

REVIEW

Aiming drug discovery
at lysophosphatidic
acid targets

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Lysophosphatidic acid (LPA, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphate) is the prototype member of a family of lipid mediators and second messengers. LPA and its naturally occurring analogues interact with G protein-coupled receptors on the cell surface and a nuclear hormone receptor within the cell. In addition, there are several enzymes that utilize LPA as a substrate or generate it as a product and are under its regulatory control. LPA is present in biological fluids, and attempts have been made to link changes in its concentration and molecular composition to specific disease conditions. Through their many targets, members of the LPA family regulate cell survival, apoptosis, motility, shape, differentiation, gene transcription, malignant transformation and more. The present review depicts arbitrary aspects of the physiological and pathophysiological actions of LPA and attempts to link them with select targets. Many of us are now convinced that therapies targeting LPA biosynthesis and signalling are feasible for the treatment of devastating human diseases such as cancer, fibrosis and degenerative conditions. However, successful targeting of the pathways associated with this pleiotropic lipid will depend on the future development of as yet undeveloped pharmacons.

Abbreviations

3CCPA, 18:1 oleoyl-3-carba cyclic phosphatidic acid; ATX, autotaxin; CHO, Chinese hamster ovary; CPA, cyclic phosphatidic acid (1-acyl-*sn*-glycerol-2,3-cyclic phosphate); CTGF, connective tissue growth factor; CYR61, cysteine-rich protein 61; DRG, dorsal root ganglion; EDG, endothelial gene; GPAT, glycerol-3-phosphate acyl transferase; GPCR, G protein-coupled receptor; HUVEC, human umbilical vein endothelial cells; iPLA2, Ca²⁺-independent phospholipase A2; IUPHAR, International Union of Basic and Clinical Pharmacology; LDL, low-density lipoprotein; LPA, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphate; LPC, lysophosphatidylcholine; LPS, lysophosphatidyl serine; MEF, mouse embryonic fibroblast; MMP, matrix metalloproteinases; NPP, nucleotide pyrophosphatase/phosphodiesterase; OMPT, D-*sn*-1-O-oleyl-2-O-methyl-glyceryl-3-phosphothionate; P2Y, purinoreceptor; PDZ domain, PSD-95/Disc-large/ZO-1 domain; PIP2, phosphatidylinositol 4,5-bisphosphate; PLD, phospholipase D; PPAR γ , peroxisome proliferator-activated receptor gamma; ROCK, Rho-associated kinase; S1P, sphingosine-1-phosphate; siRNA, small interfering RNA; TRIP6, thyroid receptor-interacting protein 6; TZD, thiazolidinedione; UDP, uridine diphosphate; UTP, uridine triphosphate; uPA, urokinase; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells

The lysophosphatidic acid-like lipid
mediator family

Lysophosphatidic acid (1-acyl-2-hydroxy-*sn*-glycero-3-phosphate; LPA) represents the minimal

glycerophospholipid based on the racyl-glycerol-phosphate scaffold. However, LPA is only one member of a family of endogenous lipid-like substances endowed with a host of biological actions that are often mediated through high-affinity

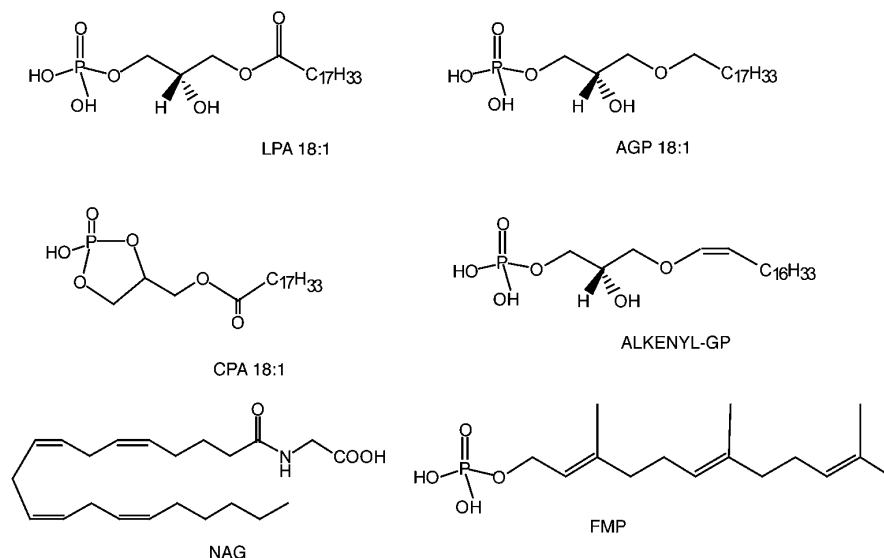


Figure 1

Naturally occurring ligands of LPA targets. LPA 18:1, oleoyl-lysophosphatidic acid; AGP 18:1, 1-O-octadecyl glycerophosphate; CPA 18:1, oleoyl-cyclic phosphatidic acid; ALKENYL-GP, alkenyl glycerophosphate; NAG, N-arachidonoyl glycine; FMP, farnesyl monophosphate.

interactions with specific cell surface and intracellular proteins (Figure 1). The specific recognition of LPA-like molecules by several receptors and enzymes provides a diversity of biological actions that have fascinated many investigators. What biomolecules constitute the LPA family? First, for the sake of brevity, we will ignore lysophospholipids built up on the sphingoid backbone. Although this creates an artificial oversimplification, it nevertheless allows for a single focus on a distinct subset of lipids, which in many instances interact with the same molecular targets. In most cases, sphingolipids, best represented by sphingosine-1-phosphate (S1P), have their own set of distinct targets that rarely overlap (but in some instances do; discussed below) with those of LPA.

Different molecular species of LPA, dominated by 16-, 18- and 20-carbon long acyl chains, have been detected in biological fluids as saturated, and mono- and poly-unsaturated variants of either the *sn1* or the *sn2* regioisomers. This natural variety within the LPA class leads to important biological and pharmacological consequences. First, most LPA G protein-coupled receptors (GPCR) show lower EC_{50} values (higher potency) for unsaturated species (Bandoh *et al.*, 2000; Fujiwara *et al.*, 2005; Williams *et al.*, 2009). An extreme case of target selectivity is found for the peroxisome proliferator-activated receptor gamma (PPAR γ), an intracellular target of LPA that is exclusively activated by unsaturated acyl species of LPA (Tsukahara *et al.*, 2006). The LPA concentration in plasma is in the low nanomolar range, whereas in

serum it increases to several micromoles (Baker *et al.*, 2001; Watanabe *et al.*, 2007b; Hosogaya *et al.*, 2008). The poly-unsaturated 18:2 (38%) and 20:4 (39%) and the mono-unsaturated 18:1 (9%) LPA species constitute the overwhelming majority (86%) of LPA in serum (Sano *et al.*, 2002). This enrichment in the unsaturated species increases the likelihood of the activation of those LPA targets that show preference for these fatty acyl species. In addition, *sn1* and *sn2* regioisomers of acyl LPA have been detected, and the LPA₃, the purinoreceptor-5 (P2Y₅) and GPR35 receptors have been suggested to show a slight preference for *sn2* over *sn1* LPA containing identical fatty acid chains (Bandoh *et al.*, 2000; Yanagida *et al.*, 2009; Oka *et al.*, 2010). One unresolved concern is the stability of these regioisomers *in vivo* due to the relatively fast rate of acyl migration towards a 9:1 (*sn1* : *sn2*) equilibrium ratio of the isomers (Wang *et al.*, 1997; Croset *et al.*, 2000).

There are two mouse phospholipase A1 enzymes that have been linked to production of unsaturated *sn-2* LPA. A phosphatidic acid-specific PLA1 α and β (Sonoda *et al.*, 2002; Hiramatsu *et al.*, 2003) from mice have been cloned, which are the equivalents of the human enzymes LIPH and LIPI respectively (Kazantseva *et al.*, 2006; Aoki *et al.*, 2007; Aoki *et al.*, 2008; Pasternack *et al.*, 2009; Shimomura *et al.*, 2009a,b). Mutations in the LIPH/mPA-PLA1 α gene have been identified as the cause of the hereditary disorder woolly hair, as its gene product supplies LPA to the P2Y₅ receptor expressed in the hair bulb (Kazantseva *et al.*, 2006; Pasternack *et al.*, 2008;

2009; Shimomura *et al.*, 2008; 2009a,b). Yet, another mouse PLA1 enzyme, designated PS-PLA1, secreted from rat platelets and specific to phosphatidylserine, has been identified by the Aoki group (Horigome *et al.*, 1987; Sato *et al.*, 1997). However, this enzyme is not found in human platelets and its role in human pathophysiology is unclear.

Alkyl-ether (Sugiura *et al.*, 1999; Nakane *et al.*, 2001) and alkenyl-ether analogues (Liliom *et al.*, 1998) of LPA have been described. These lower-abundance mediators have been shown to be weaker agonists of LPA-specific GPCR (Bandoh *et al.*, 2000; Fujiwara *et al.*, 2005; Williams *et al.*, 2009) with one exception. The LPA₅/GPR92 receptor shows a preference for 1-O-alkyl glycerophosphate over the acyl analogues with the same chain length (Williams *et al.*, 2009). Human platelets show preferential activation by 1-O-alkyl glycerophosphate over LPA; and the structure-activity relationship of the platelet response is similar, although not identical, to that of LPA₅ (Simon *et al.*, 1982; Williams *et al.*, 2009). Interestingly, 1-O-alkyl glycerophosphate shows a higher potency than LPA with respect to PPAR γ activation (Zhang *et al.*, 2004; Tsukahara *et al.*, 2006).

Cyclic phosphatidic acid (1-acyl-*sn*-glycerol-2,3-cyclic phosphate; CPA) is an additional class of acyl LPA analogues in which the *sn*-2 hydroxyl group has formed a 5-membered ring with the *sn*-3 phosphate via the elimination of a water molecule. CPA is also a naturally occurring analogue of LPA present in mammalian blood and brain, as well as in slime mold, the organism in which CPA was originally identified (Murakami-Murofushi *et al.*, 1993; Kobayashi *et al.*, 1999). CPA can be generated by autotaxin (ATX) under non-physiological conditions using a two-phase water/ether reaction system (Tsuda *et al.*, 2006), but whether this enzyme contributes to the formation of CPA in blood remains an open question. Bacterial phospholipase D (PLD) can also yield CPA production (Friedman *et al.*, 1996). In mammalian cells, CPA is generated intracellularly by PLD2 and acts as a second messenger which inhibits PPAR γ (Tsukahara *et al.*, 2010). CPA is a weak agonist of many LPA GPCR (Fujiwara *et al.*, 2005; Williams *et al.*, 2009). However, its synthetic hydrolysis-resistant 3-carba analogue (3CCPA 18:1) is a selective weak agonist of LPA₅/GPR92 that fails to activate LPA_{1/2/3/4} and also inhibits ATX (Baker *et al.*, 2006; Fujiwara, 2008). CPA and its synthetic 3CCPA analogues inhibit cancer metastasis through an as yet unidentified mechanism, which includes the inhibition of ATX (Baker *et al.*, 2006; Uchiyama *et al.*, 2007; Fujiwara, 2008).

