Lipid metabolism modulation by the P2X7 receptor in the immune system and during the course of infection: new insights into the old view

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Abstract For decades, scientists have described numerous protein pathways and functions. Much of a protein's function depends on its interactions with different partners, and those partners can change depending on the cell type or system. The P2X7 receptor (P2X7R) is one such multi-functional protein that is related to multiple partners and signaling pathways. The relationship between P2X7R and different enzymes involved in lipid metabolism represents a relatively new field in P2X7R research. This field of research began in epithelial cells and currently includes immune and nervous cells. The P2X7R-lipid metabolism pathway is related to many biological functions of P2X7R, such as cell death and pathogen clearance, and this signaling pathway may be involved in many functions that are dependent on bioactive lipids. In the present review, we will attempt to summarize data related to the P2X7R-lipid metabolism pathway, focusing on signaling pathways and their biological relevance to the immune system and infection.

Keywords P2X7 receptor · Phospholipids · Ceramide · Phospholipases · Sphingomyelinases · Lipid metabolism · Intracellular signaling

Introduction

In the cell, lipids represent 10–15% of all cellular molecules, and 5% of the genome is composed of genes responsible for synthesizing or metabolizing lipids [1]. Lipids represent a substantial energy resource, and the cell spends a large quantity of energy synthesizing them. However, early cell biology studies only analyzed lipids for their structural functions. Only recently several kinds of lipids were described as interacting with or modulating a number of signaling pathways. These lipids and pathways include arachidonic acid (AA) and its metabolites in the immune response [2], the AA-mediated smooth muscular contractions and associated regulatory genes [3], and sphingosine-1-phosphate as a bioactive lipid mediator in asthma [4] and inflammation [5, 6] that can activate a specific G protein-coupled receptor in both a paracrine and autocrine manner [4]. Other intracellular receptors translocate to the nucleus when activated by bioactive lipids, such as the peroxisome proliferator-activated receptors that are activated by fatty acids and their derivatives [7].

Since 1970 when Geoffrey Burnstock developed his purinergic theory [8, 9] and the ATP molecule was described as a neurotransmitter in non-adrenergic, non-cholinergic nerves supplying the gut and urinary bladder, novel functions for ATP and other purine derivatives have been described and huge new roles in homeostasis and pathologies have been identified [10]. For ATP to function as a signaling molecule, it must trigger other signaling mediators and interact with receptors in physiological systems, as well as pathological and biochemical signaling pathways. In general, ATP and other nucleotides bind directly to two families of cell-surface nucleotide receptors called P2 receptors. The P2X receptors are ligand-gated ion channels and, to date, seven members of this family have

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been cloned in mammals (P2X1–P2X7) while P2Y receptors are G protein-coupled receptors with eight members cloned (P2Y₁, P2Y₂, P2Y₄, P2Y₆, P2Y₁₁, P2Y₁₂, P2Y₁₃, and P2Y₁₄) [11, 12]. In addition, nucleotides can be degraded by plasma membrane ecto-ATPases [13] and indirectly, by generation of adenosine, can mediate cellular responses acting on adenosine P1 (A1, A2a, A2b, and A3) receptors (for review, see Ralevic and Burnstock [12]). In particular, ATP can activate the P2X7R, a member of the P2X receptor family [12, 14]. P2X7R is 200 amino acids longer at its C terminus than other P2X family receptors. This longer C terminus allows P2X7R to interact with different proteins than other members of the P2X family [15]. ATP triggering P2X7R activity was described as a cation channel associated with a pore activity named P2Z [16]. ATP was described as inducing a potassium efflux, caspase activation, and interleukin-1 β (IL-1 β) maturation in resident mouse macrophages [17]. Later, the activation of P2X7R was directly associated with several cellular pathways, including the following: caspase-1 activation [18] and IL-1 β maturation [19]; shedding of membrane proteins [20]; recruitment and activation of protein kinases, such as, the mitogen-activated protein kinase [21], protein kinase C (PKC) [22], Src [23], and glycogen synthase kinase 3 [24]; recruitment and activation of phosphatases [15]; and membrane blebbing via the activity of ROCK [25] and the activation of epithelial membrane proteins [26]. Despite this wide assortment of functions, this review will focus on the ability of P2X7R to modulate lipid pathways through the activation of different enzymes, such as, phospholipase A₂ (PLA2) [27, 28], phospholipase C (PLC) [29–31], phospholipase D (PLD) [32–39], and sphingomyelinases [27, 36]. Additionally, it will discuss how these enzymes modify arachidonic acid and its analogues [40–44] as well as ceramides [27, 45, 46].

