Ceramide and ceramide 1-phosphate in health and disease

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Abstract
Sphingolipids are essential components of cell membranes, and many of them regulate vital cell functions. In particular, ceramide plays crucial roles in cell signaling processes. Two major actions of ceramides are the promotion of cell cycle arrest and the induction of apoptosis. Phosphorylation of ceramide produces ceramide 1-phosphate (C1P), which has opposite effects to ceramide. C1P is mitogenic and has prosurvival properties. In addition, C1P is an important mediator of inflammatory responses, an action that takes place through stimulation of cytosolic phospholipase A2, and the subsequent release of arachidonic acid and prostaglandin formation. All of the former actions are thought to be mediated by intracellularly generated C1P. However, the recent observation that C1P stimulates macrophage chemotaxis implicates specific plasma membrane receptors that are coupled to Gi proteins. Hence, it can be concluded that C1P has dual actions in cells, as it can act as an intracellular second messenger to promote cell survival, or as an extracellular receptor agonist to stimulate cell migration.

Introduction
Sphingolipids play essential roles in normal cell and tissue homeostasis as well as in the establishment and progression of numerous diseases. In particular, ceramide is the central core in sphingolipid metabolism, but has also been involved in the regulation of signal transduction processes. Specifically, ceramides induce cell cycle arrest and promote apoptosis, a form of programmed cell death [1,2]. Also, ceramides play important roles in the regulation of autophagy, cell differentiation, survival, and inflammatory responses [3-11], and have been associated with insulin resistance through activation of protein phosphatase 2A and the subsequent dephosphorylation and inactivation of Akt (also known as protein kinase B (PKB)) [12-14]. Cell ceramides typically have long N-acyl chains ranging from 16 to 26 carbons in length [15-17]. However, in many studies short-chain analogs (N-acetylsphingosine, or C2-ceramide, N-hexanoylsphingosine, or C6-ceramide, and N-octanoylsphingosine, or C8-ceramide) have been used in experiments because these are more water soluble than long-chain ceramides. Formation of ceramide is also relevant because it is the precursor of important bioactive sphingolipids that can also regulate cellular functions, as discussed below.

A major metabolite of ceramide is ceramide-1-phosphate (C1P), which is generated through direct phosphorylation of ceramide by ceramide kinase (CerK) (Fig. 1). There is increasing evidence suggesting that C1P can regulate cell proliferation and apoptosis [7,18], and Chalfant and co-workers have elegantly demonstrated that C1P is a potent pro-inflammatory agent (Reviewed in [19,20]). In addition, C1P plays an important role in phagocytosis [21,22], and we have recently demonstrated that is a key factor in the regulation of macrophage chemotaxis. The aim of the present review is to discuss recent progress in C1P biology with especial emphasis in the context of health and disease.

Synthesis of Bioactive Sphingolipids
Although sphingosine is the simplest sphingolipid, ceramide is considered to be the central structure in sphingolipid metabolism. Fig. 1 shows that ceramide can be generated by three major mechanisms: 1) the de novo synthesis pathway is an anabolic route that begins with condensation of the amino acid serine and palmitoyl-CoA to form 3-ketosphinganine in a reaction that is catalyzed by serine palmitoyltransferase (SPT); reduction of 3-ketosphinganine to sphinganine follows immediately; acylation of sphinganine by dihydroceramide synthase
(CerS, also known as Lass) then generates dihydroceramide. The last step of this pathway is catalyzed by a desaturase through introduction of a trans-4,5 double bond in the dihydroceramide molecule to yield ceramide (Fig. 1). Concerning CerS six different genes have been identified in mammalian cells. Interestingly, the different CerS isoforms produce ceramide with different N-acyl chains. The reason why there are so many of these genes when most of the other enzymes in the sphingolipid biosynthetic pathway exist in only one or two isoforms is not known. However, it is possible that ceramides containing different fatty acids play different roles in cell biology (reviewed in [23]). For details on SPT and CerS activities the reader is referred to other excellent reviews by Hannun and Obeid [2,5,24], and Merrill and co-workers [11,25]. Also, very elegant reviews by Kolesnick et al. [26], Goñi and Alonso [27], and Cremesti et al. [28] specifically address the important roles of SMase activities, enzymology, and compartmentalization in cell biology. Once synthesized, ceramide can be used for synthesis of complex sphingolipids, through intervention of different biosynthetic enzymes, including glucosyl or galactosyl ceramide synthases to form cerebrosides or gangliosides, or it can incorporate a phosphocholine head group from phosphatidylcholine (PC) to form SM through the action of SM synthases. Formation of glucosylceramide is particularly important because of its role in conferring drug resistance to tumor cells [29]. In addition, ceramide can be directly phosphorylated by ceramide kinase (CerK) to form C1P (Fig. 1), which is a key regulator of cell homeostasis [18,30] and has been implicated in inflammatory responses [19,20,31]. 2) The second major mechanism for ceramide generation is a catabolic pathway involving activation of SMases to form phosphorylcholine and cer-

