Chapter 8

Ceramide-1-Phosphate in Cell Survival and Inflammatory Signaling

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Abstract

An important metabolite of ceramide is ceramide-1-phosphate (C1P). This lipid second messenger was first demonstrated to be mitogenic for fibroblasts and macrophages and later shown to have antiapoptotic properties. C1P is also an important mediator of the inflammatory response, by stimulating the release of arachidonic acid through activation of group IVA cytosolic phospholipase A_2, the initial rate-limiting step of eicosanoid biosynthesis. C1P is formed from ceramide by the action of a specific ceramide kinase (CerK), which is distinct from the sphingosine kinases that synthesize sphingosine-1-phosphate. CerK is specific for natural ceramides with the erythro configuration in the base component and esterified to long-chain fatty acids. CerK can be activated by different agonists, including interleukin 1-beta, macrophage colony stimulating factor, or calcium ions. Most of the effects of C1P so far described seem to take place in intracellular compartments; however, the recent observation that C1P stimulates cell migration implicates a specific plasma membrane receptor that is coupled to a G_i protein. Therefore, C1P has a dual regulatory capacity acting as an intracellular second messenger to regulate cell survival, or as extracellular receptor ligand to stimulate chemotaxis.

Introduction

Normal development of an organism requires the intervention of complex biological processes that are strictly regulated to maintain cell and tissue homeostasis. These include systems to control cell growth and survival, as well as mechanisms to prevent disease. Alteration of any of these processes can lead to metabolic dysfunction or cause illnesses such as autoimmune diseases, chronic inflammation, neural degeneration, cardiovascular disorders, or cancer.[1-3]

Many sphingolipids are crucial metabolites to control cell activation. Some of them have been described as key regulators of signal transduction processes that are essential for normal development. In particular, ceramides inhibit cell growth and are potent inducers of apoptosis, a form of programmed cell death.[4-17] In neurons however, the situation is controversial as ceramides have been shown to promote either apoptosis or cell survival.[18-22] Also, ceramides play important roles in the regulation of cell differentiation, survival and inflammation[9,13,15,23-28] and are key mediators of radiation and chemotherapy effects on tumors, bacterial and viral infections, heat or UVA injury and ischemia-reperfusion injury (Reviewed by Gulbins and Kolesnick[29]). In addition, ceramides have been associated with insulin resistance through activation of protein phosphatase 2A and the subsequent dephosphorylation and inactivation of protein kinase B (PKB).[30-32] By contrast,

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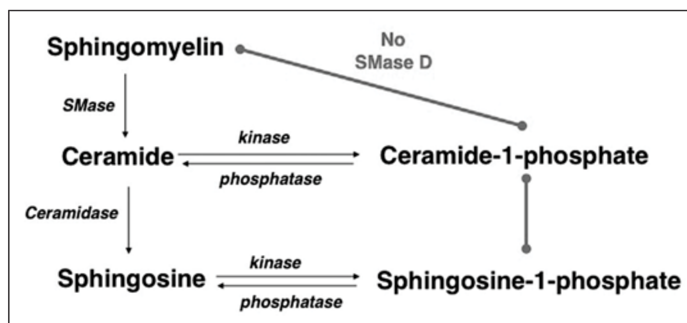

Figure 1. Biosynthesis of sphingosine 1-phosphate and ceramide-1-phosphate.

sphingosylphosphorylcholine,[33] sphingosine-1-phosphate (S1P)[8,34-37] and ceramide-1-phosphate (C1P)[24,38-40] are potent stimulators of cell proliferation. As mentioned in previous chapters of this book, ceramides are generated by de novo synthesis, or can be produced by the action of different sphingomyelinases (SMases). Details on SMase activities, enzymology and compartmentalization are reviewed in several chapters included in this book. Natural ceramides typically have long *N*-acyl chains ranging from 16 to 26 carbons in length[7,43,44] and some times longer in tissues such as skin. Many studies have used a short-chain analog (N-acetylsphingosine, or C_2-ceramide) in experiments with cells in culture because it is more water soluble than long-chain ceramides and it has been presumed that this compound did not occur in vivo. However, recent studies demonstrated that C_2-ceramide does exist in mammalian tissues. In particular, C_2-ceramide was found in rat liver cells[45,46] and brain tissue.[46]

