Histol Histopathol (2005) 20: 603-613

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Bioactive lysophospholipids and mesangial cell intracellular signaling pathways: role in the pathobiology of kidney disease

V.S. Kamanna[1], B.V. Bassa[1], S.H. Ganji[1] and D.D. Roh[2]

[1]Department of Veterans Affairs Healthcare System, Long Beach, California and the University of California, Irvine, and

[2]Nephrology Associates Medical Group, Riverside, California

Summary. Lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), and sphingosine -1-phosphate (S1P) are major biologically active lysophospholipids (LPLs) that are produced by activated platelets, monocyte/macrophages, and many types of mammalian cells. LPLs have been shown to induce a wide array of physiological and pathophysiological properties including cellular differentiation, proliferation, migration, extracellular matrix deposition, change in morphology, and chemotactic responses. The recent cloning and identification of G protein-coupled receptors as specific receptors for LPLs created a great deal of interest in LPLs signaling and diverse biological responses. The pathobiological role of LPLs has been implicated in a number of pathological states and human diseases including atherosclerosis, glomerulosclerosis, post-ischemic renal failure, polycystic kidney disease, and ovarian cancer. Although the research in this area is growing at an enormous rate, this review is specifically focused on the recent understanding of the pathophysiological properties of LPA and LPC with special reference to kidney diseases, and their specific G-protein-coupled receptors and intracellular signaling pathways.

Key words: Lysophosphatidic acid, Lysophosphatidylcholine, Sphingolipids, EDG receptors, G-protein-coupled receptors, MAP kinase, EGF receptor, G2A, GPR4, Signal transduction, Renal disease

Background

Biologically active lysophospholipids (LPLs) are generated by activated platelets, monocyte/macrophages and many types of mammalian cells, and exhibit diverse effects on growth and functions of most cells in multiple organ systems (Reviewed in Spiegel and Milstien, 1995; Moolenaar et al., 1997; Huang et al., 2002). LPLs are derived from glycerol-based or sphingosine-based phospholipids of the plasma membrane. Lysophosphatidic acid (LPA) is the major lysoglycerol-containing phospholipid and predominates quantitatively among bioactive LPLs. Cyclic- and alkenyl-forms of LPA, lysophosphatidylcholine (LPC) and sphingosyl-phosphorylcholine are other important naturally occurring biologically active LPLs. Sphingosine-1-phosphate (S1P) is the major sphingosine-based LPLs of the cell membrane, and elicits a wide range of biological effects similar to those of lysoglycerophospholipids. These LPL molecules were originally known to function as second messengers for the actions of hormones, growth factors, and cytokines. Recently, however, a number of these molecules have been identified to act as ligands to specific G-protein coupled receptors (GPCR) to stimulate signaling pathways associated with the transcription of specific genes and changes in cellular functions (reviewed in Anilker and Chun, 2004; Moolenaar et al., 2004). In the circulation all the three major LPLs, namely, LPA, LPC, and S1P are present bound to serum albumin and/or low-density lipoproteins (LDLs). The functional changes induced by LPLs in various tissues are diverse, cell type specific, and are qualitatively similar to those induced by hormones, growth factors, and cytokines. Similar to the growth factors and cytokines, LPLs play important role in the growth and development of the higher organisms . They are required for the normal functioning of the cells involved in immune defense (reviewed in Lee et al., 2002). However, increased concentrations of some of the LPLs are associated with certain pathological conditions. Experimental evidence implicated LPLs in the development of carcinogenesis, atherosclerosis, and kidney diseases (Sasagawa et al., 1998; Fang et al., 2002; Xu et al., 2003). In the following sections, we will discuss specifically the physiological/pathophysiological properties of LPA and LPC, specific G-protein coupled

Offprint requests to: Vaijinath S. Kamanna, Ph.D., Medical Research Service (151), Department of Veterans Affairs Healthcare System, 5901 East Seventh Street, Long Beach, California 90822. e-mail: vaijinath.kamanna@med.va.gov

receptors and intracellular signaling pathways, and the experimental evidence for the possible involvement of LPLs-mediated events in the development of glomerular diseases in general.

LPA

LPA (1-acyl-2-lyso-sn-glycero-3-phosphate), a ubiquitous simple glycerophospholipid, has been shown to exhibit a wide range of physiological and pathological actions. In addition to serving as a natural precursor for the formation of more complex membrane phospholipids, LPA has recently emerged as one of the important extracellular signaling phospholipid with a variety of biological responses (Moolenaar et al., 1997). A growing body of evidence indicate that LPA, through autocrine and/or paracrine pathways, serves as a potent signaling molecule involved in diverse physiological and pathological processes, such as cell proliferation, differentiation, cell-cell communication, cytoskeletal rearrangement, wound healing, and tumor cell invasion (Moolenaar et al., 1997; Balazs et al., 2001). Acting in an autocrine fashion, LPA promotes growth and migration of the immune cells and stimulates the secretion of interleukins by these cells (Lee et al., 2002). As separately discussed in the following section, LPA has also been shown to play a role in the pathogenesis of renal injury (Inoue et al., 1999). Siess et al. have indicated that LPA mediates the rapid activation of platelets and endothelial cells by mildly-oxidized low-density lipoproteins and accumulates in human atherosclerotic lesions (Siess et al., 1999). Recently, it was shown that LPA molecules present in the core region of atherosclerotic plaques trigger rapid platelet activation through the stimulation of LPA1 and LPA3 receptors (Rother et al., 2003). These studies suggest LPA as an atherothrombogenic molecule and provide a possible strategy for the treatment of atherosclerotic cardiovascular diseases.

Circulating LPA has been shown to be mainly generated by activated platelets, and the normal concentration of LPA range between 2-20 μM (Eichholtz et al., 1993). Additionally, LPA has also been shown to be produced by activated noncirculating cell types, including glomerular mesangial cells, through the actions of group II phospholipase A2 (Wada et al., 1995). The circulating LPA binds with high affinity to serum albumin while retaining its biological activity (Tigyi and Miledi, 1992; Thumser et al., 1994). In the following sections, we will discuss the recent advancement in LPA receptors and intracellular signaling, and emphasize the role of LPA-mediated pathobiological processes involved in renal disease.

LPA receptors and intracellular signaling pathways

The seminal studies reported by Moolenaar group demonstrated that LPA-induced fibroblast cell proliferation is mediated through G protein-coupled receptors (GPCR), and suggested that this signaling pathway may participate in various other LPA responses (van Corven et al., 1989; Moolenaar et al., 1997). In their studies Moolenar et al found that LPA induces calcium signals in intact cells, and LPA stimulation of the cellular functions is kinetically similar to that of growth factors. The recent cloning and identification of endothelial differentiation gene (EDG) family proteins as receptors for bioactive phospholipids further extended a great deal of interest in LPA signaling and diverse biological responses (reviewed in Fukushima et al., 2001). EDG family receptor was originally described as an orphan GPCR encoded by an immediate early response gene product cloned from human umbilical vein endothelial cells (Hla and Maciag, 1990). Currently, eight related receptors within the EDG family cluster have been cloned and identified to serve as mammalian cell receptors for bioactive lipids LPA and S1P (Fukushima et al., 2001). EDG-2 (also referred in current nomenclature as LPA1, Lynch, 2002), EDG-4 (LPA2), and EDG-7 (LPA3) have been shown to be receptors for LPA (An et al., 1998; Bandoh et al., 1999; Fukushima et al., 2001). Other five members of EDG family genes, EDG-1, EDG-3, EDG-5, EDG-6, and EDG-8 are identified as receptors for the structurally related phospholipid S1P (Fukushima et al., 2001). The LPA receptors (EDG-2, -4, -7) share approximately 55% identical amino acid sequence, and the two sub-clusters of EDG family LPA and S1P receptors share about 35% homology.

