manner. How does rapid, induced synthesis of COX-2 coordinate with $cPLA_2$, other secretory PLA_2s and downstream synthases during inflammation to produce a proinflammatory eicosanoid profile? Is there an orchestrated temporal change in lipid mediators during the resolution phase? Answers to these questions are forthcoming.

The development of specific agonists and antagonists for each of the prostaglandin and leukotriene receptors will provide important reagents for further defining the biological importance of this group of bioactive lipids. Reports on genetic variants of eicosanoid receptors and biosynthetic enzymes within the prostaglandin and leukotriene pathways have been scant. Elucidation of such variants and their potential relevance to inflammation or disease susceptibility and interindividual variations in drug response will be an area of active investigation.

Advances in eicosanoid biology certainly extend beyond the prostaglandins and leukotrienes. The hydroxy (HETE) (*59*), epoxy (EET) (*60*), and lipoxin eicosanoid molecules (*61*) are emerging areas as well. NSAIDs and coxibs may also turn out to be useful therapeutic agents in the treatment of Alzheimer's disease and certain cancers (*62–64*). Other lipoxygenase and cyclooxygenase products are implicated in atherogenesis (*65*, *66*) and will also certainly receive attention in the years to come.

The eicosanoids, like no other set of lipid mediators, possess a vast array of biological actions in many different cell types. Eicosanoid molecules are truly a conundrum, paradoxically acting as both friend and foe. New insight into their roles in pain, inflammation, and disease and the development of novel therapeutics will undoubtedly arise in the near future.

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VIEWPOINT

Lysophospholipids—Receptor Revelations

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Upon cell activation, membrane phospholipids are metabolized into potent lysophospholipid (LP) mediators, such as sphingosine 1-phosphate and lysophosphatidic acid. LPs fulfill signaling roles in organisms as diverse as yeast and humans. The recent discovery of G protein–coupled receptors for LPs in higher eukaryotes, and their involvement in regulating diverse processes such as angiogenesis, cardiac development, neuronal survival, and immunity, has stimulated growing interest in these lipid mediators. LP receptor biology has generated insights into fundamental cellular mechanisms and may provide therapeutic targets for drug development.

Glycerol-based and sphingosine-based phospholipids are abundant structural components of cellular membranes; however, they are metabolized into polar metabolites such as eicosanoids and lysophospholipids (LPs) (*1*). The latter includes lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), sphingosylphosphoryl choline (SPC), and sphingosine 1-phosphate (S1P). [1]However, in contrast to the eicosanoids, whose critical roles in normal physiology and disease are underscored by the current widespread clini-

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cal utility of eicosanoid biosynthetic enzyme inhibitors and receptor antagonists(*2*), the discovery of LPs is relatively recent. Historically, LPs have been viewed as second-messenger molecules that regulate intracellular signaling pathways (*3*). How ever, this view evolved swiftly after the discovery of plasma membrane receptors for extracellular LPs in vertebrates (*1, 4, 5*). Recent work shows that a family of G protein–coupled receptors (GPCRs) mediate the complex and diverse effects of LPs in numerous fundamental processes. For example, LPs are implicated in tumorigenesis, angiogenesis, immunity, atherosclerosis, and neuronal survival. The complexity and evolutionary conservation of LP signaling pathways also suggest the ancient nature of LPs as molecules involved in cell-to-cell signaling. The discovery of cell-surface receptors for LPs is anticipated to provide opportunities for therapeutic development of receptor agonists and antagonists to control various disease processes.

Although LPs are synthesized from the metabolism of membrane lipids including sphingomyelin and phosphatidylcholine, their subcellular sites of synthesis and the regulation of their formation are poorly understood. The biochemical pathways involved in LP synthesis can be found in several comprehensive reviews (*1*, *3*, *5*). Although intracellular synthesis of LPs is well documented, recent evidence suggests that they may also be metabolized in the extracellular milieu, suggesting an alternate mode of action for these lipids. Studies in *Drosophila* indicated that a protein containing a six-transmembrane domain and encoded by the *Wunen* gene is a critical mediator of germ cell migration during embryogenesis (*6*). This cell-surface protein is structurally similar to a lipid phosphate phosphohydrolase (LPP) that dephosphorylates LPA and S1P (*1*, *5*). Because the active site of Wunen may be located on the extracellular surface, an unidentified extracellular LP mediator of germ cell chemotaxis was suggested, and the *Wunen* gene was proposed to down-regulate the activity of this unknown LP (*6*).