Formation of ceramide is also relevant because it is the precursor of important bioactive sphingolipids that can also regulate cellular functions. For instance, stimulation of ceramidases results in generation of sphingosine (Fig. 1), which is a physiological inhibitor of protein kinase C (PKC).[14] There are numerous reports showing that PKC is inhibited by exogenous sphingosine and Merrill and coworkers demonstrated that addition of the ceramide synthase inhibitor fumonisin B1 to J774 macrophages to increase the levels of endogenous sphingoid bases, also inhibited protein kinase C.[47] Sphingosine can control the activity of other key enzymes involved in the regulation of metabolic or cell signaling pathways such as the Mg^{2+} dependent form of phosphatidate phosphohydrolase,[48,49] phospholipase D (PLD),[50] or diacylglycerol kinase (DAGK)[51,52] in different cell types. Sphingosine, in turn, can be phosphorylated by the action of sphingosine kinases to generate S1P, which is a potent mitogenic agent and can also inhibit apoptosis in many cell types.[8,34,35,37,53,54] More recently, it was demonstrated that S1P stimulates cortisol[55] and aldosterone secretion[56] in cells of the zona fasciculata and zona glomerulosa, respectively, implicating S1P in the regulation of steroidogenesis.

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 (Reviewed in refs. 24, 38) and the Chalfant laboratory group demonstrated that C1P is a key factor in inflammatory responses (Reviewed in refs. 57, 58). In addition, C1P plays a key role in phagocytosis (please see chapter by Hinkovska-Galcheva et al).[59,60] The aim of the present chapter is to review recent progress related to the control of cell survival and the inflammatory response by C1P.

Ceramide-1-Phosphate Synthesis and Degradation

The only enzyme so far identified to produce C1P in mammalian cells is ceramide kinase (CerK). CerK was first observed in brain synaptic vesicles[61] and later found in human leukemia HL-60 cells.[11] This activity was first reported to be confined to the microsomal membrane fraction, but has also been reported to be mainly located in the citosol.[62] These discrepancies might be due to

different degrees of enzyme expression in different cell types and it may also be possible that subcellular localization of this enzyme changes depending on the metabolic status of cells. In fact, Van Veldhoven's group found that tagged forms of human CerK (FLAG-HsCerK and EGFP-HsCerK fusions), upon expression in Chinese Hamster Ovary (CHO) cells, were mainly localized to the plasma membrane, whereas no evidence for an endoplasmic reticulum (ER) association was found.[63] These findings agree with those of Boath et al[64] who have recently reported that ceramides are not phosphorylated at the ER but must be transported to the Golgi apparatus for phosphorylation by CerK. Once generated, C1P traffics from the Golgi network along the secretory pathway to the plasma membrane, where it can be back-exchanged into the extracellular milieu and then bind to acceptor proteins such as albumin or lipoproteins.[64] These observations are consistent with those of Chalfant laboratory;[65] using mass spectrometry and confocal microscopy, Lamour and coworkers demonstrated that CerK utilizes ceramide transported to the trans-Golgi apparatus by ceramide transport protein (CERT). Downregulation of CERT by RNA interference resulted in strong inhibition of newly synthesized C1P, suggesting that CERT plays a critical role in C1P formation. However, this observation has been recently challenged by Boat et al[64] who reported that the transport of ceramides to the vicinity of CerK is not dependent on CERT. The reason for such discrepancy is unknown at present. However, whereas Lamour and coworkers used siRNA technology in their studies to inhibit CERT,[65] Boat et al utilized pharmacological inhibitors.[64] Also, it might be possible that different cell types might have different subcellular distribution of CerK and that expression of this enzyme may not be equal in all cell types. Concerning regulation of enzyme activity, besides its ability to move intracellularly from one compartment to another and its dependency on cations (mainly Ca^{2+} ions) for activity, CerK was proposed to be regulated by phosphorylation/dephosphorylation processes.[66] In addition, CerK is myristoylated, a feature that is related to targeting proteins to membranes. However, elimination of myristoylation did not affect the intracellular localization of the enzyme. Interestingly, CerK location and activity seem to require the integrity of its PH domain, which includes the myristoylation site.[66]