In this review, we will discuss the involvement of P2X7R in different lipid metabolism pathways, focusing specifically on the immune system and the pathogen-killing activity of the P2X7R-lipid metabolism pathway in light of current findings regarding P2X7R and the generation of bioactive lipids.

P2X7R and the lipid metabolism signaling pathway

This section will focus on the involvement P2X7R in lipid metabolism from the initial stimulus that activates P2X7R and subsequently triggers pathways of bioactive lipids (Fig. 1). P2X7R can modulate the main lipid routes inside the cell (Fig. 1c), which include the phospholipase enzymes [31], arachidonic acid metabolism enzymes [43], and sphingosine- and ceramide-metabolism enzymes [27, 45]. Additionally, P2X7R was identified in two different

domains in the plasma membrane [27], as discussed in the immune system section below.

The first identified lipid metabolism enzyme associated with P2X7R was PLD, described by Dubyak's group in 1992 [38, 39]. They demonstrated that stimulation of BAC1.2F5 macrophages with a P2Z (i.e., the former name of P2X7R) agonist resulted in PLD activation in a calcium-independent manner [38]. The same group demonstrated in 1996 that PLD activity was stimulated by either ATP or benzoylbenzoyl-ATP (BzATP), and at the same time, the combination of IFN- γ or LPS with a P2X7R agonist resulted in the synergistic induction of PLD in a human THP-1 monocytic cell line [32]. Additionally, PLD activity was inhibited by the P2X7R antagonist KN-62 and by oxidized ATP (oxATP). Moreover, the activation of PLD by P2X7R was partially dependent on calcium [32]. However, the use of calcium ionophores or the release of calcium stores by thapsigargin could not fully reproduce P2X7R signaling, and the involved signaling pathway was described as being associated with recruitment of different kinases, such as, PKC [35–37] or tyrosine kinase [35].

P2X7R has a well-described SH3 motif at proline 451 [23, 47], and this amino acid residue was suggested to act as a recruitment binding motif for a small GTPase that regulates PLD activation. However, the mutation of proline to leucine did not affect PLD activation [48] as much as it affected pore formation in P2X7R [48] (reviewed in Ref. [49]). The activation of PLD by P2X7R has been described in other cell types, including lymphocytes [48], rat parotid acinar cells [36], rat brain-derived type 2 astrocyte cells [33], primary astrocytes and microglia [30, 33, 35], as well as macrophages [32, 34, 50]. In primary and macrophage cell lines, PLD has been described as part of an important killing mechanism against *Mycobacterium* [34] and *Chlamydia* [50, 51], as discussed below.

The interaction between P2X7R and PLC remains a controversial topic. This is especially true if we combine the original published data relating to the P2X7R-PLC interaction with more recent data or with the ability of P2X7R to modulate the activity of PLC, which is classified according to the different PLC isoform-dependent phospholipids that are involved. PLC can be categorized by its phospholipid hydrolysis specificity [52, 53], and P2X7R has been associated with phosphoinositide-specific PLC (PI-PLC) [30, 36] and phosphatidylcholine-specific PLC (PC-PLC) [54].