In search of the minimal pharmacophore that activates LPA GPCR, we identified fatty alcohol phosphates that, depending on the length of the

hydrocarbon chain and the headgroup (phosphate or thiophosphate), function as either antagonists or agonists (Durgam *et al.*, 2005; Deng *et al.*, 2007; Zhang *et al.*, 2009a). Naturally occurring farnesyl phosphates are structurally similar to fatty alcohol phosphates. This similarity prompted us to confirm that farnesyl mono- and diphosphate activate various LPA GPCR targets (Liliom *et al.*, 2006). Oh *et al.* (Oh da *et al.*, 2008) reported that farnesyl monophosphate and arachidonoyl glycerol were more potent activators of LPA₅ than LPA. However, other groups have reported that LPA and 1-O-alkyl glycerophosphate are the most potent agonists of LPA₅ identified so far (Kotarsky *et al.*, 2006; Lee *et al.*, 2006; Williams *et al.*, 2009). Despite this controversy, farnesyl mono- and diphosphate are important pharmacological tools that allow for the selective activation of LPA₅ (~50 nM EC₅₀) without activation of LPA_{1,2,3,4} (in fact these reagents are weak inhibitors of LPA_{2,3,4} with IC₅₀ values in the mid-micromolar range) (Williams *et al.*, 2009).

S1P is currently classified as a specific ligand for five cognate receptors, S1P_{1,2,3,4,5}, which are not activated by physiological concentrations of LPA. It is important to recognize that LPA_{1,2,3} receptors, at least when expressed heterologously, do show activation to S1P at concentrations in excess of 1 μ M (Valentine *et al.*, 2008a). Conversely, LPA binds to (K_d = 2.3 μ M) and activates the S1P₁ receptor as a low-affinity agonist (Lee *et al.*, 1998). Because LPA concentrations can reach several micromoles in serum and tumour cell effusates (Baker *et al.*, 2001; 2002; Sutphen *et al.*, 2004), promiscuous activation of S1P₁ by LPA in pathophysiological conditions may occur. Murakami *et al.* (Murakami *et al.*, 2008) recently reported that P2Y10 is a receptor for LPA that is also activated by S1P, making P2Y10, if confirmed, a dual-specificity receptor. To make things even more interesting, heterologously expressed P2Y10 is also activated by lysophosphatidyl serine, although it is much less potent than LPA on this target.

LPA GPCRs

The LPA GPCR are the most studied and best understood targets of LPA. In mammals, LPA GPCRs have been identified in two distinct gene families (Figure 2). Chronologically, the endothelial gene (EDG) family was the first shown to encode GPCR specific for S1P and LPA. Within the EDG family, three receptors – LPA₁, LPA₂ and LPA₃ – are activated by LPA and its analogues. These three receptors share 45–56% overall amino acid identity. The overall homology of the transmembrane

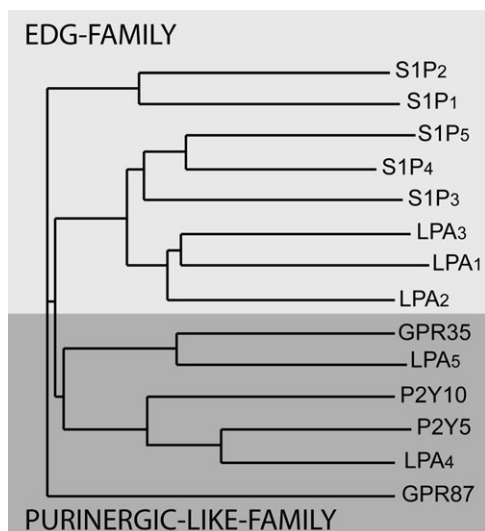


Figure 2

Phylogenetic tree of established and putative LPA GPCR generated by the NGBW program (<http://www.ngbw.org>). GPCR, G protein-coupled receptor.

domains of these three GPCR is 86%, whereas only 6% of the amino acids are conserved in their C-terminal tails. In addition, five S1P-specific receptors, S1P₁, S1P₂, S1P₃, S1P₄ and S1P₅, belong to this gene cluster. There are many excellent recent reviews on the biological function and signal transduction associated with the EDG family receptors; we refer the reader to these for a more in-depth description (Tigyi and Parrill, 2003; Choi *et al.*, 2008; Mutoh and Chun, 2008; Rivera and Chun, 2008; Ishii *et al.*, 2009; Noguchi *et al.*, 2009; Peyruchaud, 2009).

A second cluster of LPA GPCR is found within the P2Y gene cluster (Ishii *et al.*, 2009). The better-characterized LPA-sensitive members of this cluster are P2Y₉/GPR23/LPA₄ (this will be abbreviated as LPA₄) (Noguchi *et al.*, 2003; Yanagida *et al.*, 2007) and GPR92/LPA₅ (this will be abbreviated as LPA₅) (Kotarsky *et al.*, 2006; Lee *et al.*, 2006; Williams *et al.*, 2009; Yin *et al.*, 2009). There are additional related GPCR that appear to be activated by LPA. In chronological order based on the first publication, these include the GPR87 (Tabata *et al.*, 2007; Wetter *et al.*, 2009), P2Y₅ (Pasternack *et al.*, 2008; Shimomura *et al.*, 2008), P2Y₁₀ (Murakami *et al.*, 2008) and GPR35 (Oka *et al.*, 2010) gene products. The LPA GPCR nomenclature was developed by the International Union of Basic and Clinical Pharmacology (IUPHAR) nomenclature committee (Chun *et al.*, 2002), which has not met since 2002; but efforts to update this nomenclature are underway.

Hence, the assignment of numbers to LPA GPCR beyond LPA₅ has been arbitrary and confusing. According to the IUPHAR committee, numbering assignments should follow the chronological order of publication, yet some authors tend to ignore the report on GPR87 in 2007 (Tabata *et al.*, 2007) that preceded publications on P2Y₅ in 2008 (Pasternack *et al.*, 2008; Shimomura *et al.*, 2008). Now that GPR87 has been shown independently to be activated by LPA (Wetter *et al.*, 2009), it might qualify for the designation LPA₆; consequently, P2Y₅ would be LPA₇, P2Y₁₀ and GPR35, if confirmed, would be LPA₈ and LPA₉, respectively. However, these designations will have to be officially assigned by the IUPHAR pending the publication of additional confirmatory evidence concerning the identity of their most potent natural ligand. A summary of the different established and putative LPA receptors and their ligands is shown in Table 1 and Figure 3.

LPA₄ shows a wide tissue distribution with the highest density of transcripts in the ovary, uterus and placenta (Ishii *et al.*, 2009). Interestingly, this receptor was not detectable by Northern blotting in the liver, spleen and testis. LPA₄ has high transcript abundance at sites of implantation within the uterus and increased expression in the ovary during pregnancy. The ligand preference of LPA₄ is LPA 18:1 > 18:0 > 16:0 > 14:0 > 1-O-alkyl glycerophosphate > 1-O-alkenyl glycerophosphate, and the K_d for LPA 18:1 binding to the human orthologue is 77 nM (Noguchi *et al.*, 2003). The brain expresses relatively high levels of LPA₄; and it has been shown, using heterologous expression in B103 neuroblastoma cells, to cause LPA-dependent growth cone collapse and neurite retraction through G_{12/13}-RhoA-Rho-associated kinase (ROCK) activation (Lee *et al.*, 2007). Although a practical issue, it is important to note that heterologous expression of the non-EDG family receptors is often difficult and requires special plasmids and/or receptor-G protein fusion constructs (Noguchi *et al.*, 2003; Tabata *et al.*, 2007; Murakami *et al.*, 2008). Knockout mice for LPA₄ do not display a noticeable phenotype, although mouse embryonic fibroblasts (MEF) isolated from the knockouts show increased migration in response to LPA, supporting the hypothesis that this receptor inhibits cell motility (Lee *et al.*, 2008). Along with LPA₅, LPA₄ has been implicated in the inhibition of neurosphere formation and neuronal differentiation of human embryonic stem cells (Dottori *et al.*, 2008). LPA₄ shows high expression in platelets (Khandoga *et al.*, 2008), and Smyth *et al.* proposed that it might mediate LPA-induced inhibition of platelet activation (Pamuklar *et al.*, 2008). In patients whose platelets do not respond to LPA, LPA₄

Table 1

Confirmed and putative LPA receptor

Receptor	Location	Agonist preference [#]	K _D (LPA 18 : 1)	High expression	Antagonist	Signalling	References
LPA ₁	9q31.3	18 : 2 > 18 : 3 > 20 : 4 > 18 : 1 > 16 : 0 > 18 : 0	N/A	Brain, heart, gut, kidney, stomach	AP29660', Ki16425, DGPP, VPC12449, HZL5105099, HZL-5765834, tetradecyl-phosphonate, LPA α-bromophosphonate	G _i , C _q , G _{12/13} , PDZ	(Ishii <i>et al.</i> , 2004; Lee-Kwon <i>et al.</i> , 2003; Fischer <i>et al.</i> , 2001; Fujiwara <i>et al.</i> , 2005; Heise <i>et al.</i> , 2001; Ohta <i>et al.</i> , 2003; Williams <i>et al.</i> , 2009; Fells <i>et al.</i> , 2009; Zhang <i>et al.</i> , 2009a)
LPA ₂	19p12	18 : 3 > 20 : 4 > 18 : 2 = 18 : 1 > 18 : 0 > 16 : 0 >> 20 : 0	N/A	Heart, lung, gut, stomach, brain	Amgen25, tetradecyl-phosphonate, HZL-5105099, NSC47091, LPA α-bromophosphonate	C _{1q} , C _q , G _{12/13} , PDZ, LIM	(Ishii <i>et al.</i> , 2004; Beck <i>et al.</i> , 2008; Durgam <i>et al.</i> , 2005; E <i>et al.</i> , 2009; Fells <i>et al.</i> , 2009; Lin <i>et al.</i> , 2007)
LPA ₃	1p22.3	18 : 3 > 18 : 2 > OMPT > 18 : 1 > 20 : 4 >> 18 : 0 >> 16 : 0	N/A	Testis, kidney, lung, brain	NSC161613, DGPP, VPC12449, HZL-5765834, tetradecyl-phosphonate, LPA α-bromophosphonate	C _{1q} , C _q	(Ishii <i>et al.</i> , 2004; Durgam <i>et al.</i> , 2005; Fells <i>et al.</i> , 2008; Fischer <i>et al.</i> , 2001; Heise <i>et al.</i> , 2001; Zhang <i>et al.</i> , 2009a)
LPA ₄ / PR23/ P2Y9	Xq13- q21.1	18 : 1 > 18 : 0 > 16 > 0 > 14 : 0 > AGP18 : 0 > AKGP18 : 1	K _D 77 nM	Uterus, ovary, placenta, platelets, mesenchymal cells	HZL-5987411, LPA α-bromophosphonate	C _q , G _{12/13} , cAMP↑	(Noguchi <i>et al.</i> , 2003; Lee <i>et al.</i> , 2006; 2007; Williams <i>et al.</i> , 2009; Zhang <i>et al.</i> , 2009a)
LPA ₅ / CPR92	12p13.31	AGP18 : 1 > AGP 16 : 0 > 18 : 3 > 18 : 2 > 18 : 1 ≈ 16 : 0 ≈ 20 : 4 > FDP > FMP > AGP 18 : 0 > 18 : 0 > CPA 18 : 1 > CPA 16 : 0 > NAG	K _D 6.4 nM	CD8 ⁺ lymphocytes, B cells, platelets, dorsal root ganglion cells	HZL-5987411, HZL-5765834	C _q , cAMP↑	(Kotarsky <i>et al.</i> , 2006; Lee <i>et al.</i> , 2006; Williams <i>et al.</i> , 2009)
CPR87	3q25	18 : 1 > CPA18 : 1 > 2CCPA18 : 1	N/A	Squamos cell carcinomas	N/A	C _q , G ¹⁶	(Tabata <i>et al.</i> , 2007; Wetter <i>et al.</i> , 2009; Guggen <i>et al.</i> , 2008; Zhang <i>et al.</i> , 2009b)
P2Y5	13q14.2	18 : 2 > OMPT > sn2-18 : 1 > sn1-18 : 1 > 20 : 4 > 18 : 0 > 16 : 0 > 14 > 0	N/A	Henle and Huxley layers of scalp hair follicles	N/A	C _q , C _s , cAMP↑ G _{12/13}	(Pasternack <i>et al.</i> , 2008; Shimomura <i>et al.</i> , 2008)
P2Y10	Xq21.1	18 : 1 > STP > LPST18 : 1	N/A	Uterus, brain, prostate, lung, placenta, skeletal muscle, B cells	N/A	C _q , G ₁₆ , RhoA-ROCK	(Murakami <i>et al.</i> , 2008)
CPR35	2q37.3	sn2-18 : 2 > sn2-18 : 1 > sn2-20 : 4 > sn1-18 : 1 >> Zaprinast	N/A	Gastrointestinal, lymphoid and the central and peripheral nervous tissues	N/A	ERK1/2, RhoA, Ca ²⁺	(Oka <i>et al.</i> , 2010)
PPARγ	3p25	Unsaturated AGP > Unsaturated LPA	K _D 60 nM (AGP18 : 1)	Adipocytes, muscle, macrophage, vascular smooth muscle	GW9662	Genes with peroxisome proliferator response elements	(McIntyre <i>et al.</i> , 2003; Tsukahara <i>et al.</i> , 2006; Zhang <i>et al.</i> , 2004)