Figure 1 Formation of bioactive sphingolipids in mammalian cells. Ceramide can be produced by degradation of sphingomyelin (SM) by sphingomyelinases (SMase), or by de novo synthesis through the concerted action of serine palmitoyltransferase and dihydroceramide synthase. It can also be generated through metabolism of more complex sphingolipids. Ceramide can be metabolized to ceramide-1-phosphate by ceramide kinase, or to glucosylceramide by glucosylceramide synthase (GCS). The reverse reaction is catalyzed by ceramide-1-phosphate phosphatase, or by lipid phosphate phosphatases. Alternatively, ceramide can be degraded by ceramidas to form sphingosine, which can, in turn, be phosphorylated to sphingosine-1-phosphate by sphingosine kinase. The reverse reaction is catalyzed by sphingosine-1-phosphate phosphatases, or by lipid phosphate phosphatases. Sphingomyelin N-deacylase generates sphingosylphosphorylcholine.
amid directly (Fig. 1). There are three distinct forms of SMases in mammalian cells that can be discriminated *in vitro* by their optima pH: acid, neutral and alkaline SMases. Whilst acid SMase and neutral SMase are involved in signal transduction processes, the alkaline form of SMase is responsible for digestion of dietary SM in the intestine. The alkaline SMase isozyme has now been re-named NPP7 because of its similarity to the nucleotide-pyrophosphatase/phosphodiesterase (NPP) family of enzymes. In addition to its role in SM digestion, a potential implication of this enzyme in cell signaling processes has also been suggested [32]. In particular, alkaline SMase has been shown to inhibit cell proliferation in HT-29 colon carcinoma cells [33]. There is a third form of SMase. For instance, acid SMase and neutral SMase are involved in SM metabolism while the alkaline SMase isozyme has been implicated in the stimulation of steroid hormone actions [47,48]. Two sphingosine kinases (SphKs) have so far been identified in mammalian cells, SphK1 and SphK2, which exhibit different biochemical properties and regulation. The roles of S1P and SphKs in cell biology have been extensively reviewed elsewhere [42,49].