Another important observations was that bone marrow-derived macrophages (BMDM) from CerK-null mice (CerK-/-) still had significant levels of C1P, suggesting the existence of a metabolic pathway, other than ceramide/CerK, for generation of C1P.[64] In particular, formation of C16-C1P, which is a major species of C1P in cells, was not abolished in BMDM. Two alternative pathways for generation of C1P in cells might be acylation of S1P by a putative acyl transferase, or cleavage of sphingomyelin (SM) by activation of SMase D. However, work from our own lab[39] and that of others[64] demonstrated that acylation of S1P to form C1P does not occur in mammalian cells. In addition, C1P could potentially be formed by the action of SMase D, which is a major component of the venom of a variety of arthropodes including spiders of the gender *Loxosceles* (the brown recluse spider *L. reclusa*, *L. amazonica, L. arizonica*, *L. intermedia*, or *L. laeta*) and also in the toxins of some bacteria such as *Corynebacterium pseudotuberculosis*, or *Vibrio damsela*.[67] Although we found no evidence for an analogous activity of SMase D when using rat fibroblasts,[39] this possibility has not been exhaustively explored.

Human CERK was recently reported to be highly dependent on Mg^{2+} ions and less dependent on Ca^{2+}.[63] This enzyme was cloned by Sugiura and coworkers.[69] 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 Ca^{2+}/calmodulin binding domain (amino acids 124-433). Using site-directed mutagenesis, it was found that leucine 10 in the PH domain is essential for its catalytic activity.[70] 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.[71]

With regards to substrate specificity, it was reported that phosphorylation of ceramide by CERK is stereospecific.[72] A minimum of a 12-carbon acyl chain was required for normal CERK activity, whereas the short-chain ceramide analogues C_8-ceramide, C_4-ceramide, or C_2-ceramide were poor substrates for CERK. It was concluded that CERK phosphorylates only the naturally occurring D-erythro-ceramides.[72] However, Van Overloops and coworkers[46] reported that

C_2-ceramide is a good substrate for CerK, when albumin is used as a carrier and that C_2-ceramide can be converted to C_2-C1P within cells. This raises the possibility that C_2-C1P is also a natural sphingolipid, capable of eliciting important biologic effects, as previously demonstrated (i.e., stimulation of cell proliferation[39]). These observations suggest that substrate presentation is an important factor when assaying CerK activity. The importance of CERK in cell signaling was highlighted using specific RNAi to downregulate this enzyme activity. This treatment inhibited arachidonic acid (AA) release and PGE_2 production in response to ATP, the calcium ionophore A23187 and interleukin 1-β.[57,73] The relevance of this enzyme in cell biology was also highlighted in studies using CerK−/− mice; specifically, Bornancin's group found a potent reduction in the amount of neutrophils in blood and spleen of these mice, whereas de amount of leukocytes, other than neutrophils, was increased in these animals. These observations pointed to an important role of CerK in neutrophil homeostasis.[74]

Recently, a human ceramide kinase-like (CERKL) enzyme was identified in retina[75] and subsequently cloned.[76] 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 is unclear.

In a previous report, Hinkovska-Galcheva et al[60] observed that endogenous C1P can be generated during the phagocytosis of antibody-coated erythrocytes in human neutrophils that were primed with formylmethionylleucylphenylalanine and more recently, the same group established that C1P is a mediator of phagocytosis.[59] It was also reported that C1P can be formed in neutrophils upon addition of exogenous cell-permeable (^{3}H)*N*-hexanoylsphingosine (C_6-ceramide) to cells[77] and Riboni and coworkers[78] demonstrated that C1P can be generated in cerebellar granule cells both from SM-derived ceramide and through the recycling of sphingosine produced by ganglioside catabolism. C1P can be also generated by the action of interleukin 1-β on A549 lung adenocarcinoma cells,[73] or by M-CSF on bone marrow-derived macrophages[79] and plays an important role in inflammation.[57,73,80-83] We found that C1P is present in normal bone marrow-derived macrophages isolated from healthy mice[84] and that C1P levels were substantially decreased in apoptotic macrophages, suggesting that C1P plays an important role in cell survival.[38,84]

