REVIEW

Role of Platelet Activating Factor as a Mediator of Inflammatory Diseases and Preterm Delivery

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Nearly 70% of preterm deliveries occur spontaneously, and the clinical pathways involved include preterm labor and premature rupture of membranes. Prediction of preterm delivery is considered crucial due to the significant effects of preterm birth on health and the economy at both the personal and community levels. Although similar inflammatory processes occur in both term and preterm delivery, the premature activation of these processes or exaggerated inflammatory response triggered by infection or sterile factors leads to preterm delivery. Platelet activating factor (PAF) is a phosphoglycerylether lipid mediator of inflammation that is implicated in infections, cancers, and various chronic diseases and disorders including cardiovascular, renal, cerebrovascular, and central nervous system diseases. In gestational tissues, PAF is proposed to act as an inflammatory pathway that stimulates the effector mechanisms of labor, including myometrial contraction, cervical dilation, and fetal membrane rupture. Studies have shown that women with preterm labor and preterm premature rupture of membranes have increased levels of PAF in their amniotic fluid. In mice, the intrauterine or intraperitoneal administration of carbamyl PAF activates inflammation in gestational tissues, thereby eliciting preterm delivery. In this review, the authors summarize recent research on PAF as an important inflammatory mediator in preterm delivery and in other inflammatory disorders, highlighting its potential value for prediction, intervention, and prevention of these diseases. (Am J Pathol 2024, 190:1–17; https://doi.org/10.1016/j.ajpath.2024.01.018)

Preterm delivery affects around 11% of births globally, mainly in low- and middle-income countries.1 Preterm delivery is ranked fifth among the leading causes of the global disease burden and disability.2 The rate of preterm birth is between 8.1% and 11.2% in Malaysia.3 It is estimated that globally, 1 million children under 5 years of age die annually due to complications of preterm birth.4,5

Other than personal and obstetrical history, the prediction of preterm delivery depends primarily on maternal characteristics,6 cervical length measurement, and biochemical markers, such as fetal fibronectin in cervicovaginal fluid.7,8 Novel emerging tests developed for different groups of women at risk of preterm delivery are also available, including detection of inflammatory markers and cytokines in cervicovaginal fluid9 and blood,10 phosphorylated insulin-like growth factor binding protein-1 in cervical fluid,11 placental alpha-microglobulin-1 in vaginal fluid,12,13 and acetyl in cervicovaginal fluid.14 Research focusing on discovering novel markers that can be used in predictive tests to inform prophylactic interventions is also pivotal. Different triggering factors, alterations in the nature and scale of the cellular inflammatory response, and diminished coordinated regulation contribute to preterm delivery.15,16 Thus, inflammatory pathways can potentially be targets for the treatment and prevention of preterm delivery.

Platelet activating factor (PAF) participates in various physiological and pathophysiological processes. PAF has

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been implicated as a proinflammatory molecule in cardiovascular, renal, cerebrovascular, and central nervous system diseases, cancers, and infections, as well as in anaphylaxia and allergies. In pregnancy, PAF, found in the gestational tissues, including amniotic fluid, myometrium, decidua, and the cervix, is proposed to mediate the inflammatory pathways that activate the mechanisms of labor. The elevation of PAF in amniotic fluid has been demonstrated in both preterm labor (PTL) and preterm premature rupture of membranes (pPROM). Carbamyl PAF (cPAF) has also been shown to stimulate inflammation in gestational tissues, leading to preterm delivery in mice. These results suggest the potential contribution of PAF in mediating inflammation that elicits preterm delivery in both humans and mice.

### Inflammatory Pathways in Preterm Delivery

Ascending intrauterine infection is a known risk factor for preterm delivery. Different pathways have been proposed for the invasion of the amniotic cavity by microorganisms, but the most common one seems to be ascending microbial invasion from the vagina and cervix. The microorganisms frequently detected in the amniotic fluid, either by cultivation or molecular microbiologic technique, include Mycoplasma sp., Ureaplasma sp., Gardnerella vaginalis, Fusobacteria species, and Candida albicans. Infection of the amniotic cavity triggers preterm birth via the stimulation of inflammatory processes that activate the mechanisms of labor.