**Ceramides**

Besides its role as the precursor of complex sphingolipids, ceramide is a signaling molecule capable of regulating vital cellular functions including apoptosis, cell growth, differentiation, senescence, diabetes, insulin resistance, inflammation, neurodegenerative disorders, or atherosclerosis [2-5,15,35,50-56]. In this connection, it should be pointed out that the topology of ceramide generation is crucial for determination of its functions as a bioregulatory molecule, with compartmentalization being essential for separation of signaling and metabolic pools within cells. Indeed, the enzymes that regulate ceramide metabolism show distinct subcellular localization and topology (reviewed in [2]). For instance, the plasma membrane of cells contains caveolae-associated neutral SMase, and a fraction of acid SMase, and the ceramides that are generated by these enzymes may have different functions. The enzymology, and compartmentalization of sphingomyelinases have been reviewed elsewhere [26-28]. Another important aspect of ceramide action concerns its transport from the ER, where it is synthesized, to the Golgi apparatus, the primary site of SM and glycosphingolipid synthesis. Hanada et al [57] recently demonstrated the existence of a specific protein that is involved in SM biosynthesis and acts as a ceramide transfer protein (CERT) in a non-vesicular manner. This protein has two domains involved in the transport of ceramide: one that recognizes ceramide and mediates its intermembrane transfer, termed the START domain, and a phosphatidylinositol binding domain (PH domain) with selectivity towards phosphatidylinositol-4-phosphate, a lipid that is enriched in the Golgi and that could serve as the site for ceramide delivery by CERT [57]. Ceramide generation at the plasma membrane exerts distinct and specific functions including aggregation of the Fas receptor, and effects on protein kinase C (PKC), but not other effects mediated by endogenous ceramides such as apoptosis, or cell cycle arrest [2]. Although the regulation of PKC activity by ceramides has already been reported, the results are still controversial. In this regard, ceramides have been shown to activate PKC-α and to inhibit PKC-α in renal mesangial cells [58]. They have also been shown to induce the translocation of PKC-δ and PKC-ε from the cytosol to the membrane [59], the translocation of PKC-δ and PKC-ε from the membrane to the cytosol [60], and the translocation...
of PKC-δ from the cytosol to the mitochondria [61]. Also, ceramide was shown to induce apoptosis by transloca-
tion, tyrosine phosphorylation and activation of PKC-δ in
the Golgi complex [62]. Another important target of cer-
amide is phospholipase D (PLD), which is a key regula-
tory enzyme responsible for generation of phosphatidic
acid (PA), a potent mitogenic agent, and a precursor of
important second messengers including lysoPA and dia-
cylglycerol (DAG) [7]. We first reported that the cell-per-
meable ceramides N-acetylsphingosine (C2-ceramide)
and N-hexanoylsphingosine (C6-ceramide), or exogenous
bacterial sphingomyelinase, which can generate cer-
amides at the plasma membrane of cells, inhibited ago-
nist-stimulated PLD activity potently in intact rat
fibroblasts [63,64] or macrophages [65-67]. PLD inhibi-
tion by ceramides has also been demonstrated in several
other cell types [47,68-70], as well as in cell-free systems
[71,72], or digitonin-permeabilized fibroblasts that were
incubated with GTPyS [63]. However, the physiological
significance of PLD inhibition by ceramides is still
unclear.

Sphingolipids are also important because they are
implicated in atherogenic processes (reviewed by Stein-
brecher et al. [73]). In particular, ceramides, glycosphin-
golipids and S1P have been shown to accumulate in
atherosclerotic lesions, and to participate in the regula-
tion of signal transduction pathways that are implicated
in atherogenesis. Ceramides and S1P can be generated by
the action of oxidatively modified low density lipopro-
teins (LDL), or by pro-inflammatory cytokines. These
bioactive sphingolipids can upregulate the expression of
adhesion molecules and promote migration and adhesion
of monocytes to the sites of lesions. In fact, early and
intermediate atheromas are rich in macrophages and
smooth muscle cells, and show evidence of active cell
proliferation [74].