The LPA receptors are shown to differ considerably with respect to tissue distribution and G-protein coupling responses. EDG-2 receptor has been shown to be widely expressed with highest mRNA levels in brain (An et al., 1998), whereas EDG-4 was shown to be highly expressed in testis and leukocytes (An et al., 1998). The expression of EDG-7 is maximal in kidney and prostate (Bandoh et al., 1999). Using overexpression and/or heterologous expression of LPA receptors in mammalian cells, it was shown that all three LPA receptors (EDG-2, -4, -7) can mediate the inhibition of adenylyl cyclase and reduction in cAMP concentrations, and increase levels of intracellular calcium and inositol phosphate and activate MAP kinase (reviewed in Ishi et al., 2000; Fukushima et al., 2001). EDG-2 and EDG-4 are also shown to induce cell rounding via activation of the small GTPase, Rho. Furthermore, pharmacological studies suggested that both EDG-2 and EDG-4 couple to at least three types of G proteins, $G_{i/o}$, $G_{12/13}$, and G_Q, whereas EDG-7 couples with $G_{i/o}$ and G_q (reviewed in Ishi et al., 2000). However, there are some studies showing differential response with respect to specific G-protein coupling in different cell types, suggesting that the LPA receptor coupling may be dependent on the system of expression (Im et al., 2000; Fukushima et al., 2001).

Although the cellular overexpression studies are helpful in understanding generalized LPA receptor signaling, the relative contribution of specific endogenous LPA receptors in the multiple biological

activities of LPA remains poorly defined. Using stable transfection of antisense oligonucleotides, recently it was shown that EDG-2 regulates LPA-induced preadipocyte proliferation and spreading (Pages et al., 2001). These studies also indicated that the differentiation of growing preadipocytes into quiescent adipocytes led to a strong reduction in the level of EDG-2 transcripts, suggesting the differential association of EDG-2 in growing versus quiescent adipocytes. Using stereoselective agonists of the EDG receptors, Hooks et al. showed that the mitogenic activity in rat hepatoma cell line RH7777 and HEK 293 cells and platelet aggregation responses to LPA are independent of EDG-2, -4, and -7 (Hooks et al., 2001). In this study, the ectophosphatase lipid phosphate phosphatase 1 (LPP1) has been shown to down-regulate LPA-mediated mitogenesis. Additional work in this area showed that LPP1 can regulate LPA association with cells without significantly depleting bulk of LPA concentration in the extracellular medium, suggesting a novel mechanism of LPP1 for controlling EDG-2 receptor activation (Xu et al., 2000). These limited numbers of available studies suggest variable role of EDG receptors in different cell types. It is not clearly understood whether such variabilities in the involvement of specific EDG receptors for certain LPA responses are due to cell type specific or the abundance of specific EDG receptors for LPA responses in various cell types. Particularly, with reference to cell growth, the abundance of specific EDG receptors in growing and quiescent state may differ considerably for LPA-mediated cell growth responses. Addressing the above issues related to EDG receptor(s) specificity for diverse LPA responses in various cell types would be of considerable importance in understanding the role of these EDG receptors in the physiologic and pathophysiologic responses elicited by LPA.

In view of the important emerging role of EGF receptor transactivation as a mitogenic signaling mediator for various mitogenic agents (Daub et al., 1996; Hackel et al., 1999), several studies investigated the participation of EGF receptor activation in LPA signaling. A growing body of evidence indicated that LPA stimulated the transactivation of EGF receptor in various cell types including mesangial cells (Daub et al., 1996; Luttrell et al., 1997; Grewal et al., 2001; Wu et al., 2002). Using HeLa cells, NIH 3T3 cells, and B82L cells, Cunnick et al suggested that the EGF receptor tyrosine kinase contributes to the LPA-stimulated MAP kinase activation, c-fos transcription, and mitogenesis (Cunnick et al., 1998). Additionally, it was shown that LPA not only induces ligand-independent tyrosine autophosphorylation of EGF receptor but also of platelet-derived growth factor beta receptor (PDGFR, Herrlich et al., 1998). These studies also suggested that the cross-talk appears to be cell-type specific: in L cells that lack EGF receptor, LPA-induced MAP kinase activation is prevented completely by specific inhibition of PDGFR, whereas in COS-7 cells expressing only EGF receptor, the pathway via EGF receptor is chosen. In Rat-1 cells, however, that express both EGF receptor and PDGFR, the EGF receptor pathway dominates (Herrlich et al., 1998). Recently, it was shown that LPA-induced squamous cell carcinoma cell proliferation and motility involves EGF receptor transactivation, suggesting that LPA may function as tumor promoter (Gschwind et al., 2002). Phosphoinositide 3-kinase activation by LPA was also dependent on EGF receptor signaling pathway (Laffargue et al., 1999). Rho, and Rac are small GTPases that are particularly implicated in the agonist-induced changes in cellular morphology and motility. Rho GTPases are involved in the LPA stimulated invasive behavior of the cancer cells (Moolenaar et al., 2004). LPA is also a well-recognized stimulant of cellular contractions in various types of cells. The involvement of Rho activation, increased cellular calcium flux, and activation of the myosin light chain kinase are clearly established in the LPA mediated cytoskeletal changes, and cellular motility (Moolenaar et al., 2004; Yokomori et al., 2004).

Role of LPA in mesangial cell proliferation and glomerular disease

Plasma concentrations of LPA are shown to be approximately 3-fold higher in patients with renal failure on hemodialysis as compared to healthy control subjects (Sasagawa et al., 1998). Platelet aggregation has been commonly observed in the glomerular capillaries in many renal diseases (Ono et al., 1991), and the LPA released by activated platelets can enter the glomerulus to impact various responses. Extracellular LPA can also be generated through secretory phospholipase A2 through microvesicular shedding from blood cells challenged with inflammatory stimuli (Fourcade et al., 1995), suggesting the generation of LPA at the site of injury and inflammation. Indeed, such extracellular generation of LPA was observed in mesangial cells through group II PLA2 under inflammatory conditions (Pfeilschifter et al., 1993; Wada et al., 1997). These observations suggest that the local concentrations of LPA within the mesangial area may be even higher in pathological conditions associated with increased glomerular phospholipase activity.

LPA has shown to stimulate mesangial cell proliferation and contraction (Inoue et al., 1995, 1997), and implicated to play a role in the pathogenesis of renal injury (Inoue et al., 1999). Additionally, it was shown that LPA synergistically increased PDGF-mediated mesangial cell proliferation (Inoue et al., 1997). In this study, LPA (10-30 mM) alone exhibited weaker proliferative activity (about 1.4-fold) in rat mesangial cells. These studies also demonstrated that LPA activated MAP kinase, and preincubation with pertussis toxin inhibited both cell proliferation and MAP kinase activation by LPA in mesangial cells, suggesting the participation of G-protein mediated events in mesangial cell LPA signaling. Further studies indicated that LPA

serves as a survival factor for mesangial cells suppressing PDGF-induced mesangial cell death (reviewed in Inoue, 2002). Thus according to the prevailing model, LPA acts as co-mitogen with PDGF in promoting mesangial cell proliferation and progression of glomerular diseases. Interestingly by the same token of co-mitogenesis, LPA is protective against renal ischemic reperfusion injury (I/R) (de Vries et al., 2003). In our recent studies with human primary mesangial cells, we have shown that LPA increased mesangial cell proliferation by 3-3.5-fold in dense cell population (50-75% confluency) and about 1.5-fold in sparse cell density (25% confluency) as compared to the respective cell density controls (Xing et al., 2004). As noted in our study, the differences in the degree of mesangial cell proliferation by LPA between our studies in human mesangial cells and other studies in rat mesangial cells may be due to variations in cell density related processes and/or cellular species origin.