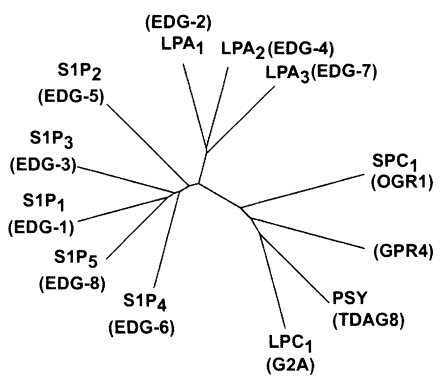

Fig. 1. Phylogenetic tree representation of LP receptors. The GPCR sequences of LP receptors were aligned and analyzed by the CLUSTAL program (*50*). The relatedness of the GPCR sequences is represented by three distinct branches of the receptor subfamilies: the S1P, the LPA, and the LPC-SPC-psychosine families. The LPA and S1P subfamilies are closer in sequence identity than the LPC-SPC-psychosine subfamily, as indicated by the closeness of the branch points of the tree (*50*). For example, the identities of the $S1P_1$ to $S1P_3$, LPA_1, and SPC_1 are 48%, 32%, and 16%, respectively. The proposed names for the receptor subtypes are followed by the orphan receptor nomenclature in parentheses.

In higher organisms, the extracellular appearance of LPs is well documented, and although the release of S1P has been shown in platelets, mast cells, and monocytic cells (*1*, *3–5*), the mechanisms involved are poorly understood. Biosynthetic enzymes for S1P, namely, sphingomyelinase, ceramidase, and sphingosine kinase are thought to function in the cytosol (*1*, *3–5*). However, recent data indicate that these enzymes are also secreted by cells, suggesting that S1P can also be formed extracellularly (*7–9*). Both S1P and LPA are found associated with serum albumin in plasma, whereas LPC is associated with oxidatively modified lipoprotein particles, particularly oxidized low density lipoprotein (LDL) (*1*, *5*). In fact, enhanced secretion of LPA may be a characteristic of some ovarian cancer cells (*10*). The potential for LPA as a plasma marker for ovarian cancer has generated great interest in how extracellular LPs modulate cell behavior.

Early reports hypothesized the presence of GPCRs for LPs, and there are 12 now identified, the best characterized of which are the receptors for S1P and LPA. It was only recently that two distinct GPCRs, VZG-1 and PSP24, were proposed as LPA receptors (*11*, *12*). Ventricular zone gene (*VZG*)–1 (also known as LPA_1) is a prototype of three LPA receptor subtypes, namely, LPA_1, LPA_2, and LPA_3, also known as endothelial differentiation gene (EDG)–2, EDG-4, and EDG-7, respectively (*13*). Whether PSP24 is an LPA receptor remains unclear at present. The identification of another *VZG-1*–related orphan receptor, $S1P_1$ (also called EDG-1), as a high-affinity receptor for S1P supports the notion that these molecules are GPCRs for the LPA and S1P branches of LPs (*14*) (Fig. 1). The primary sequences of the known LP receptors cluster into three distinct groups, although S1P and LPA receptors are much more related to each other in primary sequence than the LPC and SPC receptors.

That $S1P_1$ is a bona fide GPCR for S1P is supported by its high-affinity ($K_d \sim 8$ nM) and specificity of binding; coupling to the G_i pathway; and its regulation of cell migration, proliferation, survival, and morphogenesis in response to S1P (*14–16*) (Fig. 2). In particular, S1P interaction with $S1P_1$ on vascular endothelial cells is implicated in angiogenesis and the maturation of the vascular system in mammals (*15–17*). Its mechanism of action on endothelial cells includes the activation of $\alpha_v\beta_3$- and β_1-containing integrins through the small GTPase Rho (*15*). In addition, S1P induces a G_i- and phosphatidylinositol 3-kinase (PI3K)–dependent activation of the protein kinase Akt, which then binds to $S1P_1$ and phosphorylates the third intracellular loop at the Thr^{236} residue (*18*). This event is critical for committing the receptor to regulate the small GTPase Rac and the signaling pathways required for cortical actin assembly, lamellopodia formation, and chemotaxis (*18*). A dominant negative form of $S1P_1$ (with a substitution of Ala for Thr at position 236) inhibited endothelial cell chemotaxis and angiogenesis in mice (*18*). S1P activation of $S1P_1$ is also important for cell survival and proliferation of vascular endothelial cells and stimulation of endothelial cell nitric oxide synthase enzyme (*16*, *19*). Endothelial cell apoptosis is reversed by S1P through a G_i and extracellular signal–regulated kinase (ERK) signaling pathway (*16*). Because vascular smooth muscle cell proliferation and migration are also regulated by $S1P_1$, S1P may be a critical regulator in general of cells comprising the vessel wall (*20*). In contrast to vascular endothelial growth factor (VEGF), and unlike other LPs, S1P activation of its receptors stimulates the assembly of cadherin complexes in endothelial cells (*16*).