With regards to ceramide metabolism, the enzymes
responsible for its degradation, have recently gained par-
ticular interest because of their involvement in various
diseases. In particular ceramidas, would generate sphingosine directly, and sphingosine can be readily con-
verted to S1P, a potent mitogenic agent and tumor pro-
moter. Details on protein sequence, chromosomal
location, tissue distribution, and subcellular localizations
of the different ceramidas have been recently reviewed
by Mao and Obeid [75]. Ceramidas have been impli-
cated in the mitogenic effect of oxidized LDL (oxLDL),
probably by enhancing the production of S1P [76]. Also,
dysregulation of mesangial cell proliferation or death
involves altered ceramida activities [77-79] supporting
a role of this enzyme in diabetic nephropathy. An involve-
ment of the three different types of ceramidas (acid,
natural and alkaline) in the development of type 2 dia-
tes, insulin resistance and metabolic syndrome has also
been reported [80-84]. Ceramidas appear to also be
involved in some of the apoptotic effects promoted by
nitric oxide [58,85-87] and inflammatory cytokines [88-
99], the antipapoptotic properties of growth factors
[100,101], and in the promotion of embryo survival by
removing ceramidas from newly formed embryos, thereby inhibiting the default apoptosis pathway [102].
Moreover, ceramidas attenuate peptidoglycan-induced
COX-2 expression in macrophages [92], and the P. aerug-
inosa ceramidase enhances hemolysis induced phospholi-
pase C [103]. Increasing evidence points to important
roles of ceramidas, specially the Asah1 isoform, in the
outcome and progression of cancer, and the response of
tumors to therapy (reviewed in [33,95,104,105]. Asah1 is
overexpressed in several cancer cell lines and cancer tis-
sues [106-111], which appears to contribute to decreasing
the levels of ceramide and increasing those of S1P. Multi-
ple reports confirm the relationship between Asah1 activ-
ity and radio or chemotherapy resistance, as well as the
interest of Asah1 inhibitors as anticancer drugs. Also, in
most cases, Asah1 inhibition induces apoptosis. In fact,
high levels of Asah1 expression were found in a radiation
resistant glioblastoma cell line when exposed to gamma-
radiation, and sensitivity to radiation was achieved by
treatment with the ceramidase inhibitor N-oleylethala-
nolamine (NOE), which significantly increased ceramide
levels, caspase activation and apoptosis [60]. In search
for ceramidase inhibitors, most efforts have been directed to
Asah1 inhibition, because of their potential used as anti-
proliferative and cytostatic drugs for cancer chemother-
apy. Ceramidase inhibitors have also been used in models
other than cancer. For example, incubation of smooth
muscle cells with oxLDL increased the activities of both
acid and alkaline ceramidas and the mitogenic effect of
oxLDL was inhibited by DMAPP, suggesting a role for
ceramidas (probably through formation of S1P) in the
mitogenic effect of oxLDL [76].

