the host immune response make them particularly interesting in the context of host –
54 parasite interaction. Thanks to the sequencing of their genomes and the availability of many genomic
55 resources, we know that most protozoan parasites likely possess several *PLA* genes
56 (<https://veupathdb.org>). However, recent studies are particularly focused on apicomplexan parasites,
57 neglecting other major protozoan pathogens. Some characterized PLAs in pathogenic protozoan seem
58 to be involved in the survival of the parasite inside its host and the establishment of an infection.

59 In this review, we focus on the different PLAs and LysoPLs characterized in several protozoan
60 parasites of medical interest. We also discuss the relevance of these PLAs as potential drug and
61 vaccine targets. The different parasites and enzymes described in this review are summarized in Table
62 1 at the end of the discussion.

63 **Apicomplexan**

64 Infections by Apicomplexan parasites represent huge health burdens worldwide. These parasites are
65 characterized by the presence of the apicoplast, a plastid-like organelle which holds the lipid
66 biosynthesis pathways (7).

67 ***Toxoplasma gondii***

68 *Toxoplasma gondii* is estimated to chronically infect one third of the world's population. This parasite
69 can infect a wide range of warm-blooded animals, but its definitive hosts are felines. Although most
70 infections are asymptomatic, congenital transmission or reactivation of latent disease in
71 immunocompromised individuals such as AIDS, cancer or organ transplant patients can cause severe
72 toxoplasmosis and represent significant public health burdens. Transmission usually occurs by
73 ingestion of parasites or from an infected mother to the fetus. *Toxoplasma* parasites are present in
74 three forms: an environment-resistant oocyst, a fast-replicating tachyzoite and a latent tissue cyst
75 containing bradyzoites. *Toxoplasma* tachyzoites can replicate in a variety of nucleated cell types, while
76 the chronic-stage bradyzoites persist as cysts within neurons or muscles (9).

77 Several PLAs of *Toxoplasma* have been characterized, including some important for the survival of
78 the parasite in its host. TgLCAT (lecithin:cholesterol acyltransferase) produces cholesteryl esters
79 through the transfer of phospholipid acyl groups. The enzyme possesses both PLA2 and cholesteryl
80 esterase activities and uses phosphatidylcholine (PC) as a substrate. TgLCAT is stored in dense
81 granules and localizes to both the parasitophorous vacuole (PV) and plasma membrane following
82 secretion from the dense granules. In a first study, parasites deficient for TgLCAT displayed a delay in
83 replication and egress, suggesting a role in lipid membrane remodeling of both the parasite during
84 replication and host cell during parasite escape. Mice infected with TgLCAT-null mutants showed
85 reduced virulence, while parasites overexpressing TgLCAT were more virulent than the parental strain
86 and resulted in earlier deaths (10). In a later study using new strains, the authors confirmed its role in
87 egress but showed that it has no contribution to replication *in vitro* or virulence *in vivo* (11).

88 Other PLA2s described include patatin-like phospholipases (PLPs), Ca²⁺-independent PLA2s. Three
89 PLPs are known in *Toxoplasma* parasites. TgPL1 reduces the level of nitric oxide in activated
90 macrophages to prevent its own degradation (12). The enzyme lacks the catalytic serine and PLA2
91 activity, but the presence of other catalytic residues suggests a role in binding phospholipids or as a
92 coenzyme for other PLA2 enzymes (13). Mice infected with TgPL1-null mutants showed a greater
93 resistance to toxoplasmic encephalitis and a change in cytokine levels during the chronic infection.
94 The authors also observed a defect in bradyzoite-to-tachyzoite reactivation. The protein moves from

95 within the parasite to the cyst wall and PV during tachyzoite-to-bradyzoite differentiation. Overall,
96 TgPL1 seems to play a role in the maintenance of the infection in the host and toxoplasmosis
97 encephalitis by altering cytokine production in the chronic phase (14).

98 A second PLP, TgPL2, is located at the apicoplast. This enzyme appeared essential for apicoplast
99 maintenance and lipid homeostasis. In parasites deficient for TgPL2 or with a mutated catalytic serine,
100 the apicoplast was lost or became abnormal, leading to an impaired growth *in vitro* (15).

101 Ca^{2+} -independent PLA2 activity associated with Ca^{2+} -independent PLA1 activity was described in
102 2000, but it is unknown if one or several proteins were responsible for these activities (16).