P2X7R-dependent intracellular signaling possesses the ability to modulate PLC activity [31, 55, 56]. This modulation has been observed when PI-PLC was activated by neurotransmitters in the presence of ATP or a P2X7R agonist, which promoted the downregulation of PI-PLC activity [55, 56], as was discussed in Garcia-Marcos's review in 2006 [31]. The downregulation of PI-PLC by

Fig. 1 Lipid metabolism enzymes and P2X7R modulation. **a** Backbone of a glycerol phospholipid, indicating the biochemical site of cleavage by each phospholipase enzyme and the resulting products. **b** Backbone of sphingomyelin, indicating the biochemical site of cleavage of neutral sphingomyelinase to generate the product ceramide. The site phosphorylated by ceramide kinase is also

indicated. **c** Representation of P2X7R activation by endogenous ATP or specific purinergic agonists, triggering different lipid enzyme pathways in several cell types. The response specificity of each lipid pathway depends on cell type, and the details of these pathway steps are unclear

ATP was associated with P2X7R activation, and its activity could be provoked by the redirection of lipid metabolism [31]. Supporting the hypothesis that P2X7R does not activate PLC, but reduces its enzyme activity, Takenouchi et al. [57] demonstrated that both U73122 (a specific inhibitor of PLC) and the inactive analogue U73343 could inhibit the influx of ethidium bromide induced by ATP and

BzATP, suggesting a possible PLC-independent mechanism of U-compounds acting on P2X7R [57].

Garcia-Marcos also suggested a role for phosphatidylinositol 4-kinase (PI4K) in the process that results in the downregulation of PLC by P2X7R activation [31]. PI4K was identified, using a proteomic approach, as interacting with P2X7R [15]. PI4K is an enzyme involved in the

phosphorylation of phosphatidylinositol to phosphatidylinositol 4-phosphate (PIP) [58, 59]. PIP is used as a substrate of phosphatidylinositol 5-kinase to form phosphatidylinositol 4,5-bisphosphate (PIP₂) [58, 59] (Fig. 2a). PIP₂ is utilized as a substrate of PI-PLC to produce diacylgly-

cerol (DAG; an endogenous activator of PKC) and inositol triphosphate (a molecule responsible for the release of intracellular calcium stores) (Figs. 1a and 2).

Recently, Zhao et al. identified the amino acid sequences in P2X7R that mediate the binding of PIP₂ (i.e., R385-

Fig. 2 Hypothetical P2X7R activation and the resulting modulation of phospholipid enzyme pathways. **a** Phosphatidylinositol (PI) is phosphorylated by phosphatidylinositol 4-kinase at inositol carbon 4 to produce phosphatidylinositol 4-phosphate (PIP). PIP is phosphorylated by phosphatidylinositol 5-kinase at inositol carbon 5 to produce phosphatidylinositol 4,5-phosphate (PIP₂). **b** PIP₂ is a substrate of

phosphatidylinositol-specific phospholipase C to produce diacylglycerol (DAG) and inositol triphosphate (IP₃). **c** Activated P2X7R can interact with diverse proteins and molecules. In the presence of PIP₂, a specific motif in the C terminus of P2X7R interacts with PIP₂ and facilitates the interaction between P2X7R and PLA₂. At the same time, PLC is negatively modulated by the sequestration of PIP₂ by P2X7R

K395) [59] and related this interaction (P2X7R-PIP₂) to the ATP-induced apoptotic ability of primary T cells, macrophages and stable HEK293 cells transfected with P2X7 [59]. The authors also demonstrated that when PIP₂ was hydrolyzed or its production blocked, the apoptosis invoked by P2X7R was inhibited [59]. Updating the hypothesis presented in Garcia-Marcos's review [31], if P2X7R interacts directly with PIP₂, as demonstrated by Zhao et al. [59], then it is reasonable that P2X7R could sequester PIP₂ to make it inaccessible to PI-PLC and thus prevent PIP₂ hydrolysis (Fig. 2c).