Ceramide 1-phosphate
Phosphorylation of ceramide seems to be the major
mechanism for generation of C1P in cells. The only
enzyme so far identified to induce the biosynthesis of
C1P in mammalian cells is ceramide kinase (CerK). This
enzyme was first observed in brain synaptic vesicles
[112], and then found in human leukemia HL-60 mono-
cytes [55]. CerK was found to be present in both the
microsomal membrane fraction, and the cytosolic frac-
tion of cells [113]. It was postulated that C1P traffics from
the Golgi apparatus along the secretory pathway to the
plasma membrane, and then released into the extracellu-
ar milieu to bind to acceptor proteins such as albumin or
lipoproteins [114]. Recent work by Chalfant and co-work-
ers [115] showed that CerK specifically utilizes ceramide
transported to the trans-Golgi apparatus by ceramide
transport protein (CERT). In fact, downregulation of CERT by RNA interference resulted in strong inhibition of newly synthesized C1P, suggesting that CERT plays a critical role in C1P formation. By contrast, Boath et al. [114] recently reported that the transport of ceramides to the vicinity of CerK was not dependent on CERT. The reason for such discrepancy is unknown at present, but it might be possible that different cell types might have different subcellular distribution of CerK, and that expression of this enzyme might not be equal in all cell types. Concerning regulation of the enzyme activity, the dependency on Ca2+ ions is well established. CerK was also proposed to be regulated by phosphorylation/depolymerization processes [116]. In addition, CerK location and activity seem to require the integrity of its PH domain, which includes a myristoylation site [116]. Another interesting aspect is that although CerK is the only enzyme so far described for generation of C1P in mammalian cells, bone marrow-derived macrophages (BMDM) from CerK-null mice (CerK-/-) still produced significant levels of C1P, suggesting the existence of a metabolic pathway, other than ceramide/CerK, for generation of C1P [114]. In particular, formation of C16-C1P, which is a major species of C1P in cells, was not abolished in (CerK-/-) BMDM. We have previously speculated that two alternative pathways for generation of C1P in cells might be the transfer of a long acyl-CoA chain to S1P by a putative acyl transferase, or cleavage of SM by a PLD-like activity, similar to the existing arthropod or bacterial SMase D. However, work from our own lab [117] and that of others [114] have shown that acylation of S1P to form C1P does not occur in mammalian cells. In addition, we found no evidence for intervention of SMase D activity when using rat fibroblasts. Nonetheless, these possibilities should be further explored in other cell types. Human CerK was cloned by Sugiuira and co-workers [118]. The protein sequence has 537 amino acids with two protein sequence motifs, an N-terminus that encompasses a sequence motif known as a pleckstrin homology (PH) domain (amino acids 32-121), and a C-terminal region containing a Ca2+/calmodulin binding domain (amino acids 124-433). It was found that leucine 10 in the PH domain is essential for its catalytic activity [119]. Also, it was shown that interaction between the PH domain of CerK and phosphatidylinositol 4,5-bisphosphate regulates the plasma membrane targeting and C1P levels [120]. More recently, the existence of a conserved cysteine motif in CerK that is also essential for its function was reported [121]. Also, it has been suggested that subcellular localization of CerK requires the interplay of their PH domain-containing N-terminal regions together with the C-terminal domains [122]. Concerning substrate specificity, phosphorylation of ceramide by CerK is stereospecific [123]. It was reported that a minimum of a 12-carbon acyl chain is required for normal CerK activity, whereas the short-chain ceramide analogues C9-ceramide, C7-ceramide, or C7-ceramide were poor substrates for this enzyme. It was concluded that CerK phosphorylates only the naturally occurring D-erythro-ceramides [123]. However, Van Overloops and co-workers [124] observed that C2-ceramide is a good substrate for CerK, when albumin is used as a carrier, and that C2-ceramide can be converted to C2-C1P within cells. This raises the possibility that C2-C1P is also a natural sphingolipid, capable of eliciting important biologic effects, as previously demonstrated (i.e. stimulation of cell proliferation [125]). The importance of CerK in cell signaling was highlighted using specific RNAi to inhibit this enzyme activity. This treatment blocked arachidonic acid (AA) release and PGE2 production in response to ATP, the calcium ionophore A23187 and interleukin 1-β [19,126]. The relevance of this enzyme in cell biology was also highlighted in studies using CerK-/- mice: specifically, Bornancin and co-workers found a potent reduction in the amount of neutrophils in blood and spleen of these mice, whereas the amount of leukocytes, other than neutrophils, was increased in these animals. These observations pointed to an important role of CerK in neutrophil homeostasis [127]. Recently, a human ceramide kinase-like (CerKL) enzyme was identified in retina [128], and subsequently cloned [129]. However, this enzyme was unable to phosphorylate ceramide, or other related lipids, under conditions commonly used to measure CerK activity, and therefore its role in cell biology remains unclear. Importantly, intracellular formation of C1P was observed after challenging A549 lung adenocarcinoma cells with interleukin 1-β [126], and after treatment of bone marrow-derived macrophages with M-CSF [130]. Also of importance, C1P levels were substantially decreased in apoptotic macrophages, suggesting that C1P plays an important role in cell survival [18,117].

Role of ceramide 1-phosphate in cell growth and survival

We recently reported that the mechanisms by which C1P exerts its mitogenic effects involve stimulation of the mitogen-activated protein kinase kinase (MEK)/Extracellularly regulated kinases 1-2 (ERK1-2), phosphatidylinositol 3-kinase (PI3-K)/Akt (or PKB), and c-Jun terminal kinase (JNK) pathways [130]. We also found that C1P causes stimulation of the DNA binding activity of the transcription factor NF-kB, and increases the expression of glycogen synthase kinase-3β (GSK-3β) leading to upregulation of cyclin D1 and c-Myc, which are important markers of cell proliferation. Moreover, we have evidence suggesting that C1P-stimulated macrophage proliferation, implicates activation of SMS as well as translocation and activation of PKC-α [34], and that phospholipase D
(PLD), intracellular calcium levels, or cAMP are not involved in this process [125,131].