103 Finally, PLA2 activity possibly linked to host cell invasion was first reported in 1989, where the
104 authors showed that incubation of the parasite with exogenous PLA2 from cobra (*Naja mossambica*)
105 resulted in an increase of penetration in human fibroblasts. Meanwhile, PLA2 inhibition by 4-p-
106 bromophenacyl bromide (4-BPB) decreased cell invasion, although the authors cautioned that 4-BPB
107 seems to have a general toxic effect on parasites. Use of another PLA2 inhibitor, dihydroguaiaretic
108 acid, also resulted in a decreased host cell invasion (17). PLA2 activity could trigger arachidonic acid
109 (AA) production from host cell phospholipids, which would alter the host cell membrane and facilitate
110 parasite entry (18). Incubation of extracellular *Toxoplasma* with exogenous PLA2 increased the
111 presence of the rhoptry protein ROP1 in supernatant fractions, raising the possibility of a role of PLA2
112 in rhoptry protein secretion (19,20). Based on these results, another team confirmed the role of 4-BPB
113 on THP1 monocyte cells. Furthermore, IFN- γ treatment caused a reduction in host cell invasion, but
114 whether that effect was direct or not on PLA is unclear (21,22). A PLP contributing to host cell
115 invasion was recently reported. TgPL3 is located at the apical cap of the parasite. Δ TgPL3 parasites
116 showed defects in invasion and rhoptry secretion; the authors hypothesize that TgPL3 has a role in
117 either the mechanical release of rhoptry or in the signaling pathway leading to rhoptry release.
118 Mutation of the catalytic serine (S1409A parasites) also suppressed enzyme activity. Mice vaccination
119 with Δ TgPL3 or S1409A parasites protected them against a subsequent infection with a lethal dose of
120 *Toxoplasma* parasites (23).

121 ***Plasmodium* sp.**

122 Malaria is the most common and deadliest human parasitic disease, and one of the most prevalent
123 human infectious diseases. In 2021, 247 million cases and 619,000 deaths were reported (24). 90% of
124 deaths occur in Africa and mainly affect children under 5 and pregnant women. Parasites are
125 transmitted by female mosquitoes of the *Anopheles* genus. Most cases and deaths are attributed to *P.*
126 *falciparum*, but 4 other species of *Plasmodium* are also responsible for human infections: *P. vivax*, *P.*
127 *ovale*, *P. malariae* and *P. knowlesi*. Parasites continuously evolve inside their hosts: the sporozoite
128 insect form, once injected into a human, turns into a merozoites in hepatocytes. Merozoites are
129 released into the bloodstream where they invade red blood cells (RBCs) and produce gametocytes.
130 Mosquitoes ingest these gametocytes during a blood meal (25).

131 Cells infected by *Plasmodium* provide lipid precursors to the parasites, which are necessary for *de*
132 *novo* synthesis of phospholipids and neutral lipids. These lipids are used for membrane maintenance
133 and remodelling (26). The presence of (lyso)phospholipases is therefore essential for maintenance of
134 *Plasmodium* inside their hosts. More than 20 potential genes were identified in the genome of
135 *Plasmodium* parasites (26,27). It was first shown that use of gentamicin and amikacin,
136 aminoglycosides which bind to the 30S subunit of ribosomes and are also known as PLA1 and PLA2
137 inhibitors, repressed *P. falciparum* growth in pretreated erythrocytes (28). These enzymes could also
138 be involved in the alteration of merozoite membrane phospholipid organization in the RBCs (29).
139 Furthermore, upon *P. falciparum* infection, both PLA2 and LysoPL activities were detected in human
140 erythrocytes. LysoPL activity greatly increased in infected RBCs compared to non-infected ones and
141 was much higher than PLA2 activity, suggesting that hydrolysis by LysoPLs is an important metabolic
142 pathway for control of lysophospholipid levels. This PLA2 activity is probably of parasitic origin,
143 however, it cannot be ruled out that *P. falciparum* upregulates expression of a host *PLA2* gene, as
144 more recently described (30,32). PLA2 activity was inhibited *in vitro* by the antimalarial drugs
145 chloroquine, quinine and artemether and LysoPL activity was inhibited by quinacrine and quinine, but
146 the IC50 values for these inhibitions appear high and could be non-specific inhibitions due to high
147 concentrations of the drugs. LysoPL activity was also inhibited by the sulfhydryl reagents p-

148 hydroxymercuribenzoate and thimerosal, with IC50 values in much lower ranges (30,31). Later on, it
149 was shown that *P. falciparum* parasites internalize host RBC peroxiredoxin 6 (PRDX6), an enzyme
150 with PLA2 activity. The authors found that this host enzyme is used by the parasite for repair of lipid-
151 peroxidation damage in *P. falciparum* blood stages and is essential for hemoglobin transport to the
152 parasite food vacuole. Use of PLA2 inhibitors binding to PRDX6, such as methyl arachidonyl
153 fluorophosphonate (MAFP), ATK and Daraplabid, blocked parasite growth at the trophozoite stage.
154 Use of Daraplabid on WT mice arrested model rodent pathogen *P. yoelii* development, but had not
155 effect in transgenic *prd6x*^{-/-} mice, demonstrating a possible use of PRDX6 as a host drug target for
156 *Plasmodium* infection control (32). The PLA2 activity detected in infected RBC could therefore be
157 coming from the host and not *Plasmodium* (30,32). LysoPC from the host is also important for *P.*
158 *falciparum* differentiation during infection. Restriction of the lipid, which occurs during malaria
159 infection, led to metabolic adaptation of parasites, initiating sexual commitment and formation of
160 gametocytes (33).