In primary rat and mouse cerebellar astrocyte cultures, P2X7R was associated with the direct activation of PI-PLC and the indirect activation of PC-PLC [30]. In both cases, astrocyte cultures from wild-type and P2X7R-knockout mouse (KO) mice were stimulated by BzATP. In wild-type cultures, P2X7R-activated PI-PLC, producing DAG and stimulating a novel PKC that triggers PC-PLC [30]. The synergistic effect of PI-PLC with PC-PLC induced the activation of PKD in astrocyte cultures [30]. In microglial cells prepared from mice, ATP-stimulated P2X7R also stimulated PI-PLC, and this activation upregulated the activity of DAG lipases that produce 2-arachidonoyl glycerol, which is an endocannabinoid associated with neuroinflammation [29].

Perhaps the best-characterized association of P2X7R in the lipid metabolism pathway is the relationship between P2X7R and PLA2, which was initially proposed in submandibular gland cells by Alzola's group in 1998 [28]. These authors noted that, when stimulated with ATP or BzATP, submandibular gland cells labeled with phospholipids conjugated to [³H]-arachidonic acid (³HAA) induced the release of ³HAA from the plasma membrane [28]. In the presence of calcium, both bromoenol lactone (BEL), a specific calcium-independent PLA2 (iPLA2) inhibitor and arachidonyl trifluoromethyl ketone a specific calcium-dependent PLA2 inhibitor (cPLA2) inhibitor, inhibited the release of ³HAA. However, in the absence of calcium, only BEL was able to inhibit this release [28]. These results suggested that P2X7R could activate both calcium-dependent and calcium-independent PLA2 in submandibular gland cells and epithelial cells. The iPLA2 was also associated with P2X7R permeabilization in submandibular gland acinar cells, and this permeabilization was inhibited by BEL [60]. In submandibular gland ductal cells, P2X7R induced kallikrein secretion in an iPLA2-dependent manner [28]. However, the data related to BEL in P2X7R-iPLA2 inhibition must be evaluated with caution because Chaid et al. have described how BEL can enhance the permeabilization to ethidium bromide in submandibular acinar cells by acting directly on P2X7R [60].

PLA2 activation by P2X7R has been described in the immune system, as well. Kahlenberg and Dubyak demon-

strated in 2004 that iPLA2 activation by P2X7R was involved in IL-1 β maturation and secretion [61]. How this pertains to the immune system will be discussed below in the immune section. Usually, when PLA2 is activated, it promotes fatty acid release from the sn-2 position of the phospholipid. The most common fatty acids released are AA and it is released together with lysophosphatidic acid (LPA). Released AA can be metabolized by at least three different pathways: the lipoxygenase pathway to form leukotrienes and lipoxins [62], the cyclooxygenase pathway to form prostaglandins [62], or the cytochrome P450 pathway to form *cis*- or *trans*-epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids [63]. These three lipid metabolism pathways can use AA to generate more than 25 different bioactive lipids [64]. If P2X7R induces AA release, it is possible that P2X7R could interfere with PLA2 downstream. Indeed, P2X7R was shown to modulate PLA2 downstream, as demonstrated by Ballerini et al. They showed that P2X7R increased the production of cysteinyl leukotrienes through the activation of 5-lipoxygenase activating protein and that effect was inhibited by oxATP in rat brain cultured astrocytes [40]. P2X7R modulation of the leukotriene pathway without cysteinyl leukotriene interference was also associated with P2X7-induced apoptosis in primary cultures of mouse macrophages via an iPLA2-dependent mechanism [44].