In addition to its mitogenic effect, we also observed that C1P is a potent inhibitor of apoptosis [117,132]. This finding was further supported by Mitra and co-workers [133] who found that down-regulation of CerK in mammalian cells reduced growth and promoted apoptosis in lung epithelial cells. However, Graf and co-workers reported that exogenous addition of the cell permeable C2-ceramide to cells overexpressing CerK led to C2-C1P formation and induction of apoptosis [134]. This contradictory observation can be explained by the fact that overexpression of CerK in the presence of abnormally high concentrations of ceramide (exogenously supplied) would cause and overwhelming increase in the intracellular levels of C1P, thereby reaching C1P concentrations that are toxic for cells. In fact, we observed that in contrast to relatively low concentrations of C1P, which stimulate cell growth and inhibit apoptosis, relatively higher concentrations of C1P are toxic and can kill the cells [117,125]. Concerning apoptosis, we also found that apoptotic bone marrow-derived macrophages have high acid SMase activity and high levels of ceramides compared to healthy cells [135,136]. Investigation into the mechanism whereby C1P exerts its anti-apoptotic effects led us to demonstrate that C1P caused potent inhibition of acid SMase and subsequent depletion of ceramide levels in intact macrophages [117]. C1P also blocked the activity of acid SMase in cell homogenates suggesting that inhibition of this enzyme occurs by direct physical interaction with C1P. It was concluded that C1P is a natural inhibitor of acid SMase, and that inhibition of this enzyme is a major mechanism whereby C1P promotes cell survival [117]. Also, this observation suggests that inhibition of acid SMase by C1P is not mediated through receptor interaction. Acid SMase was also inhibited by S1P in intact macrophages [136], but the mechanism by which this action is brought about remains to be established. Recent work from our lab [137] shows that ceramide levels are also increased in apoptotic alveolar NR8383 macrophages. However, contrary to bone marrow-derived macrophages, there was little activation of neutral and acid SMases in the alveolar macrophages, suggesting that ceramides were generated through a different pathway in these cells. Investigation into the mechanisms whereby ceramide levels increased in alveolar macrophages revealed that activation of SPT, which as mentioned above is the key regulatory enzyme of the de novo pathway of ceramide synthesis, was a major factor in this process. Like for SMases, inhibition of SPT activation by treatment with C1P substantially decreased ceramide generation, and prevented the macrophages from entering apoptosis. It was concluded that C1P promoted macrophage survival by blocking ceramide accumulation through inhibition of either SMase activity, or SPT, depending on cell type. The physiological relevance of the prosurvival effect of C1P was underscored by the demonstration that the intracellular levels of C1P were substantially decreased in apoptotic macrophages. It can be hypothesized that the decrease in C1P concentration could result in the release of acid SMase, or SPT, from inhibition, thereby triggering ceramide generation and apoptotic cell death.

A well-established mechanism by which growth factors promote cell survival is through activation of phosphatidylinositol 3-kinase (PI3-K), which can lead to stimulation of the transcription factor NF-κB, and expression of antiapoptotic genes. Using two different experimental approaches, we demonstrated that PI3-K was a target of C1P in bone marrow-derived macrophages [132]. PI3-K activation was demonstrated by immunoprecipitation of the enzyme from whole cell lysates and assayed in vitro using 32P-phosphatidylinositol. In addition, an in vivo approach provided evidence of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) formation in intact cells that were prelabeled with 32P-orthophosphate [132]. Interestingly, PIP3, which is a major product of PI3-K activity, was shown to directly inhibit acid SMase [138]. Therefore, PI3-K activation may potentiate the inhibitory effect of C1P on acid SMase through generation of PIP3. We also observed that C1P stimulated phosphorylation of PKB, which is a target of kinases from different signaling pathways including PI3-K [139,140], cAMP or cAMP-dependent protein kinase (PKA) [141,142], and PKC-ζ [143]. C1P-induced phosphorylation of PKB was sensitive to inhibition by wortmannin or LY294002, which are selective inhibitors of PI3K. These two inhibitors also blocked the prosurvival effect of C1P indicating that PKB is downstream of PI3-K in macrophages, and important for the antiapoptotic effect of C1P [132]. C1P also caused IκB phosphorylation and stimulation of the DNA binding activity of NF-κB in primary cultures of mouse macrophages [132], and up-regulated the expression of antiapoptotic Bcl-XL, which is a downstream target of NF-κB. The latter results provided the first evidence for a novel biological role of natural C1P in the regulation of cell survival by the PI3-K/PKB/NF-κB pathway in mammalian cells [132].