[#]Numbers alone always refer to LPA, AKGP 18:1 denotes alkenyl glycerophosphate 18:1.

*Compounds in italics show at least a 10-fold selectivity for the receptor subtype. LPA, lysophosphatidic acid; N/A, not available.

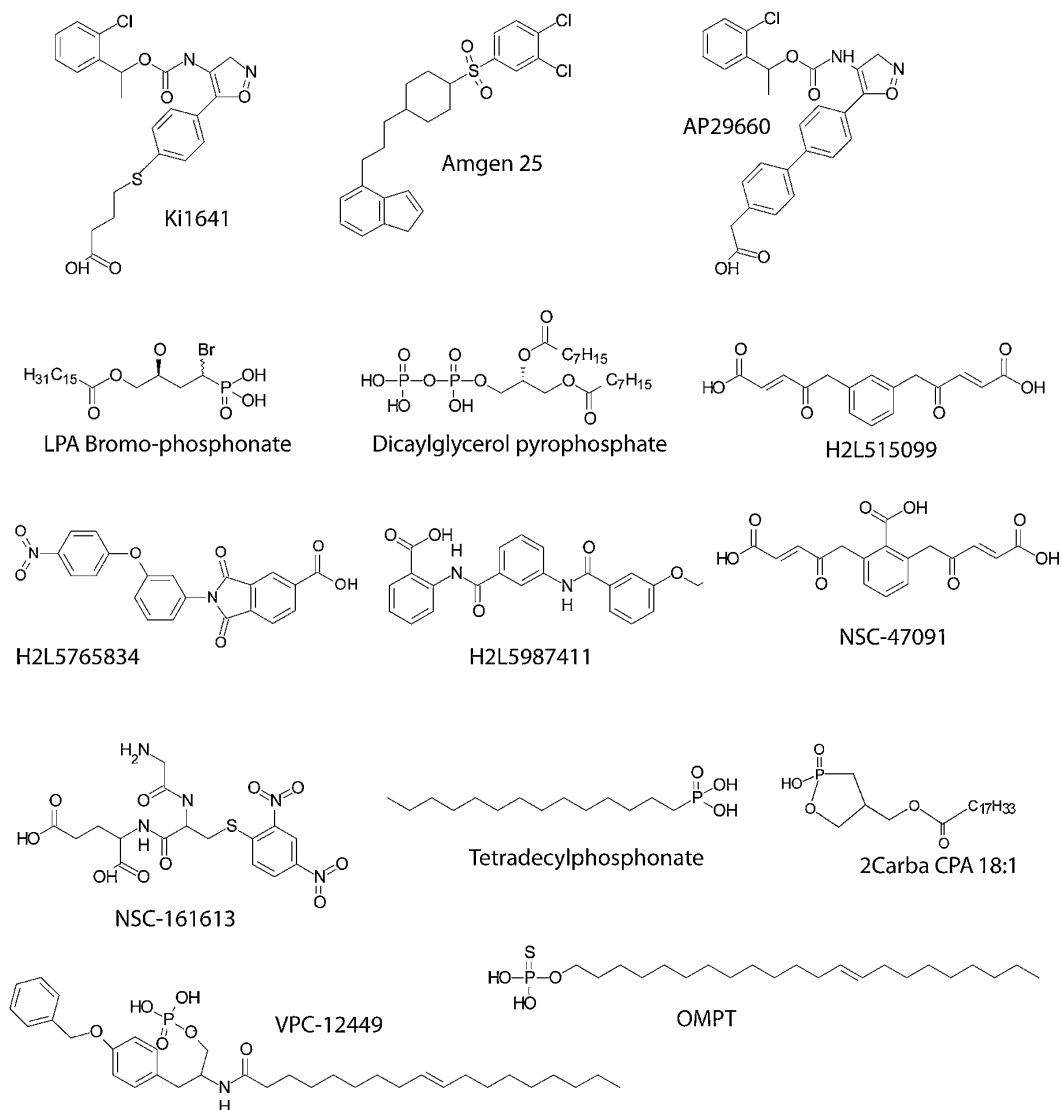


Figure 3

Synthetic ligands of LPA GPCR (see Table 1 for details). GPCR, G protein-coupled receptor; LPA, lysophosphatidic acid.

mRNA is increased sixfold over platelets from patients that are LPA-responsive. Thus, LPA₄ might be a cardioprotective receptor as patients with LPA-non-responsive platelets are less likely to suffer from coronary heart disease.

LPA₅ is present in the heart, placenta, brain, dorsal root ganglia, small intestine and spleen. Intestinal CD8⁺ lymphocytes, as well as B cells, show abundant expression of LPA₅ (Kotarsky *et al.*, 2006), suggesting an immunoregulatory role. This receptor subtype is also highly expressed in human platelets, and pharmacological evidence of its preference for 1-O-alkyl glycerophosphate suggests that it mediates platelet activation (Williams *et al.*, 2009). The ligand preference of LPA₅ is 1-O-alkyl glycerophosphate 18:1 ≥ 1-O-alkyl glycerophosphate 18:0 > LPA

18:1 > LPA 20:4 = LPA 16:0 = LPA 18:3 > farnesyl monophosphate > farnesyl diphosphate > LPA 18:0 > LPA 20:4 > CPA 18:1 > CPA 16:0 > N-arachidonyl glycine (Williams *et al.*, 2009), and the K_d for LPA 18:1 binding is 6.4 nM (Kotarsky *et al.*, 2006). The physiological/pathophysiological classification of this receptor subtype is rudimentary at the present time. Analysis of LPA₅ is currently limited to heterologous expression studies, and experiments using farnesyl phosphate as a selective, although not a specific, agonist. LPA₅ knockout mice have deficits in the response to neuropathic pain (Sheardown *et al.*, 2004; Kinloch and Cox, 2005). LPA₅-lacZ reporter gene-expressing mice showed the strongest expression in cell bodies of dorsal root ganglion cells that extend C-fibres (sensory nerves conveying pain

signals) but not in those neurons with A β fibres (sensory nerves conveying non-pain signals). In these animals, LPA₅ staining was noted in the trigeminal ganglia but not the spinal cord or brain. Behavioural testing showed that the mutant mice had no abnormalities on a wide range of standard tests, including von Frey responses. There was, however, a significant delay in the tail flick test, indicating impaired nociceptive function. *In vivo* electrophysiological recordings (Kinloch and Cox, 2005) from the dorsal horn of mutant animals revealed significantly lower numbers of action potentials in response to noxious mechanical, thermal and cold stimuli applied to the hind foot than in wild-type mice. However, there was no difference in the number of action potentials recorded in response to non-noxious brush stimulation or to temperature in the non-noxious range. Knockout mice failed to develop allodynia using the Chung model of neuropathic pain for 13 days as measured by von Frey thresholds. In contrast wild-type mice developed allodynia in this model (Sheardown *et al.*, 2004; Kinloch and Cox, 2005). In another study, LPA₅ protein was detected using immunohistology in dorsal root ganglion cells of mice and humans (Oh da *et al.*, 2008). In cultured small dorsal root ganglion (DRG) cell neurons, farnesyl diphosphate and N-arachidonoyl glycine both elicited Ca²⁺ transients that were abolished by LPA₅ small interfering RNA (siRNA) transfection, suggesting a potential role in nociception (Oh da *et al.*, 2008). These *in vitro* findings are consistent with the role of LPA₅ in pain processing. Hence, antagonists of LPA₅ might offer a therapeutic approach to the treatment of neuropathic pain.

Chronologically GPR87 was the next de-orphaned GPCR showing activation by LPA (Tabata *et al.*, 2007; Wetter *et al.*, 2009). GPR87 has a modest 27% and 25% homology with LPA₄ and LPA₅ respectively. Its sequence shows 41–48% identity with the P2Y₁₂₋₁₃₋₁₄ receptors and is nestled into this cluster of genes on human chromosome 3q25. Fujita *et al.* developed an *in silico* screening method to discover surrogate ligands for the P2Y receptor family (Hiramoto *et al.*, 2002; 2004; Nonaka *et al.*, 2005). The data supporting LPA as a ligand for GPR87 come from heterologous expression studies conducted in stably transfected Chinese hamster ovary (CHO) cells with a GPR87-G₁₆ fusion protein (Tabata *et al.*, 2007) and from using the TangoTM β -arrestin recruitment-based assay in U2OS cells (Wetter *et al.*, 2009). Expression of GPR87 alone is difficult, suggesting that the posttranslational processing of this receptor might be complex and that attaching G₁₆ to the putative receptor increases plasma membrane expression of the fusion con-

struct (Dr Norihisa Fujita – personal communication). GPR87-G₁₆-transfected CHO cells mobilize Ca²⁺ in response to LPA with an EC₅₀ of 36 nM. This response is completely abolished with a GPR87 siRNA. These cells fail to respond to uridine diphosphate (UDP) and UDP-glucose; however, uridine triphosphate (UTP) treatment of the cells elicits a desensitization-like phenomenon of the LPA response. GPR87 transcripts were detected in the brain, skeletal muscle and the reproductive organs. The initial report on GPR87 also showed that the response to LPA (100 nM) was blocked by Ki16425 (1 μ M) and diacylglycerol pyrophosphate (50 μ M), which have been previously identified as antagonists of the LPA₁₋₃ receptors (Tabata *et al.*, 2007). Clearly, more information is necessary to fully characterize this receptor's ligand selectivity and signal transduction properties, both of which should be derived from LPA-hyporesponsive cells. GPR87 is under the transcriptional regulation of p53; shows a very high expression level in squamous cell carcinomas, lung adenocarcinomas and transitional carcinomas of the urinary bladder and is involved in promoting cell survival and proliferation (Glatt *et al.*, 2008; Gugger *et al.*, 2008; Zhang *et al.*, 2009b).