In PTL and pPROM women, sterile intra-amniotic inflammation is reported to be more common than intra-amniotic inflammation with microbial trigger. Oxidative stress resulting from the imbalance between reactive oxygen species production and the level of antioxidants, can occur in response to pregnancy risk factors, such as cigarette smoke, environmental pollutants, sterility, and infection. Oxidative stress activates p38 mitogen-activated protein kinase (MAPK), causing fetal membrane senescence. Senescent cells further release senescence-associated secretory phenotypes (SASP), such as cytokines, chemokines, growth factors, and metalloproteinases (MMPs). SASP then triggers the release of damage-associated molecular patterns (DAMPs) or alarmins. Uterine overdistension caused by multiple pregnancies and polyhydramnios also stimulates inflammation, possibly by causing the secretion of DAMPs. Intracellular DAMPs include nuclear proteins, such as high mobility group box-1 (HMGB1), mitochondrial components (DNA, N-formylated peptides, ATP), cell-free DNA, uric acid, and heat shock protein 70 (Hsp70). Further, matrix proteins such as hyaluronan fragments, fetuin fibronectin, advanced glycosylation end-products (AGEs), oxyesteroids, oxidized low-density lipoprotein (oxLDL), oxidized phospholipids, and fatty acids are considered extracellular DAMPs.

### PAF Induction of Inflammatory Pathways Dependent and Independent of the PAF Receptor

PAF is produced in the blood, lungs, kidneys, myocardium, brain, liver, skin, saliva, retina, uterus, and embryo by specific stimulation of various cell types in these sites. These cell types include platelets, endothelial cells, macrophages, neutrophils, mast cells, basophils, and eosinophils. Notably, numerous structurally related phospholipids known as PAF-like lipids, which do not share structural similarity to PAF, can interact with the PAF receptor (PAFR). PAFR, encoded by PTAFR gene is a G-protein coupled receptor expressed mainly on human platelets, monocytes, neutrophils, and endothelial cells.

Both the formation of PAF and PAF-like lipids and the expression of PAFR are enhanced by numerous risk factors and are associated upstream of proinflammatory triggers, such as cytokines, oxidative stress, and PAF. The interaction between PAF and PAFR subsequently induces intracellular signaling cascades and gene-expressions. The cascade is initiated by the stimulation of PAFR signaling via a Gq-linked mechanism, which causes PLCβ-mediated hydrolysis of PIP2 to produce IP3 and DAG. This results in a transient rise in cytosolic Ca2+ released from intracellular stores and the subsequent activation of PKC. The increase in Ca2+ also stimulates cytosolic cPLA2 and the PAF biosynthetic enzyme known as LPCAT for the synthesis of PAF and other secondary lipid messengers to form a PAF cycle that will further enhance early inflammatory stimuli. The interaction between PAF and its receptor present on inflammatory cells, including macrophages, neutrophils, mast cells, basophils, and eosinophils, can induce positive feedback, leading to the escalated synthesis of PAF and subsequent inflammatory reactions, implying that PAF itself can promote PAF synthesis by these cells. Further, signaling through Gi-associated PAFR impedes the conversion of ATP to cAMP, thus blocking the activation of protein kinase A (PKA) and related signaling cascades. The translocation of NFKB, which causes the expression of genes involved in inflammatory reactions, including IL1, IL6, and tumor necrosis factor (TNF), also occurs due to the interaction between PAF and PAFR. This results in the synthesis of proinflammatory cytokines and inflammatory mediators, such as IL-1, IL-6, TNF, growth factors, lipid mediators, including PAF, PAF-like lipids, and eicosanoids, integrins, selectins, metalloproteinase, reactive oxygen species, and reactive nitrogen species (RNS). Therefore, the onset and development of chronic inflammatory diseases and disorders can be triggered PAF/PAFR signaling. The inflammatory signaling pathways mediated by PAF in these chronic conditions are depicted in Figure 1.
In vivo and in vitro experiments revealed that PAF activates the nucleotide-binding domain, leucine-rich-repeats-containing proteins 3 (NLRP3) inflammasome independently of PAFR, resulting in the synthesis of IL18 and IL1B. This activation of the NLRP3 inflammasome required NLRP3-specific-associated protein (NEK7), adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, potassium efflux, and calcium influx. The role of PAFR antagonists assessed in clinical trials against infections and different target diseases, including cardiovascular system diseases, cancers, allergies, and asthma, has been previously reviewed. Although some trials showed positive outcomes, some issues were reported regarding the efficacy of these PAFR antagonists, indicating that PAF may activate inflammation independently of PAFR.