Purinergic receptors involved in prostaglandin pathways have generally been characterized as being P2Y receptors. However, there are data that relate P2X, specifically P2X7R, to the association of COX and prostaglandins. In the field of neuroinflammation, P2X7R was associated with the enhancement of COX-2 expression in an IL-1 β -dependent manner in microglia from spinal cord lesions [43]. Additionally, in kainite-induced epileptic rat brains, P2X7R was shown to co-localize with COX-1 [42]. P2X7R was also proposed to act as an important factor in osteogenesis through a cell-autonomous mechanism by prostaglandin E2 production and LPA release in mouse osteoblasts [41]. Recently, P2X7R modulation of EET production was described in erythrocytes [65]. Rat erythrocytes were stimulated with ATP or BzATP, thereby activating P2X7R and increasing EETs released from erythrocytes without provoking hemolysis [65]. The mechanism proposed for this release of EETs is dependent on ATP secretion from erythrocytes in hypoxia, which results in blood flow regulation and could represent a new therapeutic target for arterial high blood pressure disease.

The stimulation of P2X7R also triggers the activation of the sphingolipids/ceramide pathway in lipid rafts [27, 45, 46] in a manner comparable to TNF signaling [66–68]. In cells stimulated by TNF, neutral sphingomyelinase (NSMase) is activated and hydrolyzes sphingolipids, releasing ceramide (Fig. 1b)[66–68]. Next, ceramide is

phosphorylated by ceramide kinase to ceramide-1-phosphate (C1P) (Fig. 1b). The presence of C1P stimulates cPLA2 activity, increasing both AA release and COX-2 expression, which results in prostaglandin accumulation [66–68]. This process has been described in human epithelial cell lines [69] and in the adenocarcinoma A549 cell line [70]. Garcia-Marcos et al. demonstrated in 2006 that the ceramide-stimulated PLA2 pathway is associated with P2X7R [27]. The authors showed that in submandibular gland cells, the stimulation of P2X7R activated NSMase and increased the ceramide concentration. This ceramide stimulated the activity of PLA2 [27]. In the presence of drugs that disrupted lipid rafts, PLA2 activity was inhibited, but the calcium influx was not affected [27], which suggested that lipid raft integrity is essential to PLA2 activity. Together with the ceramide-PLA2 pathway, P2X7R activation was associated with ceramide synthesis as part of the apoptosis pathways in thymocytes and macrophages [45, 46]. In both cases, P2X7R stimulated by BzATP triggered de novo ceramide synthesis and the activation of ceramide-activated caspase-3, resulting in cell death [45, 46].

Lipid pathways and the P2X7 receptor in the immune system

The immune system is a complex array of organs, tissues, and specialized cells that protect us from outside invaders, such as, bacteria, viruses, and allergens, as well as from harmful inside agents, such as, infected cells and toxins. Purinergic P2X receptors, especially P2X7R, are broadly distributed throughout immune cell types, such as, lymphocytes, monocytes, macrophages, neutrophils, eosinophils, and dendritic cells [71]. P2X7R plays an important role in various physiological processes in the immune system, some of which include killing pathogens [34, 50, 72, 73], production of reactive oxygen and nitrogen species [74], maturation and release of pro-inflammatory cytokines [17–19, 75], caspase activation [19, 75], T cell maturation [76], pore-formation activity [77], and activation of phospholipases [34, 50, 51, 72, 78].

P2X7R acts in blood and immune cells to modulate PLA2, PLD, and PLC, which leads to the control of lipid messenger levels, as discussed in the signaling pathways section [9, 14, 65]. The involvement of P2X7R in this cascade suggests it may be a therapeutic target in several diseases that involve the exacerbated production of AA-derived mediators, such as chronic inflammatory diseases [79]. Andrei et al. reported that, in human monocytes, ATP-stimulated P2X7R promoted the activation of PLA2. The authors suggested an important role for this phospholipase in the processing and secretion of IL-1 β , a key cytokine involved in the inflammatory response [80]. In murine

macrophages, the inhibition of iPLA2 blocked caspase-1 and IL-1 β processing through P2X7R stimulation [61]. Costa-Junior et al. showed that iPLA2 was involved in triggering P2X7R-induced apoptosis in intraperitoneal murine macrophages [44], which corroborates the findings of Andrei et al., which suggest that apoptosis-involved iPLA2 represents a mechanism to turn off macrophage-dependent inflammation.