The identification of C1P phosphatase in rat brain[85] and hepatocytes,[86] together with the existence of CERK suggested that ceramide and C1P are interconvertible in cells. C1P phosphatase is enriched in brain synaptosomes and liver plasma membrane fractions and appeared to be distinct from the phosphatase that hydrolyzes phosphatidic acid (PA), PA phosphohydrolase. Nonetheless, C1P can also be converted to ceramide by the action of a PA phosphohydrolase that is specifically located in the plasma membrane of cells.[87] The latter enzyme belongs to a family of at least three mammalian lipid phosphate phosphatases (LPPs).[88] LPPs have recently been shown to regulate cell survival by controlling the levels of intracellular PA and S1P pools[89] and also to regulate leukocyte infiltration and airway inflammation.[90] Dephosphorylation of C1P might be a way of terminating its regulatory effects, although the resulting formation of ceramide could potentially be detrimental for cells. Controlling the levels of ceramide and C1P by the coordinated action of CERK and C1P phosphatases, may be of crucial importance for the metabolic or signaling pathways that are regulated by these two sphingolipids. Another possibility for degradation of C1P might be its deacylation to S1P, which can then be cleaved to render a fatty aldehyde and ethanolamine phosphate by lyase activity,[4] or to sphingosine by the action of S1P phosphatases (Fig. 1). However, no C1P deacylase or lyase has so far been identified in mammalian cells.

Ceramide-1-Phosphate: A Key Regulator of Cell Growth and Survival

C1P was first found to have mitogenic properties in rat or mouse fibroblasts.[39,40] Using primary macrophages, Gangoiti and coworkers found that like for most growth factors, the mechanisms whereby C1P exerts its mitogenic effects implicate stimulation of the mitogen-activated protein kinase kinase (MEK)/Extracellularly regulated kinases 1-2 (ERK1-2), phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (PKB, also known as Akt) and c-Jun terminal kinase (JNK) pathways.[79] In addition, C1P caused stimulation of the DNA binding activity of the transcription factor NF-κB.

Another major target of PKB is glycogen synthase kinase-3β (GSK-3 β), which expression was also increased by C1P. This led to up-regulation of cyclin D1 and c-Myc, two important markers of cell proliferation that are targets of GSK-3β. Other effectors usually involved in the regulation of cell growth, such as diacylglycerol and subsequent activation of protein kinase C (PKC), phospholipase D (PLD), intracellular calcium levels, or cAMP were not affected by C1P.[39,40] However, the short-chain analogues, C_2-C1P- or C_8-C1P, induced Ca^{2+} mobilization in calf pulmonary artery endothelial (CAPE) cells,[91] thyroid FRTL-5,[92] or Jurkat T-cells.[93] By contrast, the short-chain C1Ps did not induce Ca^{2+} mobilization in fibroblasts[39,40] or neutrophils[77] and natural C_{16}-C1P failed to alter intracellular Ca^{2+} concentrations in A549 cells.[80] Whether or not natural C1P is able to affect Ca^{2+} homeostasis in any cell type still remains to be determined.

We previously demonstrated that natural C1P blocked cell death in bone marrow-derived macrophages that were incubated in the absence of macrophage-colony stimulating factor (M-CSF),[84] a condition known to induce apoptosis in these cells.[94,95] We found that C1P blocked both DNA fragmentation and the stimulation of the caspase-9/caspase-3 pathway, thereby suggesting that the prosurvival effect of C1P was due to inhibition of apoptosis.[84] Consistent with these observations, Mitra and coworkers[96] found that down-regulation of CerK in mammalian cells reduced growth, promoted apoptosis and blocked epithelial growth factor-induced cell proliferation. In addition, C1P was reported to induce the synthesis of S1P,[92] an important pro-mitogenic and anti-apoptotic sphingolipid metabolite. However, contrary to these observations, Graf and coworkers showed that addition of the cell permeable C_2-ceramide to cells overexpressing CerK led to C_2-C1P formation and apoptosis.[97] Indeed, overexpression of CerK would potently increase formation of intracellular C1P, particularly if cells were supplied with high concentration of exogenous ceramide and this would result toxic for cells. In this context, we reported previously that relatively high concentrations of C_2-C1P are less effective at stimulating cell division[39] or inhibiting apoptosis[84] than low concentrations and that C_2-C1P or natural C1P are toxic for cells at high concentrations.[84]