161 Several PLAs and LysoPLs have been identified in *Plasmodium* species. *PfLPL1* is a LysoPL
162 localized in the ER at the early stages. After erythrocyte invasion, the enzyme moves to vesicular
163 structures that are later incorporated into lipid bodies. These bodies are found next to the food vacuole,
164 where is stored hemozoin, the neutralized form of the toxic by-product heme. *PfLPL1* generates lipids
165 necessary for hemozoin formation; downregulation of the enzyme resulted in disrupted lipid
166 homeostasis and reduced levels of neutral lipids essential for conversion of toxic heme to hemozoin
167 (34). Another LysoPL from *P. falciparum*, *PfLPL20*, hydrolyzes lysophosphatidylcholine (LysoPC)
168 from the host to generate metabolites that can be used by the parasite for its growth. Like *PfLPL1*,
169 *PfLPL20* localizes to vesicular structures that later fuse in lipid bodies (35).

170 Two PLs were characterized in the rodent malaria model pathogen, *P. berghei*. First, PbPL is involved
171 in sporozoite migration, localizes at their surface and possesses both PLA2 and LCAT activity.
172 Infection of mice with null-mutant sporozoites through mosquito bite resulted in a significant decrease
173 in liver infectivity. A decrease in epithelial cell layers crossing was observed in PbPL knock-out
174 parasites. Furthermore, an hemolytic assay showed that heterologously expressed PbPL was able to

175 damage cell membranes, which the authors hypothesize could be caused by direct hydrolysis of
176 membrane PC or through the production of LysoPC species, which possess membrane lysis activities
177 (36). In infected hepatocytes, PbPL is located at the PV membrane (PVM) where it plays a role in its
178 disruption; PbPL-deficient parasites showed a defect in merozoite release from hepatocytes and
179 oocysts (37). This inefficient merozoites release was also reported in PbPL's ortholog in *P.*
180 *falciparum*, PflCAT. Lipidomics showed no significant changes in PC or LysoPC levels in PflCAT-
181 null mutants, but an accumulation of phosphatidylserine during egress was observed. PflCAT-
182 mutants also showed an inefficient egress in asexual blood stages (38). A second enzyme, PbPLA1, is
183 a phosphatidic acid preferring PLA1. The cytoplasmic enzyme is not expressed in sporozoites and is
184 not essential in both mice infection and during mosquito stages. *Plal*⁻ parasites, like PbPL-deficient
185 parasites, showed a defect in merozoite release from hepatocytes (39).

186 Just as *Toxoplasma*, *Plasmodium* species possess PLPs. PfpATPL1 is expressed in the cytosol of
187 asexual blood stage and gametocyte stages of *P. falciparum*. Depletion of the enzyme resulted in
188 reduced efficiency of rounding up, egress of gametes after gametocyte activation as well as
189 exflagellation of male gametes. Null-mutants for PfpATPL1 showed an impaired translocation of the
190 perforin-like protein PfpPLP2 to the membrane periphery. This translocation is necessary for
191 destabilization of vacuolar and RBC membranes, allowing egress of gametes (40). In a later study,
192 another group described an effect of the PLP, which they re-named PNPLA1, in gametocyte induction
193 rather than a deficiency in gametocytogenesis (41).

194 ***Cryptosporidium***

195 Cryptosporidiosis is a major diarrheal disease in young children and immunocompromised individuals
196 worldwide. Food and water contaminated by feces or contact with infected animals and people are
197 responsible for the transmission of parasites. *Cryptosporidium parvum* and *hominis* are the most
198 prevalent species found during human infections. *Cryptosporidium* can complete its life cycle in a
199 single host, and both asexual and sexual stages are found in the intestine of infected humans. The
200 parasite does not possess an apicoplast or mitochondrial DNA and relies on its host metabolism to

201 survive. After ingestion of oocysts, released sporozoites invade the small intestine and develop inside
202 epithelial cells in an extracytoplasmic niche, where they maintain the infection (42).