In 1992, el-Moatassim and Dubyak reported that P2X7R stimulation promoted the activation of PLD [38]. This P2X7R-induced activation was also observed in human lymphocytes [81] and in murine thymocytes [48]. Supporting these data, Shemon et al. used anti-P2X7R antibodies to abolish ATP-induced PLD stimulation in B-lymphocytes taken from patients with chronic lymphocytic leukemia (CLL), one of the most common types of leukemia. Also, an inhibitor of novel PKC isoforms, rottlerin, impaired the ATP-stimulated PLD activity in CLL B-lymphocytes. The authors attributed the inhibition of P2X7R-stimulated PLD activity by rottlerin to a target downstream of P2X7R activation, indicating a possible role for novel PKC isoforms in the regulation of P2X7R-mediated PLD activity [82].

P2X7R activation can modulate a variety of lipids found in the plasma membrane, thereby modulating immune responses. In addition to glycerol phospholipids, P2X7R targets ceramide sphingolipid enzymes that play a significant role in normal cell homeostasis. In particular, ceramide is considered to be vital to sphingolipid metabolism and has also been involved in the regulation of signal induction processes. Specifically, ceramides play important roles in cell differentiation, survival and inflammatory responses [83]. In thymocytes, the activation of P2X7R stimulates de novo ceramide synthesis, which is implicated in ATP-induced apoptosis in this cell type [46]. Raymond and Le Stunff demonstrated that ATP and BzATP both stimulated ceramide accumulation in macrophages, and this effect was inhibited by oxATP. Additionally, they reported that de novo ceramide biosynthesis was involved in ATP-induced macrophage death in a caspase-dependent manner, indicating a novel role for ceramide in P2X7R-regulated cell death [45]. Moreover, it is well known that ceramide is an important inducer of lipid raft formation. Ceramide produced in specific regions of the plasma membrane can form new ceramide-rich microdomains in the cell membrane, or it may join existing microdomains, and it was described that ATP induces increased levels of ceramide by activating P2X7R (Fig. 3) [45]. Through fusion and association with lipid rafts, ceramides stabilize these regions to form platforms for cellular signaling that facilitate and amplify signaling processes via receptors [84].

As described by Garcia-Marcos et al., P2X7R can be found in membrane domains either containing or lacking lipid rafts [27]. Lipid raft membrane domains are small

Fig. 3 Possible intracellular pathways triggered by ATP activation of P2X7R. After ATP coupling, P2X7R could induce (1) de novo ceramide synthesis by the endoplasmic reticulum (ER) with the ceramide then being directed to the plasma membrane to assemble existing lipid rafts

or to generate ceramide-rich domains. Likewise, (2) P2X7R can migrate to lipid raft domains. (3) Otherwise, during intracellular infections, PLD could be activated and could promote lysosome-parasitophorous vacuole fusion, leading to pathogen processing

(10–200 nm) and highly dynamic. They are specialized regions in the plasma membrane that are rich in sphingolipids, cholesterol and signaling proteins. Lipid raft membrane domains function as signaling platforms for cells, facilitating interactions between the external and internal environments of cells [85]. These regions have an important role in several cellular processes, such as endocytosis and cell–cell or cell–pathogen adhesion [86]. However, one of the most studied functions is the formation of molecular clusters of lipids, proteins and their signal transduction machinery, which enhances signaling efficiency [87]. Our group performed functional assays in murine macrophages that were either treated or untreated with drugs that disrupt membrane microdomains and described that the disruption of lipid raft integrity could inhibit both ATP-induced permeabilization and apoptosis mediated by P2X7R. This inhibition was not complete, however, suggesting that P2X7R may be only partially located in lipid microdomains