Data from $S1p_1$ null mice underscore the importance of this lipid in vascular development. Embryonic lethality at embryonic day 12.5 (E12.5) to 14.5 days of gestation results from collapse of the vascular tree and embryonic hemorrhage (*17*). However, several critical questions remain to be addressed; namely, what is the role of S1P in pathologic angiogenesis and how is the balance between the vascular maintenance function of S1P and the angiogenic function of S1P achieved? Clearly, S1P induces the proliferation of new vasculature and stabilizes the established vasculature, which is in sharp contrast to other angiogenic factors such as VEGF. A better understanding of the physiological contexts in which S1P acts should expand our understanding of vascular homeostasis and growth.

The phenotype of the $S1p_1$ null mice shows some similarities to the PDGF-β receptor null mice (*21*). For example, in both cases, there is defective ensheathment

of the nascent vascular tree by pericytes, the supportive cells related to vascular smooth muscle cells (*21*). Hobson *et al.* reported that the ability of PDGF to induce embryonic fibroblast chemotaxis depends on $S1P_1$ expression (*22*). The authors hypothesized that PDGF-mediated activation of the sphingosine kinase enzyme, resulting in secretion of S1P and subsequent activation of $S1P_1$, may be necessary for stimulation of cell migration. Although this mechanism has yet to be validated, this raises the possibility of GPCR transactivation by receptor tyrosine kinases through the sphingosine kinase pathway. An alternative possibility is that intracellular kinases activated by tyrosine kinase receptors interact with and modulate the activity of the LP receptors. Indeed, insulin-like growth factor–1, a ligand for a receptor tyrosine kinase, transactivates S1P1 through a PI3K- and Akt-dependent phosphorylation (*18*). Cross talk between S1P receptors with other receptor systems, such as integrins, cadherins, and receptor tyrosine kinases has been suggested (*15*, *16*, *18*, *22*) and may mediate the complex biological actions of this LP.

Although $S1P_2$ (also called EDG-5) was originally identified as an orphan GPCR from vascular smooth muscle cells, its expression is widespread like that of $S1P_1$, and it was eventually characterized as an S1P receptor that couples to G_i, G_q, and $G_{12/13}$ heterotrimeric G proteins (*1*, *4*, *23*). In contrast to $S1P_1$, ligand binding to $S1P_2$ inhibits Rac and growth factor–induced chemotaxis (*24*). $S1P_2$ receptor is expressed in cells in which S1P is an inhibitor of cell migration, such as melanoma cells and vascular smooth muscle cells (*24*), providing a clear example of two LP receptor subtypes that possess opposing effects on cell migration. The importance of $S1P_2$ was further revealed in the zebrafish mutant *miles apart* (*mil*), which displayed defective cardiomyocyte precursor cell migration and cardia bifida (formation of two hearts on the either side of the midline). Cloning of the *mil* gene indicated that it is a zebrafish $S1P_2$ ortholog; two mutations abolished the ability of this GPCR to signal through increase in intracellular calcium and ERK activation (*25*). The *mil* gene is not expressed in the migrating cells; rather, it is expressed in the midline region of zebrafish embryos, suggesting that it exerts an environmental field effect to regulate heart development (*25*).

$S1P_3$ (also called EDG-3) is a unique subtype of S1P receptor because it activates G_i, G_q, and $G_{12/13}$ heterotrimeric G proteins (*4*, *23*, *26*) and the small GTPase Rho (*15*) and is antagonized by the polycyclic anionic compound suramin (*26*). Although it is expressed in the vascular system, homologous recombination of the $S1P_3$ gene in the mice does not generate any phenotypic abnormalities, suggesting redundant function with other S1P receptors (*27*). Despite the fact that S1P receptor subtypes regulate unique as well as common signaling pathways, and exhibit differential expression patterns in tissues, receptor redundancy may mask the functional specificity of each receptor subtype, as determined from gene knockout studies. Two additional S1P receptors, $S1P_4$ (also called EDG-6) and $S1P_5$ (also called EDG-8), have been described in hematopoietic and neuronal cells, respectively, but their biological functions have not yet been explored (*28*, *29*).