203 Currently, not a single PLA/LysoPL gene is characterized in *Cryptosporidium*. However, secreted
204 PLA2 (sPLA2) activity was observed in parasite lysate and could be involved in the invasion of the
205 host enterocytes. Inhibition of PLA activity by 4-BPB or anti-sPLA2 antibodies resulted in a
206 significant reduction in parasite reproduction in human enterocyte cell lines. Furthermore, treatment of
207 enterocytes or *C. parvum* sporozoite with sPLA2 derived from cobra (*Naja naja*) venom before *in*
208 *vitro* infection enhanced the number of intracellular parasites (43).

209 **Kinetoplastids**

210 Trypanosomatids include major pathogenic parasites responsible for neglected tropical diseases. These
211 parasites are part of the Kinetoplastid group, organisms which possess within their single
212 mitochondrion a concatenated and compacted network of circular DNA named kinetoplast (8).

213 **African trypanosomes**

214 Human and Animal African trypanosomiasis (HAT/AAT) are neglected tropical diseases caused by
215 different species of *Trypanosoma*. Two subspecies of *Trypanosoma brucei*, namely *T. b. gambiense*
216 and *T. b. rhodesiense*, cause HAT or sleeping sickness. *T. b. brucei*, *T. vivax* and *T. congolense* are
217 responsible for Nagana or AAT. The incidence of HAT has greatly decreased and WHO estimates an
218 interruption of its transmission by 2030, but 70 million people remain at risk for the disease (44). AAT
219 cases are still very high, annually causing the death of 3 million livestock and losses of billions of US
220 dollars in Africa. Parasites are transmitted by tsetse flies (*Glossina*) (44,45). African trypanosome
221 have a complex life cycle inside their hosts. The bloodstream mammalian form differentiates into
222 procyclics when a tsetse fly takes a blood meal. In the insect, procyclics differentiate into
223 epimastigotes and finally infective metacyclics, transmitted to mammals during a blood meal. In
224 humans, infection occurs in two phases: an early hemolymphatic stage (fever, adenopathy...) and a
225 late encephalitic stage in the CNS (neurologic troubles, biological clock disorders...) which eventually

226 leads to death without adequate treatment. (44). In animals, anemia and cachexia are the main
227 symptoms, but abortion, decrease of milk production and weight loss are also observed (46).

228 Higher levels of PLAs activity were first observed in *T. brucei* and *T. congolense* compared to the
229 nonpathogenic *T. lewisi* species present in rodents (47). This activity was more important in
230 bloodstream forms (48) and increased with parasite burden in tissue fluids and blood plasma of *T.*
231 *brucei*-infected rabbits (49). Bloodstream forms also generated phospholipids from exogenous
232 lysophospholipids; this activity may help the parasite acquire fatty acids for the synthesis of the
233 variant surface glycoprotein (VSG) membrane (50). Indeed, a GPI-specific PLA may be involved in
234 fatty acid remodelling leading to the biosynthesis of glycosylphosphatidylinositol (GPI), which
235 anchors the trypanosome VSG to the plasma membrane (51). Lastly, LysoPLA1 and PLA1 activities
236 were eluted together from *T. brucei* soluble protein fractions and were indissociable, suggesting that
237 the parasite possesses an enzyme displaying both activities (52).

238 Two *PLA1* genes have been characterized so far, both in *T. brucei*. TbPLA1 is a cytosolic enzyme
239 displaying both PLA1 and LysoPLA1 activities and its preferred substrate is PC, resulting in the
240 synthesis of LysoPC metabolites (53). The enzyme levels and resulting metabolites were higher in the
241 bloodstream forms compared to procyclics. Mutants for TbPLA1 were deficient in LysoPC synthesis.
242 Homologues of this enzyme are found in the closely related *T. congolense* and *T. vivax*, but absent
243 from more distant African trypanosomes or other Trypanosomatids. Its closest homologue is a putative
244 PLA1 from *Soladis glossinidius*, a proteobacterium endosymbiont of the tsetse fly, suggesting that
245 TbPLA1 may have been acquired through horizontal gene transfer (54).

246 TbLysoPLA is a recently characterized LysoPL. This secreted enzyme is localized in both the cytosol
247 and glycosomes of bloodstream forms of *T. brucei*. TbLysoPLA showed PLA1 activity on non-natural
248 phospholipids and PLA2 activity on natural phospholipids *in vitro*. The enzyme acts on PC to produce
249 LysoPC metabolites. TbLysoPLA is not essential for parasite survival but is probably highly
250 immunogenic, since antibodies directed against it were generated during mice infection with *T. brucei*
251 *gambiense* (55,56).

252 PLA2 activity was also detected in African trypanosomes. In *T. brucei* bloodstream and procyclic
253 forms, it may regulate Ca²⁺ entry in the parasite and catalyze AA release, which could be used to
254 control eicosanoid acid production and cause changes in the host cell membrane (57,58). Furthermore,
255 AA is the precursor for prostaglandin E2 (PGE2), a lipidic inflammatory mediator involved in
256 mammalian immune responses (59). However, this AA release from phospholipids can be PLA2-
257 independent through a sequential sn-1 deacylation followed with hydrolysis by LysoPLA (60).