As mentioned above, C1P can be metabolized to ceramide by phosphatase activity, and then further converted to sphingosine and S1P by ceramidases and sphingosine kinases. Therefore, it could be speculated that the effects of C1P might be mediated through C1P-derived metabolites. However, ceramides and C1P are antagonistic signals, and C1P is unable to mimic many of the effects of sphingosine or S1P (i.e. PLD activation, adenyl cyclase inhibition, or Ca2+ mobilization) [7,64,125,131]. Also, ceramides can decrease the expres-
sion of Bcl-X₁ [19], whereas C1P causes its up-regulation [132]. Finally, no ceramidases capable of converting C1P to S1P have so far been identified, and S1P and C1P inhibit acid SMase by different mechanisms [117,136]. Therefore, it can be concluded that C1P acts on its own right to regulate cell functions. The above observations suggest that the activity of the enzymes involved in ceramide and C1P metabolism must be strictly regulated. Any alteration in the balance between ceramides and C1P could potentially result in metabolic dysfunctions, and could be fatal for cells.

Ceramide 1-phosphate and the control of inflammatory responses

C1P has been demonstrated to be proinflammatory, which in principle is beneficial for protecting the organism against infection or injury. Inflammatory mediators include chemokynes, cytokines, vasoactive amines, products of proteolitic cascades, phospholipases, different forms of eicosanoids, and some sphingolipids. Generation of proinflammatory metabolites, however, should be blocked or at least reduced when inflammation becomes out of control, so as to protect the organism from major damage. Concerning phospholipases, a key mediator of inflammatory responses is cytosolic PLA₂ (cPLA₂), an enzyme that has been involved in receptor-dependent and independent release of arachidonic acid and eicosanoid production. With regards to sphingolipids, some of them have also been described as important mediators of inflammatory responses. For instance, ceramide was initially described as pro-inflammatory for different cell types [144-147], and more recently it has been implicated in the development of allergic asthmatic responses and airway inflammation [148]. In addition, exogenous addition of the short-chain cell permeable C₂-ceramide, to cultured astrocytes upregulated the expression of 12-lipoxygenase, thereby leading to generation of reactive oxygen species (ROS) and the initiation of inflammatory responses [149]. Acid sphingomyelinase-derived ceramide has also been involved in PAF-mediated pulmonary edema [150]. Subsequently, it was proposed that at least some of the pro-inflammatory effects of ceramides might in fact be mediated by its conversion to C1P. The first report on the regulation of arachidonic acid (AA) release and the production of prostaglandins by C1P was by Chalfant’s group [126]. These authors demonstrated that C1P potently and specifically stimulated AA release and prostanoid synthesis in A549 lung adenocarcinoma cells. In the same report, the authors showed that C1P could be generated intracellularly through stimulation of CerK by the action of interleukin 1-β. In a later report, the same group demonstrated that the mechanism whereby C1P stimulates AA release occurs through direct activation of cPLA₂ [151]. Subsequently, Subramanian and co-workers [152] found that C1P is a positive allosteric activator of group IV cPLA₂, and that it enhances the interaction of the enzyme with phosphatidylcholine. The authors concluded that C1P may function to recruit CPLA₂ to intracellular membranes and that it allosterically increases the catalytic ability of the membrane-associated enzyme [152]. In addition, recent studies demonstrated that activation of group IV cPLA₂ by C1P is chain length-specific. In particular, C1P with acyl chains equal or higher than 6 carbons were able to efficiently activate cPLA₂ in vitro, whereas shorter acyl chains (in particular C₂-C1P) were unable to activate this enzyme. C1P was suggested to act in coordination with S1P to ensure maximal production of prostaglandins [153]. For details on the role of C1P in inflammatory response the reader is referred to elegant reviews by Lamour and Chalfant [115]; Wijesinghe et al [154] and Chalfant and Spiegel [19]. It should also be pointed out that C1P is involved in other inflammatory processes including stimulation of phagocytosis in neutrophils [21,22], activation of degranulation in mast cells [113], and more recently, stimulation of macrophage migration [155]. Nonetheless, apart from its clearly proinflammatory actions, C1P might act as anti-inflammatory under specific conditions. In this context, it was postulated that activation of acid SMase plays an important role in pulmonary infections as it facilitates internalization of bacteria into lung epithelial cells [156]. Therefore, the recent finding that C1P potently inhibits acid SMase [116] could be important to reduce or prevent infection in the lung, an action that would obviously result in the inhibition of inflammatory responses.