(Fig. 3 and unpublished data), which corroborates with published findings [27, 88]. P2X7R has been shown to be at least partially localized to the lipid raft fraction in T cells, and the authors suggested that lipid raft localization facilitated ADP-ribosylation of P2X7R [89]. Much evidence has indicated that rafts play an important role in T cell activation in immunological synapses between T cells and antigen-presenting cells. It appears that P2X7R and other proteins involved in an inflammation respond to agonist stimulation and migrate, in part, to raft regions. Tsukimoto and co-workers showed that blocking P2X7R with oxATP inhibited IL-2 production in murine T cells, suggesting the involvement of this receptor in T cell activation processes [90]. This evidence was strongly supported by data from Yip and colleagues that suggested an essential role for ATP release and autocrine feedback through P2X7R in T cell activation. This process may be important for T cell proliferation and for the modulation of

T cell responses [91]. Recently, Schenk et al. showed that the increase of ATP levels at inflammatory sites affected the activation state of effector T cells, and the activation of P2X7R by ATP inhibited the function and stability of regulatory T cells [92].

The lipid pathway and P2X7 receptors in infection

Mammalian cells are highly organized to optimize their functions and, particularly, to eliminate harmful infectious agents. In the intracellular space, several approaches to maximize anti-parasitic actions are taken, such as the induction or suppression of several genes [93]. This induction results in an increased capacity to eliminate pathogens and to regulate many other cells through the release of cytokines and chemokines [93, 94]. *Trypanosoma* species can downregulate genes in infected macrophages that are involved in several metabolic processes, including lipid, fatty acid and steroid metabolism [93]. In addition, it has been reported that the activation of adenosine A2A receptors can modulate the expression of pro-inflammatory molecules, such as, TNF- α , IL-1 β , IL-8, and COX-2 [95].

After being phagocytosed, pathogens are submitted to microbicidal mechanisms, beginning with acute changes in the composition of the lumen of the phagosome. The lumen becomes highly acidic and can increase the production of superoxide anions, as well as oxygen, nitrogen, and other degradative molecules [96, 97]. Additionally, other substances associated with cell killing, such as thiols, metal centers, protein tyrosine residues, nucleic acids, and lipids, are produced [98]. Most pathogens are successfully internalized and eliminated by phagocytes, while others have developed survival strategies. Pathogens such as *Salmonella typhimurium* [99], *Legionella pneumophila* [100], *Mycobacterium tuberculosis* [100], *Chlamydia* sp. [101], *Coxiella burnetii* [100], *Listeria monocytogenes* [100], *Trypanosoma cruzi* [102], *Toxoplasma gondii* [102], *Leishmania donovani* [103], as well as several viruses, such as picornaviruses [104, 105], have evolved mechanisms for survival and even growth inside macrophages. In this context, the study of P2X7R microbicidal actions has shed light on novel methods to eliminate parasites. In HEK293 cells expressing P2X7R, Kim et al. identified 11 proteins that interact with P2X7R. Among them, eight intracellular proteins showed that the P2X7R is capable of activating several signaling events through various mechanisms, including actin, which is involved in interacting with several parasites in the intracellular niche [15]. The report by Humphreys and Dubyak indicated the possible connection between infection and phospholipases because the stimulation of monocytes with LPS promoted the activation