258 As previously mentioned, GPI-PLA activity was thought to be involved in trypanosome VSG
259 remodeling (51). A protein with GPI-PLA2 activity mediating the GPI precursor fatty acid remodeling
260 was identified in procyclic *T. brucei* parasites. Deletion of the enzyme resulted in the suppression of
261 GPI-PLA2 activity. However, this enzyme was only characterized in procyclic parasites; VSG
262 remodeling is an essential feature of bloodstream parasites, and whether or not it is essential in this
263 form and its definitive role remain to be studied. The authors also caution that procyclic and
264 bloodstream form parasites may use different genes for this same activity. Moreover, the authors
265 hypothesize that another gene studied in this paper may encode for a GPI-PLA1 protein or a GPI-
266 PLA2 activity in bloodstream parasites, but did not show it (61).

267 Anemia is a major symptom of *T. congolense* infection. A PLA2 from the parasite may be directly or
268 indirectly responsible for the hemolytic activity observed during the course of infection, but the
269 enzyme(s) involved remains unidentified (62–66). ***Trypanosoma cruzi***

270 *Trypanosoma cruzi* is responsible for Chagas disease or American trypanosomiasis. This
271 anthrozoosis is endemic in Latin America, although human migrations is leading to a global
272 spread of the parasite. In endemic regions, the annual number of cases is estimated at 6 to 7 million,
273 with many more at risk. *T. cruzi* is mainly transmitted by triatomine insects, also known as kissing
274 bugs. The parasite has three main stages: a non-infective insect epimastigote, an infective
275 trypomastigote and in intracellular replicating form inside mammals, the amastigote. The acute phase
276 of infection is usually asymptomatic, with parasite invading and replicating in the peripheral
277 circulation. Parasites can migrate to cardiac and digestive tissues where they contribute to the
278 chronicity of the disease (67).

279 Phospholipases of *T. cruzi* are likely involved in global cellular lipid remodeling throughout the
280 parasite's life cycle and temperature acclimation (68). Both PLA1 and PLA2 membrane-associated
281 activities on anionic phospholipids were observed in epimastigotes and may also be implicated in
282 differentiation from trypomastigotes to amastigotes (69,70). Furthermore, the presence of exogenous
283 PLA2 significantly increased association between *T. cruzi* and macrophages, but the origin of the
284 enzyme was not specified. This association was reduced by use of PLA2 inhibitors such as 4-BPB,
285 quinacrine and phentermine. A PLA2 could therefore be involved in macrophage invasion (71).

286 The previous observation in *T. brucei* that Ca^{2+} entry was regulated by generation of AA by PLA2
287 activity was also seen in *T. cruzi* amastigotes by addition of a PLA2 activator, melittin. In parallel,
288 addition of the PLA2 inhibitor 3-(4-octadecyl)-benzoylacrylic acid (OBAA) decreased this influx (58).

289 TcPLA1 is the only PLA characterized in *T. cruzi*. It is responsible for PC degradation in autolysing
290 parasites. The enzyme possesses both PLA1 and LysoPLA1 activity and its PLA1 activity is 20-fold
291 higher in the infective trypomastigotes and amastigotes compared to non-infective epimastigotes (72).
292 In epimastigotes, TcPLA1 is found in the lysosome while in infective stages the activity of the enzyme
293 is membrane-bound. Secretion of TcPLA1 was observed during differentiation from epimastigotes to
294 trypomastigotes. *In vitro*, TcPLA1 generates DG, FFA and LPC, lipid secondary messengers that
295 activate host cell protein kinase C which leads to an upregulation of parasite invasion (73).
296 Accumulation of bioactive products such as LysoPC could be controlled by the LysoPLA activity of
297 the enzyme. Distinct levels of TcPLA1 activity were found between lethal and non-lethal strains of
298 bloodstream trypomastigotes and use of antibodies directed against TcPLA1 reduced host cell invasion
299 *in vitro* (74)

300 ***Leishmania* spp.**

301 Leishmaniasis comprises several zoonotic disease endemic in many countries of Latin America and
302 Asia. More than 20 species of *Leishmania* are responsible for the different clinical manifestations of
303 leishmaniasis. The two main clinical manifestations are visceral leishmaniasis and cutaneous
304 leishmaniasis. Hundred of millions are at risk for each disease in almost 100 endemic countries.