Another relevant finding was that apoptotic bone marrow-derived macrophages posses high acid SMase activity and high levels of ceramides compared to healthy cells.[95,98] Investigation into the mechanism whereby C1P exerts its anti-apoptotic effects demonstrated complete inhibition of acid SMase and ceramide accumulation by C1P in intact macrophages.[84] C1P also blocked the activity of acid SMase in cell homogenates suggesting that inhibition of this enzyme occurs by direct physical interaction with C1P. The concentrations of C1P needed to fully block SMase activity in intact cells were in the micromolar range (30 μM);[84] however, only 4% of C1P was incorporated by cells.[40] It was concluded that C1P is a natural inhibitor of acid SMase and that inhibition of this enzyme activity is a major mechanism whereby C1P promotes cell survival.[84] 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,[98] but unlike C1P the inhibitory effect of S1P did not involve direct interaction with the enzyme. Also of interest, activation of acid SMase plays an important role in pulmonary infections as it facilitates internalization of bacteria into lung epithelial cells.[29] Therefore, inhibition of acid SMase by C1P could be important to reduce or prevent infection in the lung. Recent work by Granado and coworkers (unpublished work) showed that ceramide levels are also increased in apoptotic alveolar NR8383 macrophages. There was only marginal activation of neutral and acidic SMases, suggesting a different source for ceramide formation in these cells. Investigation into the mechanism whereby ceramide levels increased in alveolar macrophages revealed that activation of serine palmitoyltransferase (SPT), the key regulatory enzyme of the de novo pathway of ceramide synthesis, was a major factor in this process. Interestingly, inhibition of SPT activation by treatment with C1P prevented the macrophages from entering apoptosis. It can be concluded that C1P promotes macrophage survival by blocking ceramide accumulation through inhibition of either SMases, or SPT, depending on cell type.

The physiological relevance of the prosurvival effect of C1P is underscored by the demonstration that intracellular levels of C1P are substantially decreased in apoptotic macrophages. It was hypothesized that the decrease in C1P concentration could result in the release of acid SMase from inhibition, thereby triggering ceramide generation an apoptotic cell death.[84]

A major mechanism whereby growth factors promote cell survival is 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, it was demonstrated that PI3-K was a target of C1P in bone marrow-derived macrophages.[99] PI3-K activation was demonstrated by immunoprecipitation of the enzyme from whole cell lysates and assayed in vitro using ^{32}P-phosphatidylinositol. In addition, an in vivo approach provided evidence of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) formation in intact cells that were prelabeled with ^{32}P-orthophosphate.[99] PIP3 is a major product of PI3-K activity and was shown to directly inhibit acid SMase.[100] Therefore, PI3-K activation may potentiate the inhibitory effect of C1P on acid SMase through generation of PIP3. Whether C1P and PIP3 bind to the same or different domains of acid SMase remains to be elucidated. C1P stimulated the phosphorylation of protein kinase B (PKB), which is a target of kinases from different signaling pathways including PI3-K,[101,102] cAMP or cAMP-dependent protein kinase (PKA)[103,104] and PKC-ζ.[105] C1P-induced phosphorylation of PKB was sensitive to inhibition by wortmannin or LY294002, which are inhibitors of PI3-K activity. 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.[99] Another relevant finding was that C1P caused IκB phosphorylation and stimulation of the DNA binding activity of NF-κB in primary cultures of mouse macrophages.[99] Of note, C1P up-regulated the expression of anti-apoptotic Bcl-X_L, 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.[99]

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, adenylyl cyclase inhibition, or Ca^{2+} mobilization).[24,39,40,106,107] Also, ceramides can decrease the expression of Bcl-X_L,[57] whereas C1P causes its up-regulation.[99] Finally, no ceramidases capable of converting C1P to S1P have so far been identified and S1P and C1P inhibit acid SMase by different mechanisms.[84,98] Therefore, it can be concluded that C1P acts on its own right to regulate cell functions.