An unbiased genetic search for the gene causing familial *Hypotrichosis simplex* and autosomal recessive woolly hair has identified mutations in the P2Y5 receptor gene on chromosome 13q14.2–14.3 ($Z = 17.97$) (Pasternack *et al.*, 2008; Shimomura *et al.*, 2008) underlying the disease. Woolly hair is characterized by the presence of fine and tightly curled hair and is also linked to another locus on chromosome 3q27. This region contains the lipase H (*LIPH*) gene, which has been recently shown to underlie an autosomal-recessive form of hypotrichosis (Pasternack *et al.*, 2009; Shimomura *et al.*, 2009a,b). Lipase H is the human orthologue of the PA-PLA1 enzyme that produces *sn2*-LPA from PA. The phenotypically indistinguishable autosomal-recessive woolly hair syndrome caused either by mutations in the P2Y5 or PA-PLA1/*LIPH* genes points to the signalling axis that is colocalized to the Henle's and Huxley's layers of the inner root of the hair follicle. There are differences in LPA receptor expression between hair follicles in the eyebrow and those in the scalp. The former express LPA₅ in addition to P2Y5, whereas the latter express P2Y5 only; hence, hypotrichosis manifests only in the scalp (Pasternack *et al.*, 2008). *LIPH* was originally identified by Kazantseva *et al.* (Kazantseva *et al.*, 2006) as a gene linked to hair growth deficiency in Finno-Ugric families in current-day Russia. *LIPH* is homologous to mPA-PLA1 α , the enzyme that cleaves the *sn1* fatty acid in phosphatidate to generate *sn2*-LPA (Sonoda *et al.*, 2002). *LIPH*/mPA-PLA1 α is expressed in the

skin concentrated near the hair bulb and follicle, lung, kidney and pancreas. Currently, the tissue expression of P2Y5 is not known outside the skin and the hair. P2Y5 shows a K_d of 88.6 nM for *sn1* LPA 18:1 binding and a rank order of activation of LPA 18:2 > LPA 18:1 > LPA 20:4 > LPA 18:0 > LPA 16:0 > LPA 14:0 (Yanagida *et al.*, 2009). Yanagida *et al.* also compared the activation properties of P2Y5 by *sn1* and *sn2*-LPA species and found that the *sn2*-substituted regioisomers were more potent and, in the case of arachidonoyl LPA, more efficacious than the corresponding *sn1* regioisomer (Yanagida *et al.*, 2009). *D-sn-1-O-oleyl-2-O-methyl-glycerol-3-phosphothionate* (OMPT), a previously proposed LPA₃-selective agonist, was more potent at the P2Y5 receptor than LPA 18:1 (Yanagida *et al.*, 2009). P2Y5 in heterologous expression systems has been found to couple to G_{12/13} and G_s, whereas its ability to elicit Ca²⁺ transients is disputed [(Yanagida *et al.*, 2009) vs. (Pasternack *et al.*, 2008)]. Interestingly, human umbilical vein endothelial cells (HUVEC) express LPA₁ and P2Y5; and knock-down of the latter partially inhibited LPA-induced contraction, suggesting a role for this receptor in the barrier properties of vascular endothelia (Yanagida *et al.*, 2009). Of note is the observation that transgenic mice overexpressing lipid phosphate phosphatase-1 (LPP1/PAP2a), an ectoenzyme thought to degrade LPA, show hair growth defects that is consistent with LPP1-mediated degradation of LPA in the skin (Yue *et al.*, 2004). The physiological and pathophysiological functions of P2Y5 other than in hair growth will have to be addressed in future studies.

P2Y10 was de-orphaned as an LPA-activated receptor by the Fujita group, using the same *in silico* process that led to their discovery of GPR87's responsiveness to LPA (Murakami *et al.*, 2008). The P2Y10 gene is localized to region 21.1 of the X chromosome near the gene for the LPA₄ receptor. LPA₄, LPA₅ and GPR87/LPA₆ show high 56%, 52% and 45% homology to P2Y10, which is similar to the 45% amino acid sequence identity among LPA₁, LPA₂ and LPA₃. Transcripts for this putative LPA receptor were detected in the uterus, prostate, brain, lung, placenta and skeletal muscle. P2Y10 is transcriptionally regulated by PU.1 and Spi-B of the Ets transcription factor family in B cells (Rao *et al.*, 1999) and also expressed in platelets (Amisten *et al.*, 2008). P2Y10 have been expressed functionally in Flp-In™ CHO cells either alone or as a G₁₆ fusion construct. In the stable transfectants, LPA and S1P evoke Ca²⁺ transients with EC₅₀ values of 53 nM and 130 nM respectively. Empty vector-transfected or G₁₆-transfected CHO cells do not show Ca²⁺ transients in response to S1P, and their endogenous LPA response is augmented in its

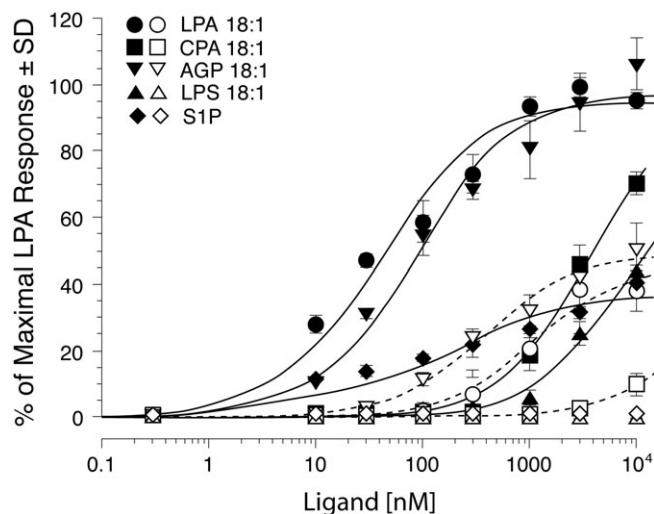


Figure 4

Activation of Ca²⁺ transients in P2Y10-transfected (filled symbols) and empty vector-transfected G_{α16}-expressing CHO cells (open symbols) by LPA, AGP, CPA, S1P and LPS. Note that P2Y10-transfected cells dose-dependently respond to low micromolar CPA, S1P and LPS. These ligands either elicit no response (S1P and LPS) or greatly diminished response (CPA) in the vector transfected G_{α16}-expressing CHO cells. All ligands display lower EC₅₀ and higher E_{max} values in P2Y10-transfected cells compared with controls. The dose-response curves were generated using Fura2 in a FLEXStation.

size with a dose-response shifted to the left. These authors found no activation by dihydro-S1P or sphingosylphosphorylcholine or other lysophospholipids. Kitamura *et al.* (Kitamura *et al.*, 2009) expressed P2Y10 in NIH3T3 and HEK293 cells and found that transfectants show morphological changes and TNF α secretion in response to lysophosphatidyl serine (LPS); the latter was inhibited by Y-27632, an inhibitor of the RhoA-ROCK signaling axis. These cells were highly responsive to LPA and S1P, and these lipids elicited similar responses in P2Y- and mock-transfected cells. In our hands, P2Y10-CHO cells respond to LPA > S1P > LPS (Figure 4), which is consistent with the findings of the Fujita and Aoki groups. GPR34, another P2Y family GPCR, has been recently de-orphaned as a receptor for LPS (Sugo *et al.*, 2006). As for GPR87, also for P2Y10: additional studies will be needed to establish whether LPA is their most potent endogenous ligand. During the redaction of this article, Oka *et al.* (2010) reported that GPR35 responds to LPA with a unique preference for *sn2*-substituted LPAs. The rank order of ligand efficacy at GPR35 was *sn2*-18:2 > *sn2*-18:1 > *sn2*-20:4 > *sn1*-18:1. GPR35 activates the Rho GTPase and the ERK1/2 mitogen activated kinase. Kynurenic acid at concentrations $\geq 100 \mu\text{M}$ activated GPR35.

Intracellular actions of LPA

Identification of its many GPCR shifted the interest to the mediator role of LPA while many early reports on its intracellular actions became ignored (Tsai *et al.*, 1989; Sando and Chertihin, 1996; Yang *et al.*, 2000; Chemin *et al.*, 2005). LPA is generated intracellularly through several pathways. Glycerol-3-phosphate acyl transferase (GPAT) links fatty acyl-CoA with glycerol-3-phosphate to yield LPA. The metabolic fate of fatty acids taken up into cells goes through this important lipid biosynthetic pathway. In cells with the ability to take up fatty acids, this LPA production pathway might have an important regulatory role through activation of the nuclear hormone receptor PPAR γ . PPAR γ plays an essential role in regulating lipid and glucose homeostasis (Evans, 2005), as well as cell proliferation (Mueller *et al.*, 1998), apoptosis (Elstner *et al.*, 1998), and inflammation (Ricote and Glass, 2007). These responses have a direct impact on human diseases, particularly diabetes (Lehmann *et al.*, 1995), atherosclerosis (Li *et al.*, 2000) and cancer (Sarraf *et al.*, 1998). Synthetic agonists of PPAR γ include the thiazolidinedione (TZD) class, widely used to treat type 2 diabetes. Physiological agonists of PPAR γ include 15d-PGJ₂ (Forman *et al.*, 1995), modified fatty acids (Baker *et al.*, 2005) (Nagy *et al.*, 1998), oxidized phospholipids (Davies *et al.*, 2001) and select forms of LPA (McIntyre *et al.*, 2003). The structure-activity relationship of PPAR γ 's activation by LPA differs from that of the GPCR (Tsukahara *et al.*, 2006). Whereas LPA GPCR are activated by both saturated and unsaturated fatty acid-substituted LPAs, PPAR γ is only activated by unsaturated LPA. LPA GPCR do not show stereoselective responses to the *S*- and *R*-isomers of LPA; in contrast, PPAR γ is only activated by the *S*-stereoisomer. Furthermore, with the exception of LPA₅, the alkyl analogues of LPA are less potent agonists of the remaining LPA GPCR; however, 1-O-alkyl glycerophosphate is considerably more potent than LPA at PPAR γ (Zhang *et al.*, 2004; Tsukahara *et al.*, 2006). The apparent K_d of 1-O-octadecenyl glycerophosphate binding was 60 nM in the PPAR γ ligand binding domain, which is similar to that of rosiglitazone at 40 nM (Tsukahara *et al.*, 2006). Some of the residues required for activation of PPAR γ by 1-O-octadecenyl glycerophosphate differ from those required by rosiglitazone. When mutated to alanine, histidines 323 and 449 within the ligand-binding domain of PPAR γ failed to abolish binding to and activation by 1-O-octadecenyl glycerophosphate but completely abolished the binding of and activation induced by rosiglitazone. Alanine mutation of arginine 288 significantly reduced binding and activation

induced by 1-O-octadecenyl glycerophosphate but not those induced by rosiglitazone. Mutation of tyrosine 273 to phenylalanine abolished binding to and activation by both agonists (Tsukahara *et al.*, 2006).

What is the physiological role of PPAR γ activation by LPA? LPA is a key intermediate of fatty acid metabolism. Fatty acids taken up by cells are activated to form acyl-CoAs. Acyl-CoAs can be targeted for β -oxidation by conversion to acyl-carnitine or converted to LPA by different isoforms of GPAT. The resulting LPA can feed either phospholipid synthesis or fuel triacylglycerol synthesis and lipid storage. The LPA formed by GPAT can up-regulate PPAR γ target genes involved in lipogenesis, lipid storage and adipocytic differentiation. In a pathophysiological context, LPA might be a key factor in non-alcoholic fatty liver disease (Nagle *et al.*, 2009; Wendel *et al.*, 2009). Thus, LPA might be a molecular link between fatty acid uptake and lipid storage. This hypothesis, although plausible, awaits experimental proof.