In contrast, the role of LPA as a regulator in neuronal and immune systems has been revealed by recent studies on LPA receptors. LPA_1 (also called EDG-2) was identified first as a high-affinity receptor in the ventricular zone of the developing cerebral cortex (*11*), and although the affinity constants for LPA binding have not been defined, nanomolar concentrations of various LPA species activate this receptor and induce G_i-dependent responses (*11*). Its expression in neurons declines after birth, but is induced again in the myelinating cells of the adult nervous system (*30*). LPA promotes the survival of myelinated Schwann cells from the peripheral nervous system through the LPA_1-dependent activation of the protein kinase Akt (*30*). However, deletion of the Lpa_1 gene in mice results in a complex phenotype characterized by 50% neonatal lethality, impaired suckling behavior, reduced growth, and craniofacial anomalies (*31*). This suggests that this receptor is critical not only in the developing nervous system but in other organ systems as well. Indeed, LPA_1 function has been implicated in LPA-induced adipocyte proliferation, protection of T cell apoptosis, and fibronectin matrix assembly in fibroblasts (*1*, *13*). LPA_2 (also known as EDG-4) is a high-affinity LPA receptor that activates the G_q pathway (*32*). It is constitutively expressed in $CD4^+$ T cells and inhibits the secretion of the cytokine interleukin-2. However, it stimulates T-lymphoma cell line migration and survival, suggesting that complex immunoregulatory properties of LPA are mediated in part by this GPCR (*33*). It is interesting that its expression is strongly induced in ovarian cancer cell lines where it regulates the tran-

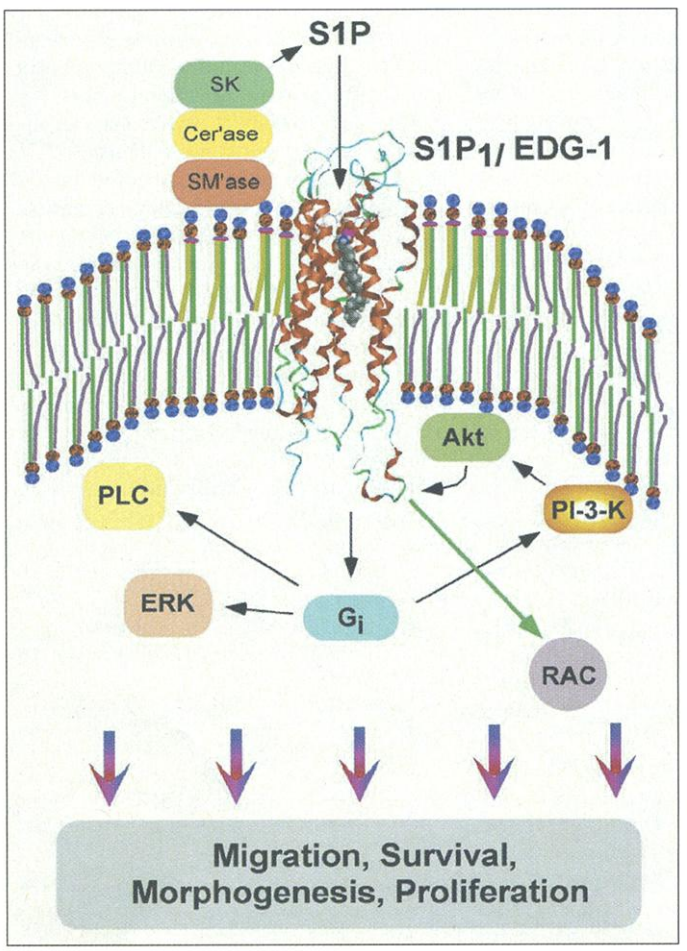

Fig. 2. Signal transduction of S1P through the $S1P_1$ receptor. The model of the $S1P_1$ receptor was adapted from (*51*). This GPCR is thought to be localized in the sphingomyelin-rich caveolar domains (*52*). Metabolism of sphingomyelin by the sphingomyelinase, ceramidase (Cer'ase) and the sphingosine kinase (SK) enzymes results in formation of S1P and receptor activation. Autocrine and paracrine modes of receptor activation have been implied but have yet to be rigorously proven. Critical signaling molecules, such as phospholipase C (PLC), ERK, PI3K, and Akt are activated. Active Akt binds to the receptor and phosphorylates the third intracellular loop, which is essential for Rac activation. These and other signaling events modulate cellular phenotypic changes outlined below.

scription of immediate-early genes and cellular proliferation (*34*). In contrast, LPA_3 (also known as EDG-7) is a high-affinity receptor that prefers G_q and that is selectively activated by LPA species with *sn-2* unsaturated fatty acid ester (*35*).