It is obvious from the above observations that the activity of the enzymes involved in ceramide and C1P metabolism must be strictly regulated so that cells can maintain appropriate levels of pro- versus anti-apoptotic metabolites. Any alteration in the balance between ceramides and C1P could potentially result in disease, or be fatal for cells. Detailed investigation into the mechanisms controlling ceramide and C1P levels may facilitate the development of new molecular strategies for counteracting metabolic disorders, or for prevention and treatment of disease.

Ceramide-1-Phosphate and the Control of Inflammatory Responses

Initially, inflammation is beneficial for protecting the organism against infection or injury, but it can be detrimental when it becomes out of control. It was proposed that inflammation evolved as an adaptive response for restoring homeostasis. In general, the acute inflammatory response triggered by infection or tissue injury involves the coordinated delivery of blood components (plasma and leukocytes) to the site of injury or infection.[108] Inflammatory mediators include chemokines, cytokines, vasoactive amines, products of proteolytic cascades, phospholipases, or lipids such as eicosanoids and sphingolipids. Concerning phospholipases, a major mediator of inflammatory responses is PLA_2. In particular, group IV cytosolic PLA_2 exhibits properties of a receptor regulated enzyme and has been involved in receptor-dependent and independent eicosanoid production. With regards to lipid metabolites, some sphingolipids have been described as key mediators of inflammatory responses. This is the case of ceramide, which was initially described as pro-inflammatory for different cell types.[109-112] More recently a role for ceramide in the development of allergic asthmatic responses and airway inflammation was established[113] and exogenous addition of C_2-ceramide to cultured astrocytes induced 12-lipoxigenase leading to generation of reactive

oxygen species (ROS) and inflammation.[114] Also, acid sphingomyelinase-derived ceramide was involved in PAF-mediated pulmonary edema.[115] Subsequently, it was proposed that at least some of the pro-inflammatory effects of ceramides may actually 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 the Chalfant laboratory.[73] 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 $cPLA_2$.[80] Subsequently, Subramanian and coworkers[83] found that C1P is a positive allosteric activator of group IV $cPLA_2$ and that it enhances the interaction of the enzyme with phosphatidylcholine. The authors concluded that C1P may function to recruit $cPLA_2\alpha$ to intracellular membranes and that it allosterically increases the catalytic ability of the membrane-associated enzyme.[83] Recent studies by Chalfant and coworkers also demonstrated that activation of group IV $cPLA_2$ by C1P is chain length-specific; in particular, C1P bearing acyl chains equal or higher than 6 carbons were able to efficiently activate $cPLA_2\alpha$ in vitro, whereas shorter acyl chains (in particular C_2-C1P) were unable to activate the enzyme. It was concluded that the biological activity of C_2-C1P does not occur via eicosanoid synthesis.[116] Also, C1P was shown to act in coordination with S1P to ensure maximal production of prostaglandins. Specifically, S1P induces cyclooxigenase-2 (COX-2) activity, which then uses $cPLA_2$-derived AA as substrate to synthesize prostaglandins.[81] For details on the role of C1P in inflammatory response the reader is referred to elegant reviews by Lamour and Chalfant;[65] Wijesinghe et al[117] and Chalfant and Spiegel.[57] Lastly, it should be pointed out that C1P is also involved in other inflammation processes including stimulation of phagocytosis in neutrophils[59,60] and activation of degranulation in mast cells.[62]