Since EDG family receptors mediate LPA signaling, we examined the expression profile of EDG family LPA receptors and the contribution of EDG receptors in LPA-mediated human mesangial cell proliferation. We have shown that human glomerular mesangial cells express all three EDG family LPA receptors in a cell-density-dependent manner. Based on semi-quantitative RT-PCR, we noted that EDG-7 was the most abundantly expressed LPA receptor, and EDG-2 was moderately abundant in mesangial cells. EDG-4 was weakly expressed in mesangial cells (Xing et al., 2004). Interestingly, expression of EDG family LPA receptors followed cell density-dependent pattern. EDG-7 maximally expressed at sparse cell density and minimally expressed in dense cell population. The EDG-2 expression pattern was opposite to the EDG-7, and no changes in EDG-4 expression as a function of cell density were noted. Additionally, we have shown that cell proliferative rate (DNA synthesis) in mesangial cell was greater in sparse cell density as compared to dense cell population, and followed a similar pattern with EDG-7 expression (Xing et al., 2004). Comparative studies in sparse and dense cell density indicated that EDG-7 was positively associated, while EDG-2 was negatively associated with mesangial cell proliferation rate (Xing et al., 2004). Furthermore, we have shown that dioctanoylglycerol pyrophosphate, an antagonist for EDG-7, almost completely inhibited mesangial cell proliferation induced by LPA. We suggest that EDG-7 regulates LPA-mediated mesangial cell proliferation. Additionally, these data suggest that EDG-7 and EDG-2 LPA receptors play diverse role as proliferative and anti-proliferative respectively in mesangial cells (Xing et al., 2004). Thus, regulation of EDG family receptors may be importantly linked to mesangial cell proliferative processes and associated glomerular proliferative diseases.

LPC

LPC is an another major biologically active LPL involved in diverse biological functions, including recruitment of monocytes, induction of adhesion molecules in endothelial cells, stimulation of smooth muscle cell proliferation, induction of growth factors, induction of smooth muscle cell migration, and inhibition of endothelium-dependent relaxation that are key pathobiological processes associated with vascular diseases (Quinn et al., 1988; Kugiyama et al., 1990; Kume et al., 1992; Kume and Gimbrone, 1994; Nakano et al., 1994; Chai et al., 1996; Kohno et al., 1998; Watanabe et al., 2001). The in-vivo generation of LPC involved in pathobiological cellular events is not completely understood; however, the hydrolysis of sn-2 fatty acid of phosphatidylcholine (PC) by plasma lecithin-cholesterol acyltransferase can produce LPC. Furthermore, LPC can also be formed during oxidative modification of low-density lipoproteins (LDL) by a LDL-associated phospholipase A2 activity (Parthasarathy and Barnett, 1990). After its formation, LPC, a relatively polar molecule, gets associated with serum albumin or LDL and is carried to various cellular sites to participate in cellular, metabolic, and pathophysiological pathways. In view of the accumulated data suggesting the pathobiologic role of oxidized LDL (ox-LDL) in atherogenesis, LPC has been considered as one of the major bioactive molecules implicated in ox-LDL-mediated cellular processes involved in the development of vascular diseases (Quinn et al., 1988; Chai et al., 1996; Watanabe et al., 2001). Impairment of endothelium-dependent relaxation in response to ox-LDL is directly attributable to the presence of LPC in these fractions (Leung et al., 1999). Increased concentrations of LPC both in serum and LDL fractions have been found in atherosclerosis and nephrosis (reviewed in Tselepis and Chapman 2002). Higher circulatory concentrations of LPC have also been found in ischemia both in animals and humans (Watanabe and Okada, 2003).

LPC receptors and intracellular signaling pathways

Platelet activating factor (PAF) receptor has been suggested to mediate the pro-inflammatory actions of LPC (Huang et al., 1999). Recent studies have described two G-protein-coupled receptors G2A and GPR4 as specific receptors for LPC (Kabarowski et al., 2001; Zhu et al., 2001). G2A (named for cell cycle G_2 accumulation) was originally discovered as a transcriptionally regulated orphan GPCR in a search for downstream targets of the BCR-ABL tyrosine kinase oncogene (Weng et al., 1998). G2A-deficient mice developed a late-onset (>1.5 year), slow-progressing autoimmune syndrome characterized by abnormal expansion of both T and B lymphocytes (Le et al., 2001). Although transcriptional induction of G2A is associated with proliferative responses, its ectopic expression inhibits transformation of B cell precursors by BCR-ABL (Weng et al., 1998). In fibroblasts, G2A overexpression leads to accumulation of cells at G2 and M phase of cell cycle and partial block in the

progression of mitosis (Weng et al., 1998). Introduction of G2A in fibroblasts by retroviral transduction or microinjection induced significant morphological alterations, including suppression of contact inhibition, foci formation, and assembly of actin stress fibers (Kabarowski et al., 2000). Intracellular signaling studies indicated that G2A-induced cytoskeletal changes require coupling to Gα13 and subsequent activation of RhoA (Kabarowski et al., 2000). Additionally, this signaling was also supported by the finding that coexpression of LscRGS, a GTPase-activating protein that suppresses signaling by Gα13, inhibited G2A-induced morphological changes (Zohn et al., 2000). In addition to Gα13, G2A has also been linked Gai (in MCF10A and Chinese hamster ovary cells, (Kabarowski et al., 2001), Gaq, and Gas (in HeLa cells, Lin P et al., 2003). It appears that coupling to G2A might be cell context-dependent.

Kabarowski et al. (2001) described G2A as a receptor with high affinity for LPC, but low affinity for sphingosylphosphorylcholine (SPC). In human breast epithelial MCF10A cells expressing G2A, LPC (0.1 μM) increased intracellular calcium concentration (Kabarowski et al., 2001). Additionally, activation of G2A by LPC induced receptor internalization, activated MAP kinase, and stimulated migratory responses of Jurkat T lymphocytes (Kabarowski et al., 2001). Recently, Radu et al. have showed that the overexpression of G2A in the T lymphoid cell line significantly enhanced chemotaxis to LPC (Radu et al., 2004). These authors have also indicated that the chronic down-regulation of G2A resulted in impaired T lymphoid cell chemotaxis by LPC. Recent studies showed that G2A was expressed predominantly by macrophages within atherosclerotic lesions at the aortic root of apolipoprotein E-deficient mice and the thoracic aortas of Watanabe heritable hyperlipidemic rabbits (Rikitake et al., 2002). In atherosclerotic plaques of human coronary arterial specimens, G2A was expressed by macrophages within the lipid-rich plaques, whereas no G2A was seen in fibrous plaques where macrophages did not present (Rikitake et al., 2002). These findings suggest that LPC-G2A interaction may be linked to inflammatory immune diseases and vascular diseases, such as atherosclerosis and potentially glomerular diseases.

Additionally, G protein-coupled receptor GPR4 has also been shown to act as low affinity receptor for LPC, and high affinity receptor for SPC (Zhu et al., 2001). In GPR4-transfected MCF10A cells, both SPC and LPC increased intracellular calcium concentration. Both SPC and LPC activate GPR4-dependent activation of serum response element reporter and receptor internalization. Swiss 3T3 cells expressing GPR4 respond to both SPC and LPC, but not sphingsine 1-phosphate (S1P) or PAF to activate MAP kinase. In GPR4 expressing Swiss 3T3 cells, both SPC and LPC induce DNA synthesis, and the MAP kinase and DNA synthesis induced by LPC and SPC are inhibited by pertussis toxin, suggesting the involvement of a Gi-heterotrimeric G-protein.

Additionally, this study indicated that the participation of GPR4 in Swiss 3T3 cell migration by SPC and LPC (Zhu et al., 2001). Unlike G2A which is predominantly expressed in hematopoietic tissues rich in lymphocytes and weaker expression in heart and lung (Weng et al., 1998), GPR4 has been shown to be highly expressed in many human tissues, including ovary, liver, lung, kidney, lymph node, and subthalamic nucleus; and the tissues with lower GPR4 expression include, aorta, placenta, bone marrow, skeletal muscle, small intestine, prostate, and skeletal muscle (Zhu et al., 2001). Since LPC is involved in diverse pathobiological processes associated with atherosclerosis and glomerular diseases, further studies on the involvement of these two LPC receptors would be important in understanding their role in vascular diseases.