Biosynthesis and Degradation of PAF

PAF biosynthesis includes two main regulatory enzymes for the remodeling and de novo pathways, namely lyso-PAFAT and dithiothreitol (DTT)-insensitive CDP-choline phosphotransferase, respectively. The remodeling enzymatic pathway of PAF synthesis involves the formation of lyso-PAF (1-O-alkyl-sn-glyceryl-3-phosphorylcholine) as a PAF precursor by PLA2 acting on phosphatidylcholine in the internal cell membrane. Lyso-PAFAT, including LPCAT-1 and LPCAT-2, then acetylates lyso-PAF in the sn-2 position of the glycerol backbone, thereby converting it to an active form of PAF.
acetyltransferase, forming alkyl-acetyl-glycerophosphate, a catalysis involving an acetyltransferase (different lyso-PAF). Alkyl-acetyl-glycerophosphate is then hydrolyzed by phosphohydrolase, forming 1-O-alkyl-2-acetyl-sn-glycerol. DTT-insensitive CDP-choline phosphotransferase subsequently mediates the transfer of CDP-choline to 1-O-alkyl-2-acetyl-sn-glycerol, forming PAF.

PAFAH, also known as phospholipase A2, transforms PAF to its inactive form of lyso-PAF by removing the acetate group from the PAF molecule. PAFAH is synthesized mainly by hepatocytes and macrophages, and is found throughout human plasma, blood, and tissues. There are three types of PAFAHs available: plasma PAFAH, intracellular PAFAH, and PAFAHII. During inflammation and oxidative stress, elevated reactive oxygen species and RNS induce the synthesis of PAF and PAF-like lipids without the participation of regulatory enzymes. For example, oxidative stress results in the oxidation of other lipids, producing PAF-like lipids, such as oxLDL that mimic the activities of PAF. The biosynthetic and enzymatic catabolic pathways of PAF are illustrated in Figure 2.

The Roles of PAF in Diseases Mediated by Inflammation

Studies in humans have documented the role of PAF as a proinflammatory molecule in cardiovascular, renal, and central nervous system diseases, tumors, anaphylaxis, allergies, and infections.

**Cardiovascular Diseases**

The crucial role of platelets in initiating the mechanisms for atherosclerotic plaque development, progression, and subsequent rupture by exerting direct effects on the endothelium and interacting with other cells of the vascular system has recently been reviewed. Immature monocyte-derived dendritic cells are among the first immune cells to infiltrate atherosclerotic plaques, and the recruitment of these cells is stimulated by PAF secreted by activated endothelial cells. PAF has been reported to promote the expression of IL8 and IL6 genes in human immature monocyte-derived dendritic cells. Macrophages are known as the most abundant leukocytes infiltrating atherosclerotic plaque and are thus recognized as the key inflammatory cell in atherosclerosis. PAF induced the transcription of various PTAFR, proinflammatory, and proatherogenic genes in human monocyte-derived macrophages in vitro. These findings indicate that PAF-activated macrophages and dendritic cells contribute to the initiation and progression of atherosclerotic plaque via the production of proinflammatory cytokines and proatherogenic mediators.

Further, macrophages have been shown to internalize various forms of oxLDL, causing the accumulation of intracellular cholesterol and the formation of foam cells.
Foam cells trigger various pathways of programmed cell death in the atherosclerotic plaques, leading to enlargement of the necrotic cores that diminish plaque stability.\textsuperscript{99} In vitro experiments revealed that human monocytes/macrophages treated with a PAFR antagonist and macrophages from \textit{Ptafr} knockout mice exhibited defective uptake of oxLDL.\textsuperscript{90} This evidence implies that the mechanism of foam cell formation in atherosclerotic plaques is mediated by PAF signaling via PAFR. Pretreatment of rats with a PAF antagonist before the induction of angina using ornipressin or epinephrine plus phentolamine, reduced ST-segment depression, but not blood pressure and heart rate.\textsuperscript{93} This study and others\textsuperscript{100} infer that PAF contributes to the development of myocardial ischemia.