Ceramide-1-Phosphate Mediates Macrophage Migration

Macrophages are an important component of both innate and adaptive immunity. They are also involved in a number of chronic diseases characterized by unregulated chronic inflammation, such as autoimmune diseases, atherosclerosis,[118] or multiple sclerosis[119] and in tumor progression and metastasis.[120] Macrophage populations in tissues are determined by the rates of recruitment of monocytes from the bloodstream into the tissue, the rates of macrophage proliferation and apoptosis and the rate of macrophage migration or efflux. Recently, our group demonstrated that exogenous addition of C1P to cultured Raw 264.7 macrophages induced cell migration.[121] This action could only be observed when C1P was applied exogenously and not by increasing the intracellular levels of C1P (i.e., through agonist stimulation of CerK). This fact led us to identify a specific receptor through which C1P stimulates chemotaxis. This is a low affinity receptor with a K_d of approximately 7.8 μM. Although relatively high concentrations (10-20 μM) of C1P are needed for optimal activation of the receptor, it should be borne in mind that C1P was added forming vesicles (sonicated in water) and therefore the concentration that was actually available to the cells was much lower than was added. In addition, C1P tightly binds to serum proteins and the bovine serum albumin that are present in the culture medium (at 0.2 and 0.1 %, respectively) making C1P even less available to the cells. The C1P receptor is coupled to G_i proteins and causes phosphorylation of ERK1-2 and PKB upon ligation with C1P. It was found that inhibition of either of these pathways completely abolished C1P-stimulated macrophage migration. In addition, C1P stimulated the DNA binding activity of NF-κB and blockade of this transcription factor resulted in complete inhibition of macrophage migration. These observations suggested that MEK/ERK1, PI3-K/PKB (Akt) and NF-κB are crucial components of the cascade of events leading to stimulation of cell migration by C1P. It can be concluded that this newly identified receptor could be an important drug target for treatment of illnesses that are associated to inflammatory processes, or to diseases in which cell migration is a major cause of pathology, as it occurs in atherosclerosis or in metastatic tumors. Recently, a C1P analogue named phosphoceramide analogue-1 (PCERA-1) did not block activation of NF-κB in Raw 264.7 macrophages and was suggested to have

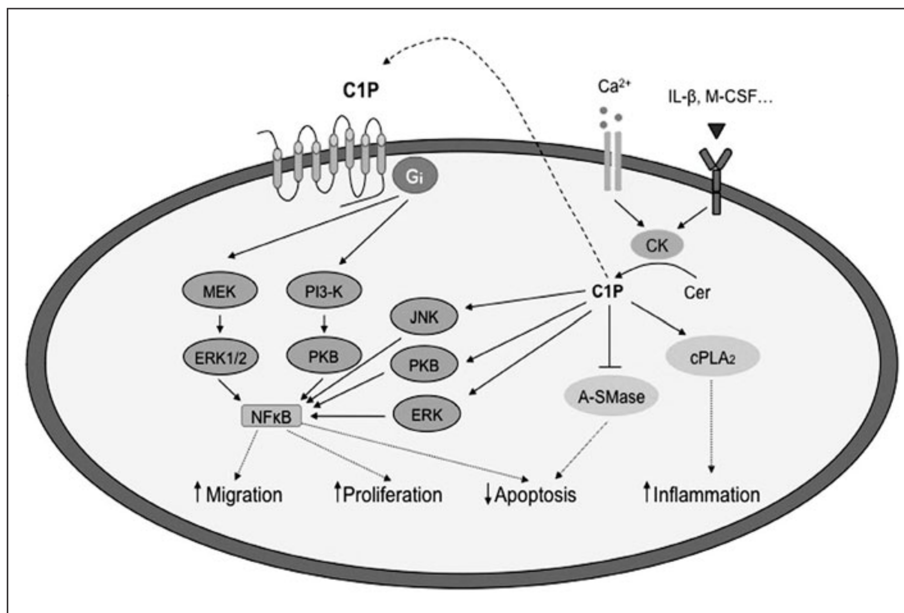

Figure 2. Working model for the induction of cell survival and inflammatory responses by ceramide-1-phosphate.

anti-inflammatory properties. The anti-inflammatory activity of PCERA-1 seems to be mediated by a cell membrane receptor that seems to be distinct to the C1P receptor. This compound, or some of its derivatives, may result in promising tools for blocking inflammation.[122]

Conclusion

In light of the pro-survival and pro-inflammatory actions of C1P, the enzyme responsible for C1P formation, CerK, might be an important target for development of novel therapeutic compounds for treatment of inflammatory illnesses, including atherosclerosis or tumorigenesis. Overexpression of $cPLA_2$, a major target of C1P, has been observed in several human cancers and downregulation of this enzyme causes reduction in the size of tumors. Blockade of $cPLA_2$ through inhibition of C1P formation might have similar effects as knocking down $cPLA_2$, thereby causing a reduction of AA release and prostanoid formation. Further characterization and cloning of the newly identified C1P receptor may provide the means for development of new pharmacological tools for treatment of these illnesses. A working model for the action of C1P is highlighted in Figure 2.

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