The activation of protein kinase C (PKC) has been the primary mechanism implicated in several actions of LPC (Kugiyama et al., 1992; Ohgushi et al., 1993; Sugiyama et al., 1994). However, the molecular basis for the activation of PKC by LPC is elusive. In glomerular mesangial cells, we have investigated the upstream signaling mechanisms of LPC-mediated PKC activation, and the involvement of PKC and/or protein tyrosine kinase (PTK) and Ras-mediated pathways in downstream MAP kinase activation by LPC (Bassa et al., 1999). In our studies with murine glomerular mesangial cells, we found that LPC and oxidized LDL transiently activated MAP kinase by three fold (Bassa et al., 1998, 1999). This activation was partially dependent on PKC, and both PKC depletion with phorbol esters and PKC inhibition by pharmacological inhibitors suppressed the activation of MAP kinase. In addition PTK inhibitors also strongly inhibited the activation of MAP kinase by LPC. In further exploring the pathway upstream of PKC, we were able to implicate phospholipase Cγ-1 (PLCγ-1) as a modulator of PKC activity. Our studies indicate that LPC-mediated PKC activation may be regulated by PTK-dependent activation of PLCγ-1, and both PKC and PTK-Ras pathways are involved in LPC-mediated downstream MAP kinase activation (Bassa et al., 1999).

LPC has later been shown to activate MAP kinase in various cell types, including endothelial cells, smooth muscle cells, and monocyte/macrophage cell line (Ozaki et al., 1999; Jing et al., 2000; Yamakawa et al., 2002). LPC-induced activation of MAP kinase was shown to be mediated by reactive oxygen species in smooth muscle cells, and the MAP kinase activation appears to involve PKC- and Ras-dependent raf-1 activation (Yamakawa et al., 2002). In monocytic THP-1 cells, LPC activated efficiently both p38- and p42/44-MAP kinase, but only the activation of p38-MAP kinase was functionally associated with LPC-induced chemotaxis (Jing et al., 2000). The LPC-induced activation of MAP kinase and c-Jun N-terminal kinase in endothelial cells was mediated by tyrosine kinase- but not PKC-dependent mechanisms (Ozaki et al., 1999). These observations in

endothelial cells differ with our studies in mesangial cells in that the MAP kinase activation by LPC was dependent on both protein tyrosine kinase and PKC-dependent pathways, suggesting cell type differences with respect to LPC signaling (Bassa et al., 1999). Additionally, it was shown that LPC-mediated activation of p38-MAP kinase was required for LPC-induced apoptosis in endothelial cells (Takahashi et al., 2002).

Role of LPC in pathobiological processes associated with kidney disease

Although LPC has been implicated in multiple pathobiological processes, the circulating and/or tissue concentrations of LPC in patients with renal disease is not clearly established. Renal patients on hemodialysis had significantly higher molar ratio of the plasma LPC - LPC/phosphatidylcholine (Sasagawa et al., 1998). The concentration of LPC in LDL was significantly greater in hypoalbuminemic nephritic patients than control subjects, suggesting that the hypoalbuminemia in combination with proteinuria causes a shift of LPC from albumin to LDL particles with greater pathobiologic properties (Vuong et al., 1999). In addition to atherosclerosis, others and we have proposed that increased levels oxidized-LDL (ox-LDL) and its components (e.g., mainly LPC) play a critical role in glomerular injury and kidney disease (Diamond et al., 1991; Kamanna et al., 1997). Since LPC has been suggested to contribute significantly ox-LDL-mediated pathophysiological processes, the increased concentrations of ox-LDL may directly implicate the pathobiological relevance of LPC in vascular pathological conditions. Several clinical studies have shown significantly increased plasma concentrations of ox-LDL in patients with renal disease (Maggi et al., 1994; Holvoet et al., 1996; van Tits et al., 2003). Multiple regression analysis of data revealed that the extent of renal disease is associated with changes in ox-LDL levels (Holvoet et al., 1996). In addition to increased circulating ox-LDL levels, increased glomerular deposition of ox-LDL (mainly in the lesions of glomerulosclerosis and mesangial areas) in diverse human renal diseases was shown to be associated with higher frequency of renal insufficiency and a greater degree of glomerulosclerosis (Lee and Kim, 1998).

Using in-vitro studies, others and we have provided direct evidence for the role of ox-LDL and LPC in pathobiological processes of glomerulosclerosis. We have shown that ox-LDL activated glomerular endothelial and mesangial cells to produce monocyte adhesion molecules and chemoattractant peptides associated with monocyte infiltration and proliferation within the mesangium (Kamanna et al., 1996, 1999a). LDL and ox-LDL with higher potency were also shown to induce the expression of extracellular matrix proteins in mesangial cells and tubular epithelial cells (Roh et al., 1998,b). Thus, LPC and its increased generation and association with ox-LDL particles may be considered as risk factors associated with the pathobiological processes of glomerular injury and subsequent glomerulosclerosis. Several other experiments with in vitro cultures of glomerular mesangial cells indirectly implicate LPC in the pathobiology of the glomeruli. For example LPC was shown to induce monocyte chemoattractant protein-1 (MCP-1) in glomerular endothelial cells (Mizobata, 2003). Mesangial cell migration is known to be a contributory factor to the pathology of glomerular diseases. It is hypothesized that activation of the mesangial cells by circulatory and glomerular ox-LDL and its major component LPC in turn would stimulate the mesangial cells to secrete growth factors and cytokines involved in monocyte infiltration and mesangial cell hypercellularity (Kamanna et al., 1997, 1998).

LPC also induces the expression of PDGF B chain in human mesangial cells and the mitogenic effects of LPC on mesangial cells may be in part attributed to the induction of PDGF BB (Tone et al., 1999). Using specific pharmacological inhibitors, our recent studies addressed the participation of epidermal growth factor receptor (EGF receptor), Src, PKC, and MAP kinase in human mesangial cell proliferation by LPC. The findings from these studies indicate that the incubation of human mesangial cells with LPC (5-25 mM) for 24-48 hours stimulated mesangial cell proliferation by 4-5 fold (Kamanna et al., unpublished data). Preincubation of mesangial cells with PKC inhibitor (bisindolylmaleimide GF109203-X) or Src inhibitor (PP-2) or EGF receptor kinase inhibitor (AG1478) significantly inhibited LPC-mediated mesangial cell proliferation. Blockage of total tyrosine kinase by genistein or blockage of MAP kinase by MEK inhibitor (PD98059) completely inhibited mesangial cell proliferation by LPC (Kamanna et al., unpublished data). These studies suggest that LPC, by activating EGF receptor, Src, and/or PKC signaling pathways, stimulates down-stream MAP kinase signaling resulting in mesangial cell proliferation.

Sphingomyelin based LPLs

Although our review focused on LPA and LPC, sphingomyelin based LPLs including sphingosine-1-phosphate (S1P), sphingosine, and ceramide also serve as biologically active phospholipids in many cell types. The biological effects of sphingomyelin products are as varied as those of growth factors, LPA, and LPC described earlier. The diverse biological properties of sphingomyelin based LPLs, identification of EDG-family proteins as specific receptors for S1P and intracellular signaling pathways have been extensively reviewed (Fukushima et al., 2001; Kluk and Hla, 2002; Spiegel and Milstein 2003).