Another mechanism regulated by LPA-mediated activation of PPAR γ is arterial wall remodelling, which can lead to plaque formation. All major cell types of the arterial wall respond to LPA. In endothelial cells, LPA has been shown to regulate the expression of adhesion molecules and stimulate proliferation, apoptosis, permeability, motility and cell-to-cell contacts responsible for transendothelial permeability (Rizza *et al.*, 1999; Lin *et al.*, 2006). LPA induces vascular smooth muscle cell (VSMC) contraction, proliferation (Tokumura *et al.*, 1994) and phenotypic transdifferentiation *in vitro* (Hayashi *et al.*, 2001; Zhang *et al.*, 2004). LPA and oxidized low-density lipoprotein (LDL) inhibit macrophage/dendritic cell egress across endothelial cell monolayers (Angeli *et al.*, 2004; Llodra *et al.*, 2004). LPA also stimulates the formation of platelet-monocyte aggregates, which are considered an early marker of acute myocardial infarction (Fueller *et al.*, 2003; Siess, 2006).

Yoshida *et al.* were the first to test the effects of lumenally applied LPA on non-injured arterial wall *in vivo* (Yoshida *et al.*, 2003). These authors infused LPA through the external carotid artery of rats into a ligated section of the common/internal carotid artery that was previously rinsed free of blood and maintained near the mean arterial perfusion pressure. In this model, which involves no mechanical injury or removal of endothelial cells within the common carotid artery, a 1-hour exposure to unsaturated but not to saturated species of LPA at low micromolar concentrations leads to neointima development. The structure-activity relationship of neointima induction does not match that of the

known LPA GPCR but matches that for PPAR γ activation by LPA. PPAR γ has long been implicated in atherogenesis (Diep and Schiffrin, 2001; Li and Glass, 2002). GW9662, a specific irreversible antagonist of PPAR γ (Leesnitzer *et al.*, 2002), completely abolished 1-O-octadecenyl glycerophosphate-, LPA- and rosiglitazone-induced neointima formation in the rat model (Zhang *et al.*, 2004). In addition to this pharmacological evidence, studies conducted with LPA_{1&2} and PPAR γ conditional knockout mice confirmed the requirement of PPAR γ and not LPA_{1&2} GPCR for arterial wall remodelling in the non-injury model (Cheng *et al.*, 2009). Several investigators have reported that systemic and chronic administration of rosiglitazone attenuates neointima in models with mechanical injury of the arterial wall, which differs from findings reported using the non-injury model (Lim *et al.*, 2006; Lee *et al.*, 2009). When 1-O-octadecenyl glycerophosphate was applied to an injured carotid artery, neointima formation was augmented, whereas rosiglitazone attenuated it. These observations underscore the differences between the non-injury and injury-induced models. It is important to note that circulating LPA bound to carrier proteins such as albumin is unable to activate PPAR γ due to limited internalization of the lipid-protein complex (Zhang *et al.*, 2004). Thus, the source of LPA is either intracellular or although the uptake of lipid particles such as oxidized LDL (Siess *et al.*, 1999; Zhang *et al.*, 2004). The role of LPA in regulating vascular wall physiology is a very promising area for future research.

The LPA-PPAR γ axis is also involved in mast cell and dendritic cell differentiation (Bagga *et al.*, 2004; Leslie *et al.*, 2008). Bagga *et al.* have shown that LPA caused mast cell hyperplasia by inducing the proliferation and maturation of these cells. These cellular responses are under dual control of LPA_{1/3} GPCR and PPAR γ as they were partially inhibited by GW9662, pertussis toxin or VPC-32179. Dendritic cells play an important role in antigen presentation. Serum lipids promote the expression of CD1d antigen-presenting molecules and enhance the activation of immune responses mediated by CD1d-restricted T cells. LPA and cardiolipin were identified as the two active ingredients in serum (Leslie *et al.*, 2008). CD1 expression in immature dendritic cells was under the transcriptional control of PPAR γ . This finding raises the hypothesis that LPA can regulate antigen presentation by dendritic cells through the expression of CD1 molecules. Although not mediated by PPAR γ , another important immunoregulatory role of LPA has been described by Kanda *et al.* (Kanda *et al.*, 2008), who found high expression of ATX in the high endothelial venules of lymph nodes. LPA

induces chemokinesis in T lymphocytes and could regulate their homing into the lymph node. These observations combined with the role of S1P in lymphocyte egress underscore the importance of lysophospholipids in lymphocyte trafficking and assign LPA and S1P to distinct steps, homing and egress respectively.

Lysophosphatidic acid is also produced in a spatially regulated fashion by the Ca²⁺-independent phospholipase A2 (iPLA2) at the leading edge of the migrating monocyte (Carnevale and Cathcart, 2001; Mishra *et al.*, 2008). Cathcart *et al.* have shown that whereas cPLA2 α generates arachidonic acid in the cytoplasm and localizes to the trailing edge in monocytes migrating towards MCP-1 chemokine, iPLA2 produces LPA and is localized to the pseudopods where actin polymerization is actively regulated. LPA GPCR have long been known to signal through the Rho-Rac-Cdc42 small GTPase-coupled pathways regulating the actin cytoskeleton (Ridley and Hall, 1992; 1994; Ridley *et al.*, 1992; Ridley *et al.*, 2004). However, there is compelling evidence for the interaction of LPA with actin-binding proteins, including gelsolin, formin, adseverin (Meerschaert *et al.*, 1998; Goetzl *et al.*, 2000b; Mintzer *et al.*, 2006) and villin, the later of which is restricted to epithelial cells (Khurana *et al.*, 2008; Tomar *et al.*, 2009). LPA generation via iPLA2 in migrating cells could regulate actin capping, severing and polymerization through direct high-affinity interactions with these actin-binding proteins. The high-affinity interaction of acting binding proteins with phosphatidylinositol 4,5-bisphosphate (PIP2) has long been known among cell biologists. In this context, findings of the Khurana group are of importance (Khurana *et al.*, 2008; Tomar *et al.*, 2009). These authors showed higher affinity binding of LPA to villin (K_d 22 μ M) than that of PIP2 (K_d 39.5 μ M) and demonstrated the LPA displaced villin-bound PIP2 *in vivo*. They also provided evidence that LPA can regulate actin nucleation, depolymerization and capping, the latter of which is not affected by PIP2. LPA has been found to enhance Src-mediated phosphorylation of villin (Khurana *et al.*, 2008; Tomar *et al.*, 2009) and gelsolin (Meerschaert *et al.*, 1998). This polarized production coupled with the direct interaction of LPA with members of the gelsolin-villin family proteins implicates it in the cellular compass mechanism underlying directional cell migration. Extracellularly applied LPA induces cell polarization, directional motility and metabolic burst in human neutrophils (Chettibi *et al.*, 1994). It is intriguing to speculate how iPLA2 could be redistributed to the leading edge of the cell exposed to a chemokinetic gradient and

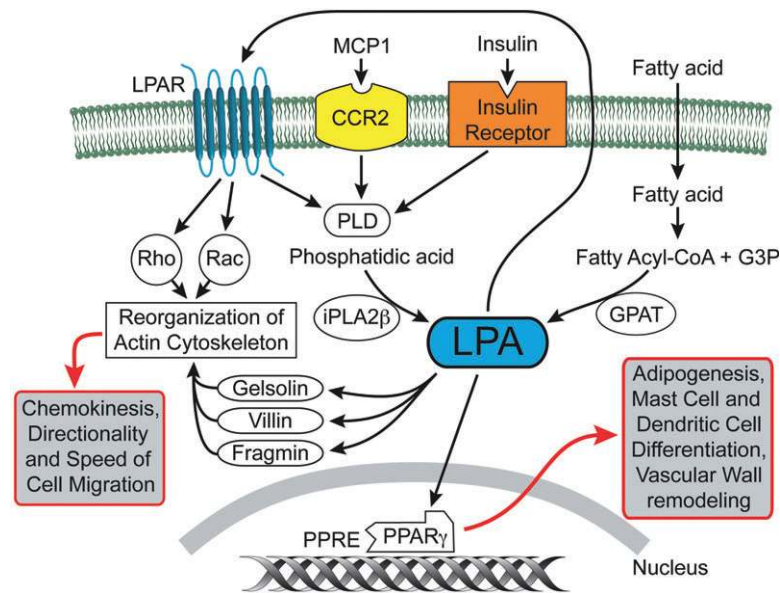


Figure 5

Intracellular sources, targets and actions of LPA. LPA can be generated by iPLA2 β in response to MCP1 or insulin stimulation at the leading-edge of migrating macrophages and regulate actin-binding/severing proteins. LPA is also generated by GPAT from fatty acids and can interact with PPAR γ , which in turn up-regulates genes involved in adipogenesis and lipid storage. LPA can potentially be excreted from cells and stimulate cell surface LPA GPCR setting up an inside-out signalling paradigm. LPA GPCR can synergize with the activation of intracellular targets as it may happen in the case of regulation of the actin cytoskeleton via Rho and Rac.

whether extracellular LPA signals originating from LPA GPCR through an inside-out mechanism could feed back into the regulation of iPLA2 and the cellular compass (Figure 5).

Lysophosphatidic acid can also be produced intracellularly by PLD from lysophosphatidyl choline and potentially be a source of ligand for intracellular and/or cell surface LPA receptors (Xie *et al.*, 2002).

Autotaxin – how to target it in vivo?

In extracellular biological fluids, LPA production is linked to ATX, which is a lysophospholipase D that cleaves the headgroup from lysophospholipids to generate LPA. ATX is a member of the nucleotide pyrophosphatase/phosphodiesterase (NPP) family and is also known as NPP2. ATX was originally identified as a secreted phosphatase in melanoma culture supernatant that promoted the motility of cancer cells (Stracke *et al.*, 1992). Ten years after its original discovery, ATX was identified as a lysophospholipase D responsible for the production of LPA in serum (Umezu-Goto *et al.*, 2002; Tokumura *et al.*, 2002a). Knocking out ATX leads to embryonic lethality due to a defect in blood vessel formation (van Meeteren *et al.*, 2006; Tanaka *et al.*, 2006) and a lack of large lysosomes in the yolk sac visceral endo-

derm cells (Koike *et al.*, 2009). This latter defect is due to a lack of LPA-mediated constitutive activation of the Rho-ROCK and LIM kinase pathways. Conditional knockout of ATX in the nervous system causes a neural tube defect that can be corrected in explants by the addition of LPA in the culture medium (Fotopoulou *et al.*, 2010). Neural tube closure involves LPA-mediated activation of the hypoxia-inducible factor HIF-1 α . In the developing nervous system, ATX also regulates the differentiation of oligodendrocytes (Fuss *et al.*, 1997; Fox *et al.*, 2003; 2004; Dennis *et al.*, 2005; 2008; Yuelling and Fuss, 2008; Nogaroli *et al.*, 2009). In adult mice, the level of ATX affects haemostasis and thrombosis (Pamuklar *et al.*, 2009).