Ceramide 1-phosphate mediates macrophage migration

Macrophages are involved in a number of chronic diseases that are characterized by unregulated chronic inflammation. These include autoimmune diseases, atherosclerosis, or multiple sclerosis [157], as well as tumor progression and metastasis [158]. Using Raw 264.7 macrophages, our group has recently demonstrated that exogenous addition of C1P potently stimulated cell migration [155]. This action could only be observed when C1P was applied exogenously, but not when C1P was generated intracellularly. The intracellular levels of C1P were enhanced using different experimental approaches, including agonist stimulation of CerK, or delivery of C1P using the photolabile caged-C1P compounds 7-(diethylamino)-coumarin (DECM), or 4-bromo-5-hydroxy-2-nitrobenzhydryl (BHNB) [159] to the cells in culture but macrophages failed to migrate (A. Ouro et al., unpublished work). These observations led to identify a specific plasma membrane receptor that stimulates chemotaxis upon ligation with C1P. This receptor had low affinity for
CIP, with a $K_d$ value of approximately 7.8 μM. In addition, studies using GTPγS, and pertussis toxin, which potently blocks Gi proteins, provided evidence that the CIP receptor is coupled to a $G_i$ protein. Interestingly, ligation of the receptor with CIP caused potent phosphorylation of ERK1-2 and PKB, suggesting that these kinases are downstream of receptor activation. Of importance, inhibition of these pathways with selective inhibitors of MEK, the enzyme that phosphorylate ERK, and selective inhibitors of PI3-K, completely abolished CIP-stimulated macrophage migration. Furthermore, CIP stimulated the DNA binding activity of NF-κB, which is downstream of PKB or ERK, and blockade of this transcription factor also resulted in complete inhibition of macrophage migration. These observations suggested that MEK/ERK1-2, PI3-K/PKB (Akt) and NF-κB are crucial components of the cascade of events leading to stimulation of cell migration by CIP. It is possible that this newly identified receptor as well as the enzymes responsible for CIP generation might be important targets for treatment of illnesses that are associated to inflammation and cell migration, such as atherosclerosis or cancer. In this connection, two inhibitors of CerK have been recently described. One of these inhibitors is an analog of a previously reported SphK inhibitor named F-12509A [160], which inhibits CerK at μmolar concentrations without affecting the activities of SphK or diacylglycerol kinases. A second compound named NVP-231 (adamantane-1-carboxylic acid (2-benzoylamino-benzothiazol-6-yl) amide) [161], inhibited CerK potently in a competitive and reversible manner at low nanomolar concentrations. Interestingly, when NVP-231 was combined with tamoxifen, a drug that is commonly used for treatment of breast cancer [162,163], it synergistically increased ceramide and blocked cell growth [161]. Also of interest, recent work by Zor and co-workers has produced a CIP analogue named phosphoceramide analogue-1 (PCERA-1), which has potent anti-inflammatory properties [164]. The activity of PCERA-1 seems to be mediated by a cell membrane receptor that is distinct to the CIP receptor described here. PCERA-1, and perhaps other compounds that may be eventually derived from modification of its original structure, might turn to also be useful tools for developing alternative strategies for treatment of inflammatory diseases.

**Concluding Remarks**

Detailed knowledge of the mechanisms controlling ceramide and CIP levels, including expression of the enzymes involved in their metabolism, and the receptors implicated in their actions, may be essential for developing molecular strategies to counteract metabolic disorders. Specifically, serine palmitoyltransferase, ceramide synthases, sphingomyelinases, ceramide kinase, cerami-


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