In reference to renal pathophysiology, sphingolipids have been shown to participate in mesangial cell growth, ischemic acute renal injury, and renal tubular injury. Recently, it was shown that S1P stimulated mesangial cell proliferation (Hanafusa et al., 2002). Further studies

on transcriptional profiling of genes in mesangial cells indicated that S1P induced gene expression patterns similar to those induced by platelet-derived growth factor (Katsuma et al., 2003). Gennero et al. have shown that S1P stimulated proliferation of mesangial cells and activation of MAP kinase at subconfluent cell density (Gennero et al., 2002). These studies also indicated that S1P induced mesangial cell apoptosis at low cell density, and this apoptotic effect was attributed to increased S1P hydrolysis. Iwata and colleagues have investigated whether sphingosine and ceramide, second messengers derived from sphingolipid breakdown, modulate kidney proximal tubular cell viability and their adaptive responses to further injury (Iwata et al., 1995). This study indicated that sphingosine (>10 mM), S1P, and ceramide induced rapid cytotoxicity in proximal tubular cells. This effect occurred in the absence of DNA laddering and apoptosis, suggesting a necrosis form of cell death by these sphingolipids. However, prolonged exposure (20 hr) to subtoxic sphingosine doses (<7.5 mM) induced substantial cytoresistance to superimposed ATP depletion/Ca^{2+} ionophore-mediated tubular cell damage (Iwata et al., 1995). In further studies Zager and associates have showed altered ceramide and sphingosine expression in the setting of ischemic and nephrotoxic acute renal failure (Zager et al., 1997, 1998). It was also shown increased concentrations of glucosylceramide and lactosylceramide in polycystic (cpk/cpk) mouse, suggesting that sphingolipids may play a potential role in the proliferative and transport abnormalities associated with cystic renal disease (Deshmukh et al., 1994).

Conclusions and the proposed model for the involvement of LPA- and LPC-mediated signaling pathways in mesangial cell proliferation and glomerular disease

Based on the in-vitro and in-vivo data discussed above, we have designed a model to understand the participation of LPA and LPC in intracellular signaling pathways associated with mesangial cell proliferation and glomerular disease (Fig. 1). As shown in Figure 1, increased plasma concentrations of LPA and LPC generated either by activated platelets and macrophages or by oxidative modification of LDL may interact with respective receptors on the mesangial cell membrane. We have recently described that mesangial cells express all three LPA receptors (EGD-2, EDG-4, and EDG-7), and blockage of EDG-7 inhibited LPA-induced mesangial cell proliferation (Xing et al., 2004). We propose that LPA through interacting with EDG receptors (specifically EDG-7) may induce the observed transactivation of tyrosine kinase growth factor receptors, including EGF receptor and PDGF receptors (Fig. 1). This would trigger the activation of various cytoplasmic signaling molecules leading to the activation of Ras-MAP kinase pathway. Activated cytoplasmic MAP kinase translocates into the nucleus and activate various transcription factors and protooncogenes associated with cell growth and proliferation.

Increased concentrations of LPC associated with oxidized LDL within the glomerulus may interact with specific G2A or GPR4 LPC receptors on the mesangial

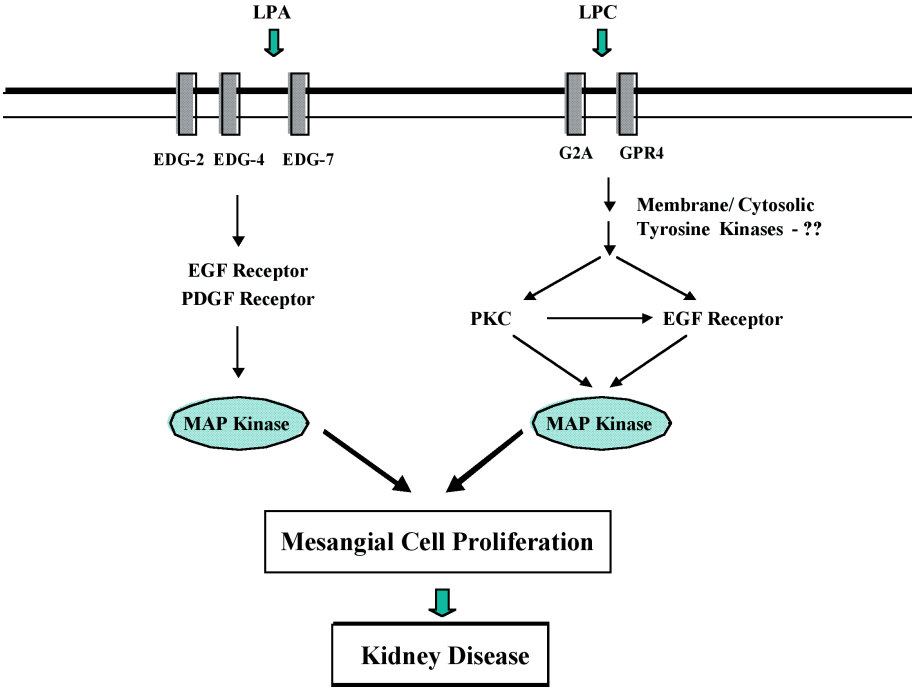

Fig. 1. Proposed model for the involvement of LPA- and LPC-mediated signaling pathways in mesangial cell proliferation and kidney disease. LPA and LPC interact with specific GPCR receptors on the mesangial cell membrane leading to the activation of membrane/cytosolic tyrosine kinases and/or PKC. These signaling events lead to the activation of down-stream MAP kinase involved in mesangial cell proliferation. Increased mesangial cell proliferation by LPA and LPC subsequently stimulate extracellular matrix deposition leading to glomerular injury and kidney disease.

cell membrane. Since G2A is mainly expressed in hematopoietic tissues rich in lymphocytes and its overexpression is associated with blockage of mitosis, it is unlikely that G2A mediates LPA-induced mesangial cell proliferation. Alternatively, highly expressed GPR4 in kidney may be involved in mesangial cell proliferation by LPC. The interaction of LPC with receptors (such as GPR4) on the mesangial cell membrane, through yet unidentified tyrosine kinase-mediated events, may activate PLCγ-1 and in turn PKC (Fig. 1). Alternatively, LPC by PKC-dependent and/or PKC-independent pathways may stimulate the transactivation of EGF receptor (Kamanna et al., 1999b). In concert, these signaling by PKC and/or PKC-EGF receptor-mediated events would activate MAP kinase and mesangial cell proliferation. In addition to LPA and LPC, sphingolipid-based LPLs may also influence and cross-talk with the above signaling processes involved in mesangial cell proliferation. Mesangial cell proliferation induced by LPLs may in turn influence mesangial cell extracellular matrix production and deposition leading to glomerular injury and renal disease. As discussed in our previous review in this Journal (Kamanna et al., 1998), LPLs-mediated activation of glomerular cells and the generation of adhesion molecules and chemokines will be involved in monocyte infiltration into the mesangium and transformation into lipid-rich macrophages, characteristic features involved in the development of diverse forms of renal diseases.

Thus, understanding the pathophysiological properties of LPLs in glomerular cells, the expression patterns of LPL receptors and signaling pathways, and the involvement of specific LPL receptors in biological responses in glomerular cells would be important in discerning their role in the pathogenesis of renal disease. In view of the increased relevance of lipids, inflammation, and oxidative modification of LDL in renal disease, future research on LPLs and their receptors in glomerular cells and in in-vivo renal disease experimental models may play important role in defining certain pathobiological mechanisms associated with renal disease.

Acknowledgements. This work was supported in part by Merit Review grants from the Department of Veterans Affairs, and by the Southern California Institute for Research and Education, Department of Veterans Affairs Healthcare System, Long Beach, California.

References

An S., Bleu T., Hallmark O.G. and Goetzl E.J. (1998). Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. J. Biol. Chem .273, 7906-7910.

Anilker B. and Chun J. (2004). Lysophospholipid G protein-coupled receptors. J. Biol. Chem. 279, 20555-20558.

Balazs L., Okolicany J., Ferrebee M., Tolley B. and Tigyi G. (2001). Topical application of the phospholipid growth factor lysophosphatidic acid promotes wound healing in vivo. Am. J. Physiol. 280, R466-72.

Bandoh K., Aoki J., Hsono H., kobayashi T., Murakami K., Tsujimoto M., Aria H. and Inoue K. (1999). Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. J. Biol. Chem. 274, 27776-27785.

Bassa B.V., Roh D.D., Kirschenbaum M.A. and Kamanna V.S. (1998). Atherogenic lipoproteins stimulate mesangial cell p42 mitogen-activated protein kinase. J. Am. Soc. Nephrol. 9, 488-496.