Many cancers secrete ATX, which contributes to their invasive properties. Gene copy numbers increase in ovarian cancer in chromosomal region 8q24, which also contains the genes encoding the Myc oncogene (Dimova *et al.*, 2006). This raises the possibility that the ATX gene might be amplified in ovarian cancer. Ectopic expression of ATX in mice has recently been shown to lead to the development of chronic mastitis, hyperplasia, mammary intraepithelial neoplasia, and invasive and metastatic tumours (Liu *et al.*, 2009). Ovarian cancer cells produce high levels of LPA in tumour ascites (Baker *et al.*, 2002; Sutphen *et al.*, 2004). ATX inhibits paclitaxel-induced apoptosis in breast cancer cells

(Samadi *et al.*, 2009), and LPA renders ovarian cancer cells chemoresistant to cisplatin and adriamycin (E *et al.*, 2009). ATX is overexpressed in patients with recurrent disease after prior treatment with chemotherapy (Jazaeri *et al.*, 2005). An siRNA screen has identified ATX as a candidate drug-resistance gene in ovarian cancer (Vidot *et al.*, 2010).

Autotaxin shows product feedback inhibition, meaning that LPA and S1P inhibit its activity (Durgam *et al.*, 2005; van Meeteren *et al.*, 2005). This feedback inhibition of ATX by LPA has been proposed to be a factor in maintaining plasma levels of LPA in the low nanomolar range (van Meeteren *et al.*, 2005). However, the rate of ATX production seems to be an overriding factor because ATX knock-out heterozygous mice have half the normal plasma level of LPA (van Meeteren *et al.*, 2006; Tanaka *et al.*, 2006). Nevertheless, the search for ATX inhibitors is intensifying due to the potential therapeutic applicability of such compounds in cancer therapy (Clair *et al.*, 2005; Baker *et al.*, 2006; Ferry *et al.*, 2008; van Meeteren *et al.*, 2008; Parrill and Baker, 2008; Parrill *et al.*, 2008; North *et al.*, 2009; Zhang *et al.*, 2009a). As proof of this concept, we and others have shown that CCPA analogues inhibit ATX-mediated melanoma invasion *in vitro* and metastasis *in vivo* (Baker *et al.*, 2006; Uchiyama *et al.*, 2007). However, inhibition of ATX does not lead to an anti-tumour effect, as cancer cells continue to proliferate although their metastasis is reduced. Zhang *et al.* (Zhang *et al.*, 2009a) have developed alpha-bromophosphonate analogues of LPA that inhibit ATX activity and also are antagonists of LPA_{1,2,3,4,5} GPCR and showed that these dual-action compounds not only reduce breast cancer metastasis but also inhibit tumour growth. Hence, the challenge now is to combine receptor antagonism with the inhibition of ATX to obtain anti-tumour and anti-metastatic drug candidates. Pure ATX inhibitors also face another challenge; intravenously injected recombinant ATX is very rapidly >10-min cleared within the liver sinusoids (Jansen *et al.*, 2009). This rapid clearance might also apply to endogenous ATX. Another new direction is to explore agonists of LPA₄ and LPA₅ that also inhibit ATX. LPA₄ inhibits invasion and migration elicited by the EDG-family LPA receptors LPA₁ and LPA₂ (Lee *et al.*, 2008). Hence, selective agonists of this receptor subtype that inhibit ATX might prove to be useful in limiting cancer invasion and metastasis (Baker *et al.*, 2006; Williams *et al.*, 2009). LPA receptors of the EDG family are dysregulated in ovarian cancer. In a study with 30 ovarian cancer patients, LPA₁ expression was decreased, whereas LPA₂ and LPA₃ were increased (Murph *et al.*, 2008) in the tumour tissue. siRNA-mediated knock-down of LPA₂ and LPA₃ in SKOV and OVCAR-3 ovarian cancer cell lines reduced

aggressiveness and increased survival after xenografting (Yu *et al.*, 2008).

LPA in malignant transformation, cancer metastasis, and radiation- and chemo-resistance

Lysophosphatidic acid is a mitogen, motogen and anti-apoptotic agent, all of which combined provide survival advantages to cells that generate LPA and utilize it in an autocrine or paracrine fashion. In the last decade, many reports have confirmed that several types of cancer cells secrete ATX, which in turn generates LPA in the immediate vicinity of the cell, so long as lysophosphatidylcholine is available. Biological fluids are rich in lysophosphatidylcholine. Perhaps the best-established example for the role of LPA in cancer pathobiology is ovarian cancer. Ovarian cancer ascites has highly elevated levels of LPA (Xu *et al.*, 1995; Baker *et al.*, 2002; Sutphen *et al.*, 2004) due to increased ATX expression (Dimova *et al.*, 2006). Ovarian cancers predominantly express LPA₂ receptors, whereas this receptor is barely detected in ovarian epithelial cells (Goetzl *et al.*, 1999a). In addition to LPA₂, LPA₄ is highly expressed in normal ovarian tissues (Noguchi *et al.*, 2003). LPA₂ might play an important role in the aggressive behaviour of ovarian cancer in at least two ways. First, LPA₂ promotes the production of vascular endothelial growth factor (VEGF), urokinase (uPA) and matrix metalloproteinases (MMP) (Zebrowski *et al.*, 1999; Hu *et al.*, 2001; Huang *et al.*, 2004; So *et al.*, 2004; 2005). LPA increases VEGF production, and VEGF in turn up-regulates ATX production, which increases LPA levels (Ptaszynska *et al.*, 2008) – a potential feed-forward loop. LPA-induced uPA production promotes invasion by several ovarian cancer cell lines (Pustilnik *et al.*, 1999; Gil *et al.*, 2008). uPA levels carry a prognostic value in ovarian cancer and correlate with poor prognosis (Schmalfeldt *et al.*, 2001; Murthi *et al.*, 2004). LPA conveys resistance to chemo- and radiation-therapy through its anti-apoptotic action (Fang *et al.*, 2000; Deng *et al.*, 2007; E *et al.*, 2009). The LPA₂ receptor plays a unique role in chemoresistance, mediated through its C-terminal interaction with the thyroid receptor-interacting protein 6 (TRIP6), the pro-apoptotic transcription factor Siva-1 and the PSD-95/Disc-large/ZO-1 domain (PDZ domain)-binding proteins NHERF2 and MAGI-3 (Yamada *et al.*, 2005; Lin *et al.*, 2007; Zhang *et al.*, 2007a,b; E *et al.*, 2009). LPA₂ binds Siva-1 through a-C₃₁₁XXC motif and the complex becomes polyubiquitinated and degraded by the proteasome, which leads to the attenuation of the DNA-damage-

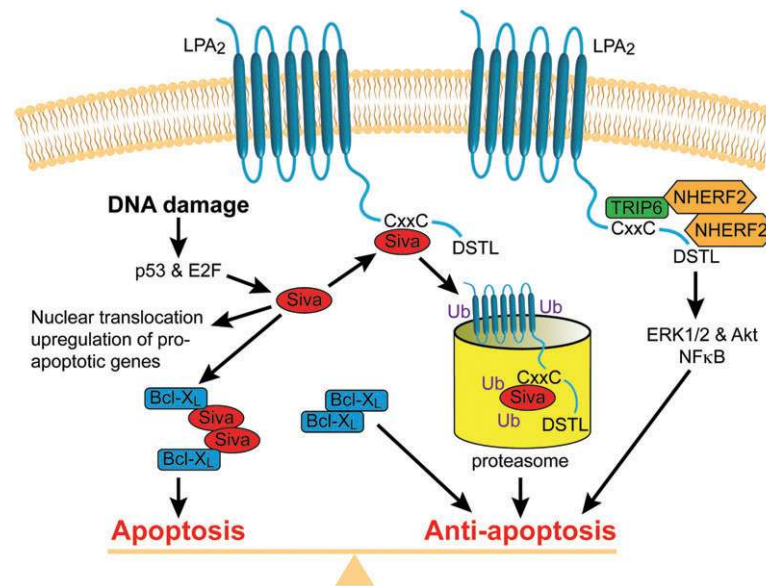


Figure 6

Macromolecular complex-mediated anti-apoptotic signalling by the LPA₂ receptor. The C-terminal tail of LPA₂ contains docking sites for PDZ-binding proteins (NHERF2) and LIM family proteins (Siva-1 and TRIP6). LPA₂ activation captures the pro-apoptotic Siva-1 and targets it to proteasomal degradation. LPA₂ receptor activation recruits a ternary complex formed with NHERF2 and TRIP6, which augments anti-apoptotic signals mediated via ERK1/2 and Akt kinase pathways.

induced apoptosis. Siva-1 has also been shown to bind and sequester the anti-apoptotic BCL-X_L protein and promote the progression of apoptosis via the mitochondrial pathway. LPA-induced molecular complex between LPA₂ and Siva-1 plays a major role in the anti-apoptotic effect of LPA. LPA₂ can form a ternary complex with TRIP6 and NHERF2 in such a way that TRIP6 and NHERF2 also attach to each other. This ternary complex is formed via the ⁻³⁴⁷DSTL PDZ motif of LPA₂ and the PDZ2 domain of NHERF2. NHERF2 homodimerizes with an other NHERF2 via its PDZ1 binding domain, leaving the second PDZ2 domain available to bind the -TTDC PDZ motif of TRIP6. TRIP6 also binds to the -CXXC motif in LPA₂ via its LIM3 domain completing the ternary complex (Figure 6). The ternary complex is required for the full activation of ERK1/2 and AKT kinases and through them essential for LPA-induced protection against radiation- and DNA-damage-induced apoptosis. LPA₂-associated macromolecular signalosomes have been detected in ovarian cancer cells and for this reason also represent novel targets for prevention of therapeutic resistance of cancer (Figure 6). MMPs represent yet another group of important effectors of LPA in cancer cells (Fishman *et al.*, 2001; Meng *et al.*, 2004; So *et al.*, 2004; 2005; Hope *et al.*, 2009). LPA up-regulates MMP production, which in turn promotes the proliferation of cancer cells and invasion. Increased

MMP production correlates with poor prognosis of ovarian cancer (Davidson *et al.*, 1999a,b; Schmalfeldt *et al.*, 2001). In view of the mitogenic, motogenic, pro-invasive and anti-apoptotic effects of LPA, receptor antagonists and ATX inhibitors offer potential for anti-cancer therapeutics.

LPA in the nervous system

The LPA₁ receptor was originally identified from neuronal progenitor cells in the ventricular zone of the developing brain (Hecht *et al.*, 1996). LPA is important in myelination and Schwann cell survival (Weiner *et al.*, 1998; Moller *et al.*, 1999; Li *et al.*, 2003; Nogaroli *et al.*, 2009). Perhaps the most profound morphological effect of LPA is on the development of cortical folds that lead to the development of gyri in the cerebral cortex (Kingsbury *et al.*, 2003; Estivill-Torrus *et al.*, 2008; Matas-Rico *et al.*, 2008). There are several reviews dedicated to the topic of LPA in the brain, and the reader is referred to those for a more in-depth coverage of this exciting aspect of LPA developmental neurobiology (Birgbauer and Chun, 2006; Herr and Chun, 2007; Choi *et al.*, 2008; Rivera and Chun, 2008; Noguchi *et al.*, 2009).

One significant therapeutic effect of LPA appears to be its mediator role in neuropathic pain.

Intrathecal injected lysophosphatidylcholine (LPC) has been shown to elicit neuropathic pain and cause demyelination (Wallace *et al.*, 2003; Inoue *et al.*, 2008b). These effects of LPC require ATX; and the bioactive compound is LPA, the product of ATX-mediated LPC hydrolysis (Inoue *et al.*, 2008b; Ahn *et al.*, 2009). LPA initiates neuropathic pain and regulates the production of pain-related molecules through the LPA₁ receptor (Inoue *et al.*, 2004; 2008a,b; Ueda, 2006; Fujita *et al.*, 2007; Xie *et al.*, 2008). Recently, Ueda *et al.* proposed a feed-forward loop that involves LPA₃ receptor-mediated production of lysophosphatidylcholine, which supplies the substrate to ATX, rich in the cerebrospinal fluid. These authors showed that LPA produced by ATX in turn can lead to LPA₁-mediated neuropathic pain (Ma *et al.*, 2009).