305 700,000 to 1 million cases are reported every year. Parasites are transmitted by femal plhebotomine
306 sandflies. *Leishmania* exists in two forms: the mammalian stage amastigote and the insect stage
307 promastigote. After infection, promastigotes parasites are phagocytosed by host macrophages, where
308 they differentiate into infective amastigotes and multiply inside a parasitophorous vacuole. Parasites
309 can persist for decades and reactivate in immunocompromised patients (75).

310 A first report showed that infection of macrophages by *L. amazonensis*, causative agent of cutaneous
311 leishmaniasis, resulted in a significant increase of LysoPC in the host cell. LysoPC and AA are
312 products of PLA2 activity on PC; production of PGE2 from AA is known to exacerbate *Leishmania*
313 infection in macrophages (76,77). However, the origin of this activity (parasite or macrophage) was
314 not specified (76). PLA2 activity was detected in the supernatant and lysate of *L. amazonensis*.
315 Treatment of amastigotes with PLA2 from *Crotalus durissus collilineatus* snake increased the growth
316 of parasites within macrophages. Infection of BALB/c mice after promastigote treatment with PLA2
317 led to an increase in lesion size and larger regions of necrosis, as well as a higher density of
318 inflammatory infiltrate. PLA2 activity inhibited IL-2 levels, a cytokine associated with a protective
319 Th1 response and PGE2 generation. PLA2 activity in *Leishmania* parasites thus may be a mediator of
320 cutaneous leishmaniasis (78). In parallel, addition of the PLA2 inhibitors bromoenol lactone (BEL) or
321 MAFP)affected the survival of *L. (L.) amazonensis* promastigotes in culture. Treatment of intracellular
322 *L. (L.) amazonensis* amastigotes with the same inhibitors resulted in decreased macrophage parasitism.
323 Finally, BALB/c mice infected with *L. (L.) amazonensis* and treated with BEL presented a reduction
324 of lesion size after 6 weeks of infection compared to non-treated infected mice. This decrease was
325 associated with decreased skin parasitism (79).

326 Similarly to *T. cruzi*, addition of melittin increased Ca^{2+} influx in in *L. donovani* promastigotes.
327 Inhibition by OBAA reduced this influx (58).

328 *Leishmania* parasites possess in their genome a *LmHydroLPAFAH* gene (platelet-activating factor-
329 acetylhydrolase) encoding a Ca^{2+} -independant PLA2 which hydrolyzes platelet-activating factors
330 (PAF), phosphoglycerides involved in inflammation. The enzyme is found in the ER of promastigotes
331 and amastigotes. Null-mutants for the enzyme showed no phenotype *in vitro* but a reduced virulence

332 was observed in *L. major*-infected BALB/c mice. LmPAFAH may regulate levels of host platelet-
333 activating factor (PAF), which could inhibit *Leishmania* survival in macrophages by NO activation. It
334 may also be involved in mediation of levels of LysoPC to facilitate parasite survival (80). The PAFAH
335 protein from *L. major*, along with its ortholog in *T. cruzi* and *T. brucei*, is therefore an interesting drug
336 and vaccine target (81).

337 LbPLA1 from *L. braziliensis* is responsible for PC hydrolysis in infective amastigotes and insect
338 promastigotes. The purified recombinant protein exhibited PLA1 activity. The enzyme had a higher
339 activity in amastigotes (82).

340 LdLip3 is a secreted lipase from *L. donovani*. The enzyme, found in both amastigote and promastigote
341 forms, could act on host fatty acids in order to synthesize lipids for parasite growth and survival, as
342 well as tissue damage associated with leishmaniasis (83).

343 **Other protozoan parasites of medical relevance**

344 Beside the more popular Apicomplexan and Trypanosomatids, some protozoan parasites such as
345 amoebas or other flagellates cause severe disease worldwide.

346 ***Entamoeba histolytica***

347 Intestinal amebiasis caused by *Entamoeba histolytica* is a prevalent disease in developing countries
348 with lower standards of sanitation. With 100 million cases each year, it is the fourth highest infectious
349 parasite. 90% of infections remain asymptomatic. Two stages have been described: an active
350 pathogenic trophozoite and a disseminating environmental amebic cyst. After ingestion of cysts
351 through contaminated stools, released trophozoites invade the intestine and colonize the colon. In
352 symptomatic cases, parasites interact with the epithelial cells and cause tissue damages (84).