Bassa B.V., Roh D.D., Vaziri N.D., Kirschenbaum M.A. and Kamanna V.S. (1999). Lysophosphatidylcholine activates mesangial cell PKC and MAP kinase by PLC-1 and Tyrosine kinase-Ras pathways. Am. J. Physiol. 277, F328-F337.

Chai Y.C., Howe P.H., DiCorleto P.E. and Chisolm G.M. (1996). Oxidized low-density lipoprotein and lysophosphatidylcholine stimulate cell cycle entry in vascular smooth muscle cells: evidence for release of FGF-2. J. Biol. Chem. 271, 17791-17797.

Cunnick J.M., Dorsey J.F., Standley T., Turkson J., Kraker A.J., Fry D.W., Jove R. and Wu J. (1998). Role of tyrosine kinase activity of epidermal growth factor receptor in the lysophosphatidic acid-stimulated mitogen-activated protein kinase pathway. J. Biol. Chem. 272, 14468-75.

Daub H., Weiss F.U., Wallasch C. and Ullrich A. (1996) Role of transactivation of the EGF receptor in signaling by G-protien-coupled receptors. Nature 379, 557-560.

Deshmukh G.D., Radin N.S., Gattone V.H. and Shayman J.A. (1994). Abnormalities of glycosphingolipid, sulfatide, and ceramide in the polycystic (cpk/cpk) mouse. J. Lipid Res. 35, 1611-1618.

de Vries B., Matthijsen R.A., van Bijnen A.A., Wolfs T.G. and Buurman W.A. (2003). Lysophosphatidic acid prevents renal ischemia-reperfusion injury b by inhibition of apoptosis and complement activation. Am. J. Pathol. 163, 47-56.

Diamond J.R. (1991). Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis. Kidney Int. 39, S29-S34.

Eichholtz T., Jalink K., Fahrenfort I. and Moolenaar W.H. (1993). The bioactive phospholipid lysophosphatidic acid is released from activated platelets. Biochem. J. 291, 677-680.

Fang X., Schummer M., Mao M., Yu S., Tabassam F.H., Swaby R., Hasegawa Y., Tanyi J.L., LaPushin R., Eder A., Jaffe R., Erickson J. and Mills G.B. (2002). Lysophoaphaatidic acid is a bioactive mediator in ovarian cancer. Biochim. Biophys. Acta 1582, 257-264.

Fourcade O., Simon M.F., Viode C., Rugani N., Leballe F., Ragab A., Fournie B., Sarda L. and Chap H. (1995). Secretory phospholipase A2 generates the novel lipid mediator lysophosphatidic acid in membrane microvesicles shed from activated cells. Cell 80, 919-927.

Fukushima N., Ishii I., Contos J.A. Weiner J.A. and Chun J. (2001) Lysophospholipid receptors. Annu. Rev. Pharmacol. Toxicol. 41, 507-534.

Gennero I., Fauvel J., Nieto M., Cariven C., Gaits F., Briand-Mesange F., Chap H. and Salles J.P. (2002). Apoptotic effect of sphingosine 1-phosphate and increased sphingosine 1-phosphate hydrolisis on mesangial cells cultured at low density. J. Biol. Chem. 277, 12724-12734.

Grewal J.S., Luttrell L.M. and Raymond J.R. (2001). G protein-coupled receptors desensitize and down-regulate epidermal growth factor receptors in renal mesangial cells. J. Biol. Chem. 276, 27335-27344.

Gschwind A., Prenzel N. and Ullrich A. (2002). Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation.

Cancer Res. 62, 6329-6336.

Hackel P.O., Zwick E., Prenzel N. and Ullrich A. (1999). Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Curr. Opin. Cell Biol. 11, 184-189.

Hanafusa N., Yatomi Y., Yamada K., Hori Y., Nangaku M., Oduda T., Fujita T., Kurokawa K. and Fukagawa M. (2002). Sphingosine 1-phosphate stimulates rat mesangial cell proliferation from outside the cells. Nephrol. Dial. Transplant. 17, 580-586.

Herrlich A., Daub H., Knebel A., Herrlich P., Ullrich A., Schultz G. and Gudermann T. (1998). Ligand-independent activation of platelet-derived growth factor receptor is a necessary intermediate in lysophosphatidic, acid-stimulated mitogenic activity in L cells. Proc. Natl. Acad. Sci. USA 95, 8985-8990.

Hla T. and Maciag T.J. (1990). An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to g protein coupled receptors. J. Biol. Chem. 265, 9308-9313.

Holvoet P., Donck J., Landeloos M., Brovwers E., Luijtens K., Arnout J., Lesaffre E., Vanrenterghem Y. and Collen D. (1996). Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. Thromb. Haemost. 76, 663-669.

Hooks S.B., Santos W.L., Im D.S., Heise C.E., Macdonald T.L. and Lynch K.R. (2001). Lysophosphatidic acid-induced mitogenesis is regulated by lipid phosphate phosphatases and is EDG-receptor independent. J. Biol. Chem. 276, 4611-4621.

Huang M.C., Graeler M., Shankar G., Spencer J. and Goetzl E.J. (2002). Lysophospholipid mediators of immunity and neoplasia. Biochim. Biophys. Acta 1582, 161-167.

Huang Y.H., Schafer-Elinder L., Wu R., Claesson H.E. and Frostegard J. (1999). Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. Clin. Exp. Immunol. 116, 326-331.

Iwata M., Herrington J. and Zager R.A. (1995). Sphingosine: a mediator of acute renal tubuloar inury and subsequent cytoresistance. Proc. Natl. Acad. Sci. USA 92, 8970-8974.

Im D.S., Heise C.E., Harding M.A., George S.R., O'Dowd B.F., Theodorescu D. and lynch K.R. (2000). Molecular characterization of a lysophoaphatidic acid receptor, EDG-1, expressed in prostate. Mol. Pharmacol. 57, 753-759.

Inoue N. (2002). LPA as a determinant of mesangial growth and apoptosis. Semin. Nephrol. 5, 415-22.

Inoue C.N., Forster H.G., and Epstein M. (1995). Effects of lysophosphatidic acid, a novel lipid mediator, on cytosolic Ca^{2+} and contractility in cultured rat mesangial cells. Cir. Res. 77, 888-896.

Inoue C.N., Epstein M., Forster H.G., Hotta O., Kondo Y. and Iinuma K. (1999). Lysophosphatidic acid and mesangial cells: Implications for renal diseases. Clin. Sci. (Lond) 96, 431-436.

Inoue C.N., Ko Y.H., Guggino W.B., Forster H.G. and Epstein M. (1997). Lysophosphatidic acid and platelet-derived growth factor synergistically stimulate growth of cultured rat mesangial cells. Proc. Soc. Exp. Biol. Med. 216, 370-379.

Ishi I., Contos J.J., Fukushima N. and Chun J. (2000). Functional comparisions of the lysophosphatidic acid receptors, LPA1/V2G-1/EDG-2, LPA2/EDG4, and LPA3/EDG-7 in neuronal cell lines using a retrovirus expression system. Mol. Pharmacol. 58, 895-902.

Jing Q., Xin S.M., Zhang W.B., Wang P., Qin Y.W. and Pei G. (2000). Lysophosphatidylcholine activates p38 and p42/44 mitogen-activated protein kinases in monocytic THP-1 cells, but only p38 activation is involved in its stimulated chemotaxis. Cir. Res. 87, 52-59.

Kabarowski J.H., Feramisco J.D., Le L.Q., Gu J.L., Luoh S.W., Simon M.I. and Witte O.N. (2000). Direct genetic demonstration of G alpha 13 coupling to the orphan G protein-coupled receptor G2A leading to RhoA-dependent rearrangement. Proc. Natl. Acad. Sci USA 97, 12109-12114.

Kabarowski J.H., Zhu K., Le L.Q., Witte O.N. and Xu Y. (2001). Lysophosphatidylcholine as a ligand for the immunoregulatory receptor G2A. Science 293, 702-705.

Kamanna V.S., Pai R., Roh D.D. and Kirschenbaum M.A. (1996). Oxidative modification of low-density lipoprotein enhances the murine mesangial cell cytokines associated with monocyte migration, differentiation and proliferation. Lab. Invest. 74, 1067-1079.