LPA₁ up-regulates the voltage-gated Ca²⁺ channel in the dorsal root ganglion cell, through a Rho-Rho kinase-coupled mechanism, causes demyelination of the fibres originating from the ganglion cells and leads to the reorganization of A β -fibres (Inoue *et al.*, 2004; Fujita *et al.*, 2007; Xie *et al.*, 2008). In addition to the LPA₁ receptor, LPA₅ has also been implicated in neuropathic pain, although the mechanism responsible remains to be elucidated (Sheardown *et al.*, 2004; Kinloch and Cox, 2005). Antagonists targeting LPA₁ and LPA₅ receptors might provide new ways to treat this currently difficult-to-manage neurological condition. Perhaps more than a curiosity is the fact that B lymphocytes from patients with bipolar disorders are hyper-responsive to LPA (Wasserman *et al.*, 2004; Perova *et al.*, 2008; 2009); and in patients with schizophrenia, LPA₁ is among 18 genes that show close association with the disease (Bowden *et al.*, 2006).

LPA in bone metabolism

LPA₁ knockout mice show decreased bone density, shorter bones (J.P. Salles – personal communication) and a craniofacial dysmorphic phenotype due to abnormal development of the frontal bones of the skull (Contos *et al.*, 2000). This abnormality might represent only one manifestation of defects in osteoclast and osteoblast function regulated by the LPA₁ receptor. LPA has been identified as a mitogen for osteoblasts (Caverzasio *et al.*, 2000; Grey *et al.*, 2001; Lyons and Karin, 2001); it prevents osteoblast apoptosis (Grey *et al.*, 2002) and stimulates alkaline phosphatase expression (Dziak *et al.*, 2003) and cell migration. Osteocytes develop a mechanosensory network within the bone matrix and communicate through gap junctions established at their dendritic termini. LPA is a potent stimulator of dendrite out-

growth, which is blocked by the LPA₁/LPA₃ antagonist Ki16425 and pertussis toxin (Karagiosis and Karin, 2007). Resting zone chondrocytes generate LPA, which in turn promotes osteoblast differentiation, proliferation and survival (Hurst-Kennedy *et al.*, 2009). Osteolytic metastasis of breast cancers also utilizes LPA as a mediator. LPA₁ regulates the secretion of IL-6 and IL-8, which are potent bone resorption stimulators. Silencing expression or utilizing pharmacological inhibition of LPA₁ in cancer cells reduces bone metastasis progression. Future pharmacological manipulation of LPA receptors might provide opportunities to prevent bone loss and reduce the bone metastasis of cancers.

LPA in reproductive disorders

The effects of LPA on cells from the female reproductive system have been known since the late 1980s. We previously identified and purified LPA as a serum factor based on its activation of oscillatory Cl⁻ currents in *Xenopus* oocytes (Tigyi *et al.*, 1990; Tigyi and Miledi, 1992). LPA enhances embryonic development from the pronuclear stage to the blastocyst stage in mice (Kobayashi *et al.*, 1994). In luteal cells, LPA stimulates type V adenylyl cyclase, which is important for the maintenance of pregnancy (Budnik and Mukhopadhyay, 1997). This response is likely to be mediated through LPA₄, which, in contrast to the EDG family receptors, elevates cAMP and is highly expressed in the ovary (Noguchi *et al.*, 2003). LPA stimulates contraction in uterine smooth muscle cells (Tokumura *et al.*, 1980) and increases embryo transport in the oviduct of mice (Kunikata *et al.*, 1999; Tokumura *et al.*, 1999). In the follicular fluid, LPA concentration is as high as 25 nmol·mL⁻¹, which is nearly double its serum concentration of *c.* 15 nmol·mL⁻¹ (Tokumura *et al.*, 1999). The LPA₃ receptor, which is up-regulated by progesterone and down-regulated by oestrogen, is required for embryo implantation and affects embryo spacing in the womb (Ye *et al.*, 2005; Hama *et al.*, 2007). In view of its effects on implantation, it is plausible that LPA is involved in placental pathologies, including non-receptive endometrium, decidualization, embryo crowding, placenta previa and placenta accreta, any of which could lead to sterility or loss of pregnancy (Ye, 2008). There are some selective LPA₃ antagonists available that include diacylglycerol pyrophosphate (Fischer *et al.*, 2001), VPC12449 (Heise *et al.*, 2001) and Ki16425 (Ohta *et al.*, 2003). However, these antagonists also inhibit LPA₁. Recently, a specific non-lipid antagonist, NSC161613, has been identified showing an IC₅₀ of 24 nM (Fells *et al.*, 2008).

LPA_{1/2/3} are highly expressed in the male reproductive system. Mice deficient in all three receptors show a testosterone-independent reduction of mating activity and sperm production, with an increased prevalence of azoospermia in aging animals. These triple knockouts show a significant increase in germ cell apoptosis, leading to a reduction in germ cell proliferation (Ye *et al.*, 2008). Similar azoospermia and reduced germ cell numbers were found in transgenic animals that overexpress lipid phosphate phosphatase, an enzyme that degrades LPA (Yue *et al.*, 2004). There is a wealth of evidence that links LPA, LPA₃ and LPA₁ to the regulation of prostate cancer cells (Qi *et al.*, 1998; Prenzel *et al.*, 1999; Im *et al.*, 2000; Daaka, 2002; Guo *et al.*, 2006; Hwang *et al.*, 2006). In contrast, little is known about the physiological role of LPA in the normal prostate. Selective ligands of the LPA₃ receptor would likely offer novel drug candidates to modulate both male and female reproduction.

LPA in vascular pathologies

In 1978, Tokumura *et al.* reported that soy lipid extract contains a vasopressor lipid that they identified as LPA (Tokumura *et al.*, 1978a; Tokumura *et al.*, 1978b). LPA had hypertensive effects in rats and guinea pigs and hypotensive effects in cats and rabbits. Schumacher *et al.* discovered that factors developed during 'aging' of heparinized plasma kept at 36°C for 18–24 h and that these factors activated platelet aggregation and caused constriction of pulmonary vessels (Schumacher *et al.*, 1979). These authors identified LPA as being one of the active factors and noted that it caused desensitization of the platelet response, a hallmark of GPCR action. Simon *et al.* discovered that the alkyl-ether analogue of LPA is a potent activator of human platelets at concentrations as low as 10⁻¹⁰ M, making this analogue nearly as potent as platelet-activating factor (Simon *et al.*, 1982; 1984). The barrier function of vascular endothelial cells is altered by LPA (Schulze *et al.*, 1997; Alexander *et al.*, 1998; English *et al.*, 1999), and LPA induces the expression of VCAM-1 and E-selectin on the surface of cultured human endothelial cells (Rizza *et al.*, 1999). LPA induces endothelin-1 production *in vitro* and *in vivo* (Chua *et al.*, 1998; Yakubu and Leffler, 1999). LPA is a mitogen for VSMC (Tokumura *et al.*, 1994) and also promotes their phenotypic dedifferentiation from a contractile phenotype to a secretory phenotype (Hayashi *et al.*, 2001; Yoshida *et al.*, 2003). Topical application of unsaturated LPA species into the non-injured carotid artery of rodents induces arterial wall remodelling, and this response requires PPARγ

but not LPA₁ or LPA₂ (Hayashi *et al.*, 2001; Yoshida *et al.*, 2003; Zhang *et al.*, 2004; Cheng *et al.*, 2009).

Lysophosphatidic acid is required for normal vascular development, as indicated by the embryonic lethality of ATX knockout mice at a stage when vascular stabilization begins (van Meeteren *et al.*, 2006; Tanaka *et al.*, 2006). In adult mice, ATX overexpression leads to haemorrhages due to an inhibitory effect of LPA on platelets, whereas ATX ± heterozygotes, which have a reduced plasma LPA concentration, are more prone to thrombosis (Pamuklar *et al.*, 2009). LPA and ATX have been implicated in wet-type macular degeneration by promoting angiogenesis in the retina.

Nanomolar concentrations of LPA and minimally oxidized LDL induce shape change of washed platelets through LPA receptor-linked signal transduction pathways that presumably involve the activation of the heterotrimeric G₁₂/G₁₃ protein, the small GTPase Rho and Rho kinase, the Rho kinase-mediated inhibition of myosin light-chain phosphatase, and stimulation of myosin light-chain phosphorylation (Bauer *et al.*, 1999; Retzer and Essler, 2000; Retzer *et al.*, 2000). It seems that there are two types of LPA receptors that regulate LPA responsiveness in human platelets. One subtype inhibits platelet responses by elevating cAMP levels, which in turn attenuates activation, as proposed by Pamuklar *et al.* (Pamuklar *et al.*, 2008). These authors ascribed this effect to LPA₄. Khandoga *et al.* could not detect elevation in cAMP in LPA-treated human platelets (Khandoga *et al.*, 2008). However, these authors found that the structure-activity relationship, characterized by the preference for alkyl over acyl LPA analogues, is similar to that of LPA₅, which makes this receptor a candidate for the activating LPA receptor subtype in platelets (Williams *et al.*, 2009). Williams *et al.* identified two non-lipid antagonists of LPA₅, H2L 5987411 and H2L 5765834, that also blocked platelet activation by LPA (Williams *et al.*, 2009). The former compound but not the latter is also a weak antagonist of LPA₄ (K_i 741 nM), yet both inhibited platelet responses to LPA. LPA₄ and LPA₅ transcripts are both abundant in human platelets mixed from multiple donors (Amisten *et al.*, 2008). Tokumura *et al.* described human donors whose platelets failed to respond to alkyl glycerophosphate while maintaining normal responsiveness to acyl analogues of LPA (Tokumura *et al.*, 2002b). This observation lends support to the hypothesis that multiple receptors are involved in platelet responses to LPA.

Lysophosphatidic acid also activates haptotactic migration in monocytes/macrophages (Zhou *et al.*, 1995), inhibits the egress of dendritic cells from the vessel wall (Llodra *et al.*, 2004), and promotes the formation of monocyte/platelet aggregates that are

an early marker of acute myocardial infarction (Fueller *et al.*, 2003). Acyl and alkyl LPA accumulate in human atherosclerotic plaques and likely activate platelets and initiate thrombus formation upon plaque rupture (Siess *et al.*, 1999; Rother *et al.*, 2003). LPA production and the LPA receptor subtypes expressed by different subsets of blood cells and cells of the vessel wall are undoubtedly important potential drug targets for manipulating pathological angiogenesis and thrombosis.