353 It was first shown that antagonists of PLAs such as the PC analog Rosenthal's inhibitor and
354 hydrocortisone were able to inhibit the cytolytic effects of trophozoite parasites *in vitro*, suggesting the
355 involvement of PLAs in host cells lysis. (85). The authors raised the hypothesis that *Entamoeba* PLA
356 could directly act on the membrane of the target cell, or indirectly by the generation of cytotoxic
357 lysocompounds. A more speculated argument could be the insertion of PLA in membranes, resulting

358 in the activation of intracellular host PL and inducing autolysis in the presence of extracellular Ca^{2+}
359 (85). The same team also described Ca^{2+} -dependent and Ca^{2+} -independent PLAs. The first one was
360 associated with membrane fractions while the second was mainly found in soluble fractions. A link
361 was then established between these activities and cytolytic effects on host cells (86). Hemolytic
362 activity of *E. histolytica* was associated with a vesicular subcellular fraction called P30 and most likely
363 due to a PLA (87–89). In this fraction, both PLA1 and PLA2 as well as LysoPLA activities were
364 identified (90). Finally, it was observed that long-term axenic culture of *Entamoeba* caused a
365 diminution of erythrophagocytosis, PLA2 and hemolytic activities. This was correlated with an
366 impaired virulence of parasites (91). The role of these PLAs in the virulence and pathogenicity of the
367 parasite remains to be described. Although the genome of *E. histolytica* was sequenced in 2005 (92),
368 no PLA-encoding gene has been characterized yet.

369 *Naegleria fowleri*

370 Primary amoebic meningoencephalitis by the brain-eating amoeba *Naegleria fowleri* is a deadly,
371 fulminating disease with a 98% mortality rate. The parasite possesses three forms: a pathogenic
372 trophozoite, a transitory flagellated form in the presence of water and an environment-resistant cyst.
373 After the parasite enters its host, most commonly through the nose when swimming, replicating
374 trophozoites migrate to the olfactory bulb, where they enter the brain. Once in the CNS, the parasites
375 proliferate and destroy tissue, ultimately causing death in 7 to 10 days (93).

376 In the early eighties it was shown that PLA, LysoPL and sphingomyelinase activities were
377 substantially increased in virulent *N. fowleri* cell line compared to non-pathogenic *Naegleria* spp.
378 These activities were associated with cells and cell-free culture media. Moreover, the authors showed
379 evidence that lipolytic activities were released in the media of the virulent cell line but not in culture
380 media of non-pathogenic attenuated cells (94). In a later study, the authors showed that PL-enriched
381 culture of pathogenic *Naegleria* induced the degradation of human phospholipids (95). This secreted
382 activity could be at least partially responsible for the lipolytic activity observed in primary amoebic
383 meningoencephalitis (96). A Ca^{2+} -independent PLA2 associated with membrane fraction could play a
384 role in the parasite metabolism (97). However, no *PLA* gene has been characterized yet.

385 *Giardia*

386 With a prevalence up to 30% in developing countries and 300 million cases reported each year,
387 giardiasis is one of the most common diarrheal disease worldwide. *G. duodenalis*, also known as *G.*
388 *intestinalis* or *G. lamblia*, can infect all mammals. Infection occurs via the fecal-oral route, most
389 commonly by ingestion of contaminated water. *Giardia* exists in two forms: a replicating trophozoite
390 and a form of dissemination, the cyst. Once ingested cysts reach the stomach, the acidic pH and gastric
391 enzymes induce an excystation. Parasites then migrate to the duodenum and differentiate into
392 trophozoites. They adhere to intestinal epithelial cells where they multiply. Trophozoite can encyst
393 back into quadrinucleated cysts, which are shed in the environment through the feces (98).
394 PLA activity has been detected in *in vitro* culture of *Giardia* (99,100). Like for *Entamoeba*, PLA2
395 activity was found in the P30 subcellular fraction. Cell fractionation suggested that two isoforms of PL
396 sensitive to Rosenthal's inhibitor could be separated, one being membrane-bound and the other soluble
397 (99). The authors hypothesized that these activities could be responsible for the cytotoxicity of the
398 parasite (99). Interestingly, *Giardia intestinalis* is sensitive to the lipase inhibitor Tetrahydrolipstatin
399 (Orlistat) *in vitro*, a drug which is also active on trypanosomes and *Plasmodium*. The target of Orlistat
400 could be enzymes with a GX SXG motif, which is found in both PLA and fatty acid synthases (101).

401 ***Trichomonas vaginalis***

402 Trichomoniasis is the most common non-viral sexually transmitted infection in the world, with more
403 than 250 million cases per year. *Trichomonas* only exists as a trophozoite. After infection, the parasite
404 colonizes the human lower urogenital tract and adhere to host genital epithelial cells. Men are more
405 commonly asymptomatic than women, but both are carriers and can manifest symptoms. (102).
406 Several studies suggest the role of a PLA2 in the pathogenicity of this parasite. PLA2 activity was
407 measured in the vaginal fluids of pregnant women, which correlated with *Trichomonas* infection
408 (103). A material found in the soluble lytic fraction of *Trichomonas* had hemolytic activity and was
409 able to degrade PC with lysed profiles similar to those obtained with a PLA2, suggesting that this
410 lipase activity is PLA2-like (104). PLA activity was detected in a subcellular fraction of *Trichomonas*.
411 An hemolytic activity was correlated with the fraction, which decreased with addition of Rosenthal's
412 inhibitor (105). This observation is consistent with PLA1 and PLA2 activities from other parasites
413 being involved in hemolysis (62–65,87–89). Indeed, PLA1 and PLA2 activities associated with