Kamnna V.S., Bassa B.V. and Kirschenbaum M.A. (1997). Atherogenic lipoproteins and human disease: extending concepts beyond the heart to the kidney. Curr. Opin. Nephrol. Hyperten. 6, 205-211.

Kamanna V.S., Roh D.D. and Kirchenbaum M.A. (1998). Hyperlipidemia and kidney disease: concepts derived from histopathology and cell biology of the glomerulus. Histol. Histopathol. 13, 169-179.

Kamanna V.S., Pai R., Ha H., Kirshcenbaum M.A. and Roh D.D. (1999a). Oxidized low-density lipoprotein stimulates monocyte adhesion to glomerular endothelial cells. Kidney Int. 55, 2192-2202.

Kamanna V.S., Bassa B.V., and Vaziri N.D. and Roh D.D. (1999b). Atherogenic lipoproteins and tyrosine kinase mitogenic signaling in mesangial cells. Kidney Int. 56, S70-S75.

Katsuma S., Hada Y., Shiojima S., Hirasawa A., Tanoue A., Takagaki K., O Yano J. and Tsujimoto G. (2003). Transcriptional profiling of gene expression patterns during sphingosine 1-phosphate-induced mesangial cell proliferation. Biochem. Biophys. Res. Commun. 300, 577-84.

Kluk M.J. and Hla T. (2002). Signaling of sphingosine-1-phosphate via the S1P/EDG-family of G protein-coupled receptors. Biochim. Biophys. Acta 1582, 72-80.

Kohno M., Yokokawa K., Yasunari K., Minami N., Kano H., Hanehira T. and Yoshikawa J. (1998). Induction of lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration. Circulation 98, 353-359.

Kugiyama K., Ohgushi K., Ohgushi M., Sugiyama S., Murohara T., Fukunaga K., Miyamoto E. and Yasue H. (1992). Lysophosphotidyl-choline inhibits surface receptor-mediated intracellular signals in endothelial cells bya pathway involving protein kinase C activation. Cir. Res. 71, 1422-1428.

Kugiyama K., Kerns S.A., Morrisett J.D., Roberts R. and Henry P.D. (1990). Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins. Nature 344, 160-162.

Kume N. and Gimbrone M.A. Jr (1994). Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human vascular endothelial cells. J Clin Invest 93, 907-911.

Kume N., Cybulsky M.I. and Gimbrone M.A. Jr (1992). Lysophosphatidyl-choline, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J. Clin. Invest. 90, 1138-1144.

Laffargue M., Raynal P., Yart A., Peres C., Wetzker R., Roche S., Payrastre B. and Chap H. (1999). An epidermal growth factor receptor/Gab1 signaling pathway is required for activation of

phosphoinositide 3-kinase by lysophosphatidic acid. J. Biol. Chem. 274, 32835-32841.

Le L.Q., Kabarowski J.H., Weng Z., Satterthwaite A.B., Harvill E.T., Jensen E.R., Miller J.F. and Witte O.N. (2001). Mice lacking the orphan G protein-coupled receptor G2A develop a late-onset autoimmune syndrome. Immunity 14, 561-571.

Lee H.S. and Kim Y.S. (1998). Identification of oxidized low density lipoprotein in human renal biopsies. Kidney Int. 54, 848-856.

Lee H., Liao J., Graeler M., Huang Mei-Chuan. and Goetzl E.J. (2002). Lysophospholipid regulation of mononuclear phagocytes. Biochim. Biophys. Acta 1582, 175-177.

Leung S.W., Teoh H., Quan A. and Man R.Y. (1999). Endothelial dysfunction exacerbates the impairment of relaxation by lysophosphatidylcholine in porcine coronary artery. Clin. Exp. Pharmacol. Physiol. 24, 984-986.

Lin P. and Ye R.D., (2003). The lysophospholipid receptor G2A activates a specific combination of G proteins and promotes apoptosis. J. Biol. Chem. 278, 14379-14386.

Luttrell L.M., Della Rocca G.J., van Biesen T., Luttrell D.K. and Lefkowitz R.J. (1997). Gbetagamma subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for tG protein-coupled receptor-mediated Ras activation. J. Biol. Chem. 272, 4637-4644.

Lynch K.R. (2002). Lysophospholipid receptor nomenclature. Biochim. Biophys. Acta 1582, 70-71.

Maggi E., Bellazi R., Gazo A., Seccina M. and Bellamo G. (1994). Autoantibodies against oxidatively-modified LDL in uremic patients undergoing dialysis. Kidney Int. 46, 869-876.

Mizobata R. (2003). Effects of lysophosphatidylcholine on expression of monocyte chemoattractant protein-1 in glomerular endothelial cells. Nippon Jinzo Gakkai Shi. 45, 76-83.

Moolenaar W.H., Kranenburg O., Postma F.R. and Zondag G.C. (1997). Lysophosphatidic acid: G-protein signalling and cellular responses. Curr. Opin. Cell Biol. 9, 168-173.

Moolenaar W.H., van Meeteren L.A. and Giepmans B.N. (2004). The ins and outs of lysophosphatidic acid signaling. Bioessay 26, 870-881.

Nakano T., Raines E.W., Abraham J.A. Klagsburn M. and Ross R. (1994). Lysophosphatidylcholine upregulates the level of heparin-binding epidermal growth factor-like growth mRNA in human monocytes. Proc. Natl. Acad. Sci. USA 91, 1069-1073.

Ohgushi M. Kugiyama K., Fukunaga K., Murohara T., Sugiyama S., Miyamoto E. and Yasue H. (1993). Protein kinase C inhibitors prevent impairment of endothelium-dependent relaxation by oxidatively modified LDL. Arteriosc. Thromb. 13, 1525-1532.

Ono T., Kanatsu K., Doi T., Sekita K., Onoe C., Nagai H., Muso E., Yoshida H., Tamura T. and Kawai C. (1991). Relationship of intraglomerualar coagulation and platelet aggregation to glomerular sclerosis. Nephron 58, 429-36.

Ozaki H., Ishii K., Arai H., Kume N. and Kita T. (1999). Lysophosphatidyl-choline activates mitogen-activated protein kinases by a tyrosine kinase-dependent pathway in bovine aortic endothelial cells. Atherosclerosis 143, 261-266.

Pages C., Daviaud D., An S., Krief S., Lafontan M., Valet P. and Saulnier-Blache J.S. (2001). Endothelial differentiation gene-2 receptor is involved in lysophosphatidic acid-dependent control of 3T3F442A preadipocyte proliferation and spreading. J. Biol. Chem. 276, 11599-11605.

Parthasarathy S. and Barnett J. (1990). Phospholipase A2 activity of low density lipoprotein: evidence for an intrinsic phospholipase A2 activity of apoprotein B-100. Proc. Natl. Acad. Sci. USA 87, 9741-9745.

Pfeilschifter J., Schalkwijk C., Briner V.A. and van den Bosch H. (1993). Cytokine stimulated secretion of Group II phospholipase A2 by rat mesangial cells. J. Clin. Invest. 92, 2516-2523.

Quinn M.T., Parthasarathy S. and Steinberg D. (1988). Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherosclerosis. Proc. Natl. Acad. Sci. USA 85, 2805-2809.

Radu C.G., Yang L.V., Riedinger M., Au M. and Witte O.N. (2004). T cell Chemotaxis to lysophosphatidylcholine through the G2A receptor. Proc. Natl. Acad. Sci. USA 101, 245-250.

Rikitake Y., Hirata K., Yamashita T., Iwai K., Kobayashi S., Itoh H., Ozakai M., Ejiri J., Shiomi M., Inoue N., Kawashima S. and Yokoyama M. (2002). Expression of G2A, a receptor for lysophosphatidlycholine, by macrophages in murine, rabbit, and human atherosclerotic plaques. Arterioscler. Thromb. Vasc. Biol. 22, 2049-2053.