LPA as a mediator of organ fibrosis

Lysophosphatidic acid has long been known for its mitogenic effect on fibroblasts (van Corven *et al.*, 1989; 1992). In addition to being a mitogen, LPA increases connective tissue growth factor production, which promotes tissue fibrosis (Hahn *et al.*, 2000; Muehlich *et al.*, 2004; Vial *et al.*, 2008). LPA induces the expression and activation of the CIC-3 chloride channel that is required for myofibroblast dedifferentiation during wound healing (Wang *et al.*, 2002; Yin and Watsky, 2005; Yin *et al.*, 2008). In light of these findings, it is perhaps not surprising that LPA has been implicated in renal (Pradere *et al.*, 2007), hepatic (Watanabe *et al.*, 2007a,b) and pulmonary fibrosis (Tager *et al.*, 2008; Xu *et al.*, 2009). In several models, inhibition of the LPA₁ receptor inhibits the progression of fibrosis (Pradere *et al.*, 2007; Tager *et al.*, 2008), but LPA-induced α V β 6 integrin-mediated TGF- β activity is mediated via the LPA₂ receptor (Xu *et al.*, 2009). Tager *et al.* showed that LPA levels increase in bronchoalveolar lavage fluid following lung injury in the bleomycin model of pulmonary fibrosis and that mice lacking LPA₁ are protected from fibrosis in this model. These investigators showed that patients with idiopathic pulmonary fibrosis, had increased LPA levels in bronchoalveolar lavage fluid and that inhibition of LPA₁ reduced fibroblast responses to the chemotactic activity of the lavage fluid. A recent study using plasma and serum samples from systemic scleroderma patients found elevated LPA and S1P levels. Furthermore, serum LPA : LPC ratios of the 18:2 and 20:4 molecular species, and also the ratio of all species combined, were significantly higher in systemic scleroderma patients versus controls (Tokumura *et al.*, 2009). Currently, a diagnosis of organ fibrosis leaves the physician with very limited therapeutic options that often come with severe side effects. Exploration of the role of LPA- and LPA receptor antagonist-based therapies might offer an entirely new therapeutic avenue of treatment.

LPA in infection and immunity

Activation of the RhoA GTPase and phospholipase D-dependent acidification of phagolysosomes can have profound effects on viral and bacterial entry into cells and also on the intracellular killing of bacteria. The cysteine-rich protein 61 (CYR61) and connective tissue growth factor (CTGF) regulate adhesion, migration, extracellular matrix deposition and cell differentiation and play a role in wound healing. Bacterial lipid extracts derived from *Yersinia*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* or *Staphylococcus aureus* induce expression of CYR61 and CTGF in epithelial cells. Wiedmaier *et al.* have shown that Ki16425, a selective antagonist of LPA_{1/3} receptors (Ohta *et al.*, 2003), inhibits Cyr61 and CTGF expression (Wiedmaier *et al.*, 2008). LPA-induced CYR61 and CTGF mRNA expression requires Rho GTPases because it is abolished by *Clostridium difficile* toxin B, which inhibits RhoA and Rac-1. These authors raised the hypothesis that LPA GPCR could be involved in sensing bacterial lipid products, thereby regulating the host's response to infection via CYR61 and CTGF expression.

Tsurudome *et al.* reported that LPA promotes cell-cell fusion in parainfluenza virus-infected Vero cells (Tsurudome *et al.*, 2008). This process was sensitive to the Rho kinase inhibitor Y-27632. Thus, LPA, at least theoretically, could promote the cytopathic effect of parainfluenza virus infection. This hypothesis awaits experimental testing. Inhibition of RhoA activation elicited by respiratory syncytial virus infection is implicated in the antiviral mechanism of the macrolide antibiotics bafilomycin and clarithromycin (Asada *et al.*, 2009). RhoA activation is associated with entry and exocytosis of viruses, and LPA might synergize with these events and also attenuate the antiviral efficacy of macrolide antibiotics.

Recent reports assign anti-microbial activity to LPA in *Mycobacterium tuberculosis* infection (Garg *et al.*, 2006; Greco *et al.*, 2010). LPA and S1P exert a cytoprotective effect in *Mycobacterium tuberculosis*-infected type II alveolar cells and activate PLD, which in turn leads to phagolysosome maturation, leading to mycobacterial killing and inhibition of bacterial dissemination *in vitro* (Greco *et al.*, 2010). Garg *et al.* have shown that LPA enhances anti-mycobacterial activity *in vitro* and *ex vivo* in cells derived from the bronchoalveolar lavage of patients with tuberculosis. LPA activated PLD-dependent acidification of the phagolysosomes in these cells that had been chronically infected with endogenous mycobacteria in the lungs of the patients. The molecular target(s) of LPA underlying its anti-mycobacterial effect remain to be identified. None-

theless, LPA is known to activate PLD (Qi *et al.*, 1998; Zhao *et al.*, 2005) through a Ca^{2+} -dependent mechanism. Furthermore, ATX is important for normal lysosomal morphology and function (Koike *et al.*, 2009). These exciting studies on the role of LPA in infection are at a very early stage; hence, it is difficult to predict whether LPA or LPA-based pharmacons might have therapeutic value in infection control.

The role of LPA in immune cells cannot be underestimated, and research in this field will likely lead to therapeutically important new discoveries. T lymphocytes (Goetzl *et al.*, 2000a), B cells (Roskopf *et al.*, 1998; Satoh *et al.*, 2007; Perova *et al.*, 2008), eosinophils (Idzko *et al.*, 2004), neutrophils (Chettibi *et al.*, 1994), macrophages (Hornuss *et al.*, 2001), mast cells (Bagga *et al.*, 2004) and dendritic cells (Panther *et al.*, 2002; Llodra *et al.*, 2004; Chan *et al.*, 2007) express functional LPA receptors. Natural killer cells respond to LPA with increased $\text{IFN}\gamma$ production and chemotaxis (Jin *et al.*, 2003; Maghazachi, 2003; Jo *et al.*, 2008), although it was also found to inhibit their cytotoxic response through a cAMP-PKA-dependent mechanism. Lipopolysaccharide treatment of T_H -1 lymphocytes increases ATX expression that can supply LPA upon contact of the T cell with other immune cells (Li and Zhang, 2009). These findings lend support to the role of LPA in innate as well as acquired immunity. In the preceding sections, we have already described some effects of LPA on mast cell and dendritic cell differentiation and the regulation of T cell homing regulated by ATX-LPA GPCR and $\text{PPAR}\gamma$ (Bagga *et al.*, 2004; Llodra *et al.*, 2004; Kanda *et al.*, 2008; Leslie *et al.*, 2008; Nakasaki *et al.*, 2008). LPA has been shown to stimulate proliferation, migration and survival of T cells and macrophages (Koh *et al.*, 1998; Goetzl *et al.*, 1999b; Zheng *et al.*, 2000; 2001). LPA regulates chemokine and cytokine production by immune cells that include IL-1 β , IL-2, IL-8, IL-13, TNF α , MCP-1 and MIP-1 β (Zheng *et al.*, 2000; Lee *et al.*, 2002; Panther *et al.*, 2002; Gobeil *et al.*, 2003; Lin and Boyce, 2005; Rubinfeld *et al.*, 2006). Perhaps the most compelling evidence for the immunomodulatory role of LPA comes from Leslie *et al.* (Leslie *et al.*, 2008), who showed that it differentially regulates the development of group 1 (CD1a, CD1b and CD1c positive) and group 2 (CD1d) dendritic cells. LPA, in a completely reversible $\text{PPAR}\gamma$ -dependent mechanism, inhibited the transcription and cell surface expression of group 1-specific CD1 markers, and conversely increased CD1d, involved in lipid antigen presentation by dendritic cells to T cells. Group 1-reactive T cells display a cytotoxic phenotype and secrete T helper type 1 cytokines, suggesting their role in microbial

infection (Brigl and Brenner, 2004). CD1 molecules are also involved in T cell recognition of self-lipids (Leslie *et al.*, 2002; Vincent *et al.*, 2002; 2003). Thus, CD1-restricted T cells mediate not only host defense mechanisms but also pro-inflammatory responses in human autoimmune diseases, including systemic lupus erythematosus (Sieling *et al.*, 2000), multiple sclerosis (De Libero *et al.*, 2002) and autoimmune thyroiditis (Roura-Mir *et al.*, 2005). Based on these reports, LPA through its effect on dendritic cell maturation might promote the expansion of T helper 2 type cells relative to T helper 1 cells (Panther *et al.*, 2002; Leslie *et al.*, 2008), which has far-reaching implications for the physiological regulation of autoimmunity and host defense.

Conclusion

In recent years, there has been an explosion in the number of reports describing the numerous actions of LPA, making it impossible to write a completely up-to-date and comprehensive review of this rapidly changing field. The objective of the present article was not to be comprehensive but to highlight only certain aspects of the LPA story. The reader is referred to the many excellent and more in-depth reviews on specific aspects of LPA biology. Our aim in this article was to generate additional interest for this exciting and novel field of lipid research.

Lysophosphatidic acid, with its pleiotropic effects and simple structure, provides an enticing opportunity for therapeutic exploration. LPA engages targets both as a mediator and a second messenger in almost every cell type in the body. The many LPA receptors represent an unparalleled redundancy of cellular signalling. The multiplicity of cell surface and intracellular LPA targets/receptors, often co-expressed in the same cell type and coupling to overlapping signal transduction pathways, poses an unmet challenge to designing LPA-based therapeutics.

A fundamental problem often neglected is the endogenous expression of LPA receptor transcripts in every mammalian cell line. Although there are a few cell lines that do not respond to LPA with Ca^{2+} transients (RH7777 and B103) and although heterologous overexpression of $\text{LPA}_{1,2,3,4,5}$ in these cell lines conveys LPA-elicited Ca^{2+} responses, these cells still express low levels of LPA GPCR transcripts and show endogenous signalling responses to LPA (Valentine *et al.*, 2008b). Functional expression of LPAR of the purinergic subcluster often requires plasmids with strong promoters such as pCXN2.1 (Niwa *et al.*, 1991) and/or co-expression of promiscuous G

protein α -subunits (G_{16}) due to low coupling efficiency of some LPAR. However, overexpression of $G_{\alpha_{16}}$ can increase the coupling efficiency of the endogenous LPAR and introduce Ca^{2+} transients into RH7777 and B103 cells even in the absence of heterologous LPAR (Valentine and Tigyi, unpublished). This complicates the characterization of the novel LPAR and emphasizes the need for 'cleaner' assay platforms (Wetter *et al.*, 2009).

The S1P field has surpassed the LPA field in therapeutic exploration because the S1P receptor agonist FTY720 is now near completion of phase III clinical trials for the treatment of multiple sclerosis, whereas no LPA-based therapeutic has advanced this far. What might be the reason for such disparity between the two lysophospholipid fields? Perhaps one key difference lies in the obligatory role of S1P₁ in lymphocyte egress. Thus far, no matching (patho-)physiologically essential cellular response has been linked to a single LPAR, although the role of LPA₃ in embryo implantation offers some degree of similarity. Thus, the greater redundancy and overlapping signalling pathways that prevail among the LPAR families might be the reason that no LPA-based drug has emerged thus far. Perhaps, the redundancy of LPA signalling is an indicator of the fundamental necessity of this mediator/second messenger for normal cell function. Indeed the S1P₁ receptor knockout mouse is the only embryonic lethal lysophospholipid receptor knockout, whereas none of the LPAR knockouts cause embryonic lethality. This contrasts with the ATX knockout, which also causes embryonic lethality and indicates the essential requirement for LPA during development. More thorough characterization of LPAR knockouts under different pathophysiological challenges will likely pinpoint distinct and unique functions associated with a single or a select few LPAR.

The field still lacks drug-like compounds with the desired specificity or ubiquity for LPA targets. Currently, identified lipid-like LPA analogues lack the necessary potency and selectivity, and they have suboptimal pharmacokinetic profiles. With the identification of the novel LPAR, the selectivity and specificity of the previously developed and presumed 'selective' pharmacons begs further characterization. From now on, any new compound will have to be tested on the nine established and putative LPAR and the five S1P receptors. While the opportunity to control LPA-mediated pathophysiology is apparent, harnessing the LPA system through pharmacological tools remains a stimulating task for the academic community and a lucrative opportunity for the pharmaceutical industry.

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Conflicts of interest

The author is a shareholder in RxBio Inc.

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