The work on S1P and LPA receptors has stimulated interest in identifying GPCRs for other LPs. A new subfamily of orphan GPCRs was recently identified that includes receptors for SPC, LPC, and the glycolipid psychosine (galactosylsphingosine). However, it is important to point out that much less is known about their pharmacology and biological functions. The orphan receptor OGR-1, originally isolated from ovarian cancer cells, but later shown to have a broader tissue distribution, was identified as a high-affinity SPC receptor (SPC_1) that inhibits cellular proliferation (*36*). Although the function of OGR-1 is not clear at present, either in normal physiology or in ovarian cancer, the affinity of this receptor to SPC and its signaling properties suggest a potentially important function. A related GPCR, TDAG8 responds to micromolar concentrations of psychosine (*37*). It is interesting that TDAG8 overexpression results in a multinucleated cellular phenotype, a phenomenon associated with elevated psychosine levels in Krabbe's disease (*37*). This phenotype can be explained by nuclear replication followed by defective cytoplasmic division in mitosis. It is known that Rho and the Rho-activated kinase Citron control cytokinesis (*38*), suggesting that psychosine interaction with TDAG8 may regulate this process. Another related receptor, G2A, originally isolated as a gene whose expression is induced during the G_2 to M transition of the cell division cycle, was recently shown to be activated by nanomolar concentrations of LPC (LPC_1) (*39*). This GPCR is normally expressed in immune cells and transforms NIH 3T3 fibroblasts when overexpressed (*39*). It is noteworthy that deletion of the *G2A* gene results in adult-onset autoimmune disease, similar to the human syndrome of systemic lupus erythematosus (*40*). These data suggest that LPC and/or related LPs may play a previously unappreciated role in autoimmunity. In addition, a related orphan receptor, GPR4, was recently identified as a high-affinity receptor for LPC (*41*). LPC, as a component of oxidized LDL, is known to impair normal endothelial cell function and to induce vascular injury (*42*). These data warrant further investigation into the possible regulatory role of LPC_1 or related GPCRs in cardiovascular pathophysiology. However, the interactions of these LP receptors with their proposed ligands need to be confirmed. In particular, both phosphorylated amphipathic ligands (SPC and LPC) and glycolipid ligands (psychosine) were proposed to interact with them and, in some cases, with relatively low affinity, suggesting that there may be yet unidentified physiologically relevant ligands for these GPCRs.

In conclusion, it is clear that metabolism of membrane phospholipids results in the formation of a novel branch of LP mediators. The possible intracellular second-messenger functions of LPs (or their nonphosphorylated derivatives), as exemplified by studies in yeast and slime molds (*5, 43–46*), suggest that these polar metabolites are ubiquitous regulators of cell signaling throughout evolution. However, the proposal that LPs act as second messengers is tenuous, and intracellular targets and receptors require further characterization. In contrast, in higher organisms, extracellular action of LPs on cell-surface GPCRs is supported by abundant data, particularly for S1P and LPA. It is likely that GPCRs for LPs coevolved with sophisticated cardiovascular, immune, and nervous systems in bony fishes and higher organisms. Indeed, S1P and LPA receptors have been found in bony fishes and amphibians but not in lower organisms (*25, 47, 48*). Pharmacological tools to selectively block or stimulate LP receptors may have utility in the control of angiogenesis, vascular diseases, autoimmunity, cancer, and neurodegeneration, among others. Numerous analogies exist between the LP and eicosanoid lipid signaling systems (*1, 2, 4*) as both classes of lipids are produced by the regulation of critical enzymes and act as extracellular signaling molecules through GPCRs. Eicosanoid function appears to be more relevant in late developmental stages and adulthood, whereas LPs appear to be involved in both early and late stages of embryonic development and adulthood. If experience from eicosanoid biology and therapeutics (*49*) can be extrapolated to the LP system, therapeutic tools of widespread utility may well result from this endeavor.

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