Roh D.D., Kamanna V.S. and Kirschenbaum M.A. (1998a). Oxidative modification of low density lipoprotein enhances mesangial cell protein synthesis and gene expression of extracellular matrix proteins. Am. J. Nephrol. 18, 344-350.

Roh D.D., Kamanna V.S. Kirschenbaum M.A. (1998b). Atherogenic lipoproteins enhance murine cortical epithelial cell fibronectin protein synthesis and gene expression. Am. J. Nephrol. 18, 83-88.

Rother E., Brandl R., Baker D.L., Goyal P., Gebhard H., Tigyi G. and Siess W. (2003). Subtype-selective antagonists of lysophosphatidic acid receptors inhibit platelet activation triggered by lipid core of atherosclerotic plaques. Circulation 108, 741-747.

Sasagawa T., Suzuki K., Shiota T., Kondo T. and Okita M. (1998). The significance of plasma lysophospholipids in patients with renal failure on hemodialysis. J. Nutr. Sci. Vitaminol. (Tokyo) 44, 809-818.

Siess W., Zangl K.J., Essler M., Bauer M., Brandl R., Cominty C., Bittman R., Tigyi G. and Aepfelbacher M. (1999). Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions. Proc. Natl. Acad. Sci. USA 96, 6931-6936.

Spiegel S. and Milstein S. (1995) Sphingolipid metabolites: members of a new class of lipid second messengers. J. Membr. Biol. 46, 225-237.

Spiegel S. and Milstien S. (2003). Sphingosine-1-phosphate: an enigmatic signaling lipid. Nat. Rev. Mol. Cell Biol. 4, 397-407.

Sugiyama S., Kugiyama K., Ohgushi M., Fujimoto K. and Hirofumi Y. (1994). Lysophosphatidylcholine in oxidized low-density lipoprotein increases endothelial susceptibility to polymorphonuclear leukocyte-induced endothelial dysfunction in porcine coronary arteries. Circ. Res. 74, 565-575.

Takahashi M., Okazki H., Ogata Y., Takeuchi K., Ikeda V. and Shimada K. (2002). Lysophosphatidylcholine induces apoptosis in human endothelial cells through a p38-mitogen- activated protein kinase-dependent mechanism. Atherosclerosis 161, 387-394.

Thumser A.E., Voysey J.E. and Wilton D.C. (1994). The binding of lysophospholipids to rat liver fatty acid-binding protein and albumin. Biochem. J. 301, 801-806.

Tigyi G and Miledi R. (1992) Lysophosphatidates bound to serum albumin activate membrane currents in Xenopus oocytes and neurite retraction in Pc12 pheochromocytoma cells. J. Biol. Chem. 267, 2136-21367.

Tone Y., Yukawa S., Varghese Z. and Moorhead J.F. (1999).

Lysophosphatidylcholine induces platelet-derived growth factor expression in a human mesangial cell line. Kidney Int. Suppl. 71, S182-S185.

Tselepis A.D. and John Chapman M. (2002). Inflammation, bioactive lipids, and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor-acetylhydrolase. Atheroscler Suppl. 3, 57-68.

van Corven E.J., Groenink A., Jalink K., Eichholtz T. and Moolenaar, W.H. (1989). Lysophosphadate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell 59, 45-54.

Van Tits L., De Graaf J., Hak-Lemmers H., Bredie S., Demacker P., Holvo Stalenhoef A. (2003). Increased levels of low-density lipoprotein oxidation in patients familial hypercholesterolemia and in end-stage renal disease patients on hemodialysis. Lab. Invest. 83, 13-21.

Vuong T.D., Stroes E.S., Willekes-Koolschijn N., Rabelink T.J., Koomans H.A. and Joles J.A. (1999). Hypoalbuminemia increases lysophosphatidylcholine in low-density lipoprotein of normocholesterolemic subjects. Kidney Int. 55. 1005- 1010.

Wada A., Sugiura T., Arai M., Tojio H., Okamoto M., Fujiwara Y., Ueda N. and Kamada T. (1995). Group II phospholipase AII promotes mesangial cell proliferation via phospholipids. J. Am. Soc. Nephrol. 5, 704A.

Wada A., Tojo H., Sugiura T., Fujiwara Y., Kamada T., Ueda N. and Okamoto M. (1997). Group II phospholipase A2 as an autocrine growth factor mediating interleukin-1 action on mesangial cells. Biochim. Biophys. Acta 1345, 99-108.

Watanabe M. and Okada T. (2003). Lysophosphatidylcholine-induced myocardial damage is inhibited by pretreatment with poloxamer 188 in isolated rat heart. Mol. Cell Biochem. 248, 209-215.

Watanabe T., Pakala R., Koba S., Katagiri T. and Benedict C.R. (2001). Lysophosphatidylcholine and reactive oxygen species mediate the synergistic effect of mildly oxidized LDL with serotonin on vascular smooth muscle cell proliferation. Circulation 103, 1440-1445.

Weng Z., Fluckiger A.C., Nisitani S., Wahl M.I., Le L.Q., Hunter C.A., Fernal A.A., Le Beau M.M. and Witte O.N. (1998). A DNA damage and stress inducible G protein-coupled receptor blocks cells in G2/M. Proc. Natl. Acad. Sci. USA 95, 12334-12339.

Wu J. and Cunnick J.M. (2002) Trans-regulation of epidermal growth factor receptor by lysophosphatidic acid and G protein-coupled receptors. Biochim. Biophys. Acta 1582, 100-106.

Xing Y., Ganji S.H., Noh J.W. and Kamanna V.S. (2004). Cell density-dependent expression of EDG family receptors and mesangial cell proliferation: Role in lysophosphatidic acid-mediated cell growth. Am. J. Physiol. Renal Physiol.

Xu Y.J., Aziz O.A., Bhugra P., Arneja A.S., Mendis M.R. and Dhalla N.S. (2003). Potential role of lysophosphatidic acid in hypertension and atherosclerosis. Can. J. Cardiol. 13, 1525-36.

Xu J., Love l.M., Singh I., Zhang Q.X., Dewald J., Wang D.A., Fischer D.J., Tigyi G., Berthiaume L.G., Waggoner D.W. and Brindley D.N. (2000). Lipid Phosphatase-1 and Ca^{2+} control lysophosphatidate signaling through EDG-2 receptor. J. Biol. Chem. 275, 27520-27530.

Yamakawa T., Tanaka S., Numaguchi K., Motley E.D., Inagami T. and Eguchi S. (2002). Lysophosphatidylcholine activates extracellular signal-regulated kinases 1/2 through reactive oxygen species in rat vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 22, 752-758.

Yokomori H., Yoshimura K., Funakoshi S., Nagai T., Fujimaki K., Nomur M., Ishii H. and Oda M. (2004). Rho modulates hepatic sinusoidal endothelial fenestrae via regulation of the actin cytoskeleton in rat endothelial cells. Lab. Invest. 84, 857-864.

Zager R.A., Iwata M., Conrad D.S., Burkhart K.M. and Igarashi Y. (1997). Altered ceramide and sphingosine expression during the induction phase of ischemic acute renal failure. Kidney Int. 52, 60-70.

Zager R.A., Conrad S., Lochhead K., Sweeney E.A., Igarashi Y. and Burkhart K.M. (1998). Altered sphingomyelinase and ceramide expression in the setting of ischemic and nephrotoxic acute renal failure. Kidney Int. 53, 573-82.

Zhu K., Baudhuin l.M., Hong G., Williams F.S., Cristina K.L., Kabarowski J.H., Witte O.N. and Xu Y. (2001). Sphingosylphosphorylcholine and lysophosphatidylcholine are ligands for the G protein-coupled receptor GPR4. J.. Biol. Chem. 276, 41325-41335.

Zohn I.E., Klinger M., Karp X., Kirk H., Symons M., Chrzanowska-Wodnicka M., Der C.J. and Kay R.J. (2000). G2A is an oncogenic G protein-coupled receptor. Oncogene 19, 3866-3877.

Accepted November 25, 2004