414 soluble and membrane-bound fractions could induce indirect and direct hemolytic activities (105). A
 415 PLA2 was isolated from a subcellular fraction, but solid functional genomics proofs need to be added.
 416 This eluted fraction caused hemolysis and was inhibited by Rosenthal's inhibitor (106). The genome
 417 of *T. vaginalis* was sequenced in 2007 (107), but no *PLA* gene has been identified since then.

418 **Conclusion**

419 Phospholipases and lysophospholipases play a crucial role in the host-parasite interactions by
 420 performing a variety of functions. For the last few years, there has been a clear interest in the study of
 421 these enzymes, with the discovery of new genes and new functions paving the way for further
 422 investigations. Studies have revealed their importance in the development and maintenance of
 423 protozoan pathogens inside their host, notably in common processes such as the invasion, host
 424 damages, production of active compounds and scavenging of metabolites for parasite survival. As of
 425 now, no single protozoan PLA/LysoPL has been shown to be essential. This could be explained by the
 426 fact that these parasites possess not only one but an arsenal of PLs that could compensate for the loss
 427 of one. Their roles in the virulence of these pathogens make them potential targets for drugs, vaccine
 428 or diagnostic development as was demonstrated for several protozoan or even host PLAs
 429 (23,32,55,56,80,81). The genome of protozoan parasites contains several different PL and LysoPL
 430 genes, but only a few have been characterized and studied yet, as summarized in Table 1, making their
 431 study a future necessity. Moreover, as we saw in this review there is, in some pathogens, a complete
 432 lack of knowledge at the gene level such as in intestinal parasites. It is crucial to continue deciphering
 433 the complexity of (lyso)phospholipases to improve our understanding of protozoan parasites and
 434 possibly eliminate them.

435

436 **Table 1. Characterized PLAs and LysoPLs of protozoan parasites.**

Protozoan parasite	Genome Database	Enzyme	Database Entry	Putative function(s)	References
<i>Toxoplasma gondii</i>	ToxoDB	TgLCAT	TGME49_272420	Facilitates egress from host cells	(10,11)
		TgPL1	TGME49_232600	Supresses NO in macrophages,	(12–14)

				bradyzoite-to-tachyzoite reactivation	
		TgPL2	TGME49_231370	Apicoplast maintenance, regulation of fatty acids generation and PC/LPC levels	(15)
		TgPL3	TGME49_305140	Host cell invasion, virulence in mice	(23)
<i>Plasmodium</i> sp.	PlasmoDB	PfLPL1	PF3D7_1476700	Conversion of heme to hemozoin	(34)
		PfLPL20	PF3D7_0702200	Hydrolysis of LPC	(108)
		PbPL	PBANKA_1128100	Sporozoites migration through the epithelial layers, merozoite release from hepatocytes	(36,37)
		PfLCAT	PF3D7_0629300	Merozoite release from hepatocytes, egress in asexual blood stage	(38)
		PbPLA1	PBANKA_1423100	Merozoite release from hepatocytes	(39)
		PfPAPTL1/PNPLA1	PF3D7_0209100	Gametocyte induction and maturation, translocation of PfPLP2 to membrane periphery	(40,41)
<i>Trypanosoma brucei</i>	TriTrypDB	TbPLA1	Tb927.1.4830	Synthesis of LysoPC metabolites	(53,54)
		TbLysoPLA	Tb927.8.6390	Synthesis of	(55,56)

				LysoPC metabolites	
		TbGPI-PLA2	Tb927.7.6110	GPI precursor fatty acid remodeling	(61)
<i>Trypanosoma cruzi</i>		TcPLA1	TcCLB.510679.100	Plasma membrane remodeling	(72–74)
<i>Leishmania</i> sp.		LmPAFAH	LmjF35.3020	Regulation of PAF levels, survival in host macrophages, mediation of LysoPC levels	(80,81)
		LbPLA1	LBRM2903_340037900	Hydrolysis of PC	(82)
		LdLip3	LdBPK_310860.1	Host fatty acids scavenging for lipid synthesis, tissue damage	(83)
<i>Cryptosporidium</i>	CryptoDB	No enzyme characterized			
<i>Entamoeba histolytica</i>	AmoebaDB				
<i>Naegleria fowleri</i>					
<i>Giardia</i>	GiardiaDB				
<i>Trichomonas vaginalis</i>	TrichDB				

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