of PLD in response to ATP [32]. The role of P2X7R in controlling target enzymes in phospholipid metabolism indicates possible alternative pathways influencing or favoring infections [34, 51]. P2X7R appears to be a necessary component of hepatitis B virus entry into susceptible cells during infection [106]. In the endothelium, cytomegalovirus infection significantly upregulates P2 receptors, which might enable the virus to escape from an important host defense response [107]. In HIV infections, it was suggested that PLC is a key enzyme in the host's response to secrete IL-1 β from human monocytic cells [108], while in murine cytomegalovirus infection, PLC signaling plays a central role in the virus pathogenesis in vivo [109]. In human bocavirus, the parvovirus was discovered to possess a secretory PLA2-like enzymatic activity, demonstrating that this enzyme may be linked to the action of different virus types and that the phospholipase activated through P2X7R may be a potential target in the virus-controlling mechanisms [110]. In certain bacterial infections, such as *Mycobacteria* and *Chlamydia*, activated P2X7R has been shown to be capable of controlling the infection [73, 78]. Specifically, in *Mycobacteria* [111, 112] and *Chlamydia* [50, 73, 113] infections, P2X7R activation mediates inhibition of the infection through fusion of vacuoles with lysosomes, promoting vacuole acidification, a process that requires the activation of host-cell phospholipases [50, 113, 114]. In macrophages infected with *T. gondii*, ATP mediates parasite elimination through acidification of the parasitophorous vacuole [115], which suggests a role for PLD in this infection (Fig. 3). Conversely, macrophages infected with *Leishmania amazonensis* upregulate P2X7R, which, when activated by ATP, induces reduction of parasitic load and consequent cell death [116]. Unlike other pathogens, *L. amazonensis* maintains the acidification of the parasitophorous vacuole to survive, and so it is possible that the role of P2X7R in parasite elimination is through an alternative pathway. Because activated P2X7R can couple to lipid messengers in intracellular signaling, including phospholipase activation and modulation, and because several pathogens modulate lipid metabolism, there is a potential link between these components and intracellular infection.

Future perspectives

In this review, we focused on P2X7R and lipid metabolism pathways in the immune system and infection. The P2X7R field is growing rapidly, and new information is added daily. The view of ATP solely as a simple molecule related to energy in cells is outdated. ATP is an important signaling molecule in a variety of biological systems, triggering

multiple receptor activities and provoking different organismal responses.

Cellular signaling pathways are organized to follow sequential events that result in specific and/or varied biological functions. It is clear that P2X7R can interact with different lipid metabolism enzymes in a variety of cell systems and in different ways, but the precise interaction mechanism that specifies one pathway over another is still unclear. It is well established that microenvironments determine protein interactions. However, it is intriguing that P2X7R in one situation would prefer to interact with PLA2 but not with PLD. Perhaps the P2X7R interaction with PI4K could be one cause of this result (Fig. 2). PI4K is involved in PIP₂ synthesis (Fig. 2a), PIP₂ anchors PLA2 into the plasma membrane [117] and binding with P2X7R [59]. Structural analysis of the P2X7R-PIP₂ binding motif (R385-K395) [59] reveals that this motif is close to the first cysteine-palmitoylated group found in the C terminus of P2X7R. These modified residues are associated with P2X7R membrane localization [88]. The cysteine-palmitoylated group could potentially anchor P2X7R in lipid rafts, concurrently facilitating an interaction between P2X7R and PIP₂. The localization of P2X7R in lipid rafts would present an ideal condition for interaction with PLA2 (Fig. 2c). In this case, P2X7R could indirectly modulate PLC activity (Fig. 2c), but more experimentation is required to prove this speculative hypothesis.

P2X7R can regulate a variety of cellular events in the immune system through the activation of downstream effectors; however, its association with these downstream effectors remains to be elucidated. The activation of phospholipid signaling by P2X7R could be a link to this coupling. The evidence that phospholipases can regulate P2X7R-induced apoptosis in addition to processing and releasing IL-1 β indicates the importance of phospholipases and lipid mediators in the immune response and inflammation. Although recent advances have been made in the study of P2X7R with regard to controlling infections, several questions still remain concerning the exact mechanism by which the host's lipid metabolism fulfills the pathogen's sabotage mechanisms. Because both host and pathogens can synthesize phospholipases, and P2X7R has a dubious role in pathogen survival, it is possible that in the future the innovation gap that relies on receptor lipid signaling will lead to more efficient drugs for combating infections (Fig. 3). Despite a scarcity of direct data supporting a role for P2X7R and the lipid metabolism pathway in modulating immune response and/or parasite infection, the field of studying P2X7R interactions with lipid metabolism enzymes is growing, and this interaction represents new targets for pharmacological interventions and therapeutics.

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