Renal Disorders

In vitro experiments utilizing human mesangial cells revealed the role of PAF in enhancing the expression of \textit{PTAFR}, as well as lipid accumulation, in these cells.\textsuperscript{101} Lipid accumulation in human mesangial cells is known as to be the mechanism that causes glomerulosclerosis and thus renal injury. The genetic ablation of \textit{Ptafr} ameliorated renal dysfunction, the development of acute tubular damage, and segmental interstitial fibrotic lesions in mice induced with renal injury using folic acid.\textsuperscript{102} Induced renal injury in \textit{Ptafr} knockout mice also showed decreased expression of \textit{Tnf} and \textit{C-C motif chemokine ligand 2 (CCL2)} genes, with the subsequent diminished recruitment of neutrophils and macrophages into the injured kidney and chronic interstitial fibrotic lesions, respectively.\textsuperscript{103} Thus, the interaction between PAF and PAFR is implicated in the development of renal injury and fibrosis by stimulating inflammation.

\textit{Ptafr}\textsuperscript{−/−} mice that underwent unilateral ureter obstruction failed to develop the two main characteristics of chronic kidney diseases, which are renal dysfunction and fibrosis.\textsuperscript{103} These mice exhibited decreased formation of extracellular matrix (ECM), gene expression of adhesion molecules, and synthesis of proinflammatory cytokines in the kidneys.\textsuperscript{104} In this model of obstructive nephropathy, PAF signaling via PAFR potentiates inflammation in the kidneys, leading to the development of fibrosis, renal dysfunction, and chronic kidney disease.

Both desialylated and degalactosylated polymeric IgA and aberrantly glycosylated IgA1 obtained from sera of patients with IgA nephropathy stimulated mesangial cells to secrete PAF, which caused a decrease in nephrin expression associated with cytoskeleton reorganization by the cultured human glomerular epithelial cells.\textsuperscript{105} Furthermore, glomerular epithelial cells transfected with a vector carrying the cDNA for human plasmatic PAFAH did not exhibit a substantial loss of nephrin when incubated with supernatants from mesangial cells pretreated with aberrantly glycosylated IgA isolated from patients with IgA nephropathy.\textsuperscript{104} Patients with IgA nephropathy who developed proteinuria were also observed to have diminished nephrin expression in renal biopsy samples and elevated PAFAH activity in the serum.\textsuperscript{104} These findings in IgA nephropathy patients can explain the contribution of PAF in causing proteinuria and loss of nephrin, which accelerates the progression toward renal failure.

An in vitro model of diabetic nephropathy experiments revealed that PAF stimulated the deposition of ECM molecules, such as fibronectin and collagen, when human mesangial cells were treated with both high glucose and lysophosphatidylcholine (LPC).\textsuperscript{105} In patients with glucose and lipid metabolism disorders, an elevation in ECM deposition is known to be a risk factor for the development of glomerular fibrosis and diabetic nephropathy. In rats induced with diabetic nephropathy, the development of the disease was ameliorated by rupatidine, a dual antagonist of histamine 1 and PAF receptors.\textsuperscript{106} The development of diabetic nephropathy was demonstrated by the elevation in oxidative stress markers, inflammatory cytokines, premature senescence markers, renal injury markers, and fibrosis markers, as well as morphological damage of the renal glomeruli and tubules in rats treated with streptozotocin.\textsuperscript{107} These results substantiate the role of PAF/PAFR signaling in the pathogenesis of diabetic nephropathy.

Central Nervous System Disorders

Following global cerebral ischemia and reperfusion, \textit{Ptafr} knockout mice showed improved neurological outcome, associated with a decrease in the size of the infarcted brain area, neuropathological lesions, caspase-3 activation, vascular permeability, brain edema, and levels of inflammatory cytokines in the brain.\textsuperscript{108} The administration of recombinant PAFAH (rPAFAH) to mice that underwent similar transient cerebral ischemia resulted in reduced volume of the cerebral infarct and decreased levels of the neurovascular injury mediators MMP2 and MMP9 in the brain.\textsuperscript{109} However, elevated levels of vascular endothelial growth factor (VEGF) in the brains of these mice given rPAFAH suggest that VEGF potentially has a neuroprotective effect by promoting neurogenesis and angiogenesis.\textsuperscript{108} These studies reflect the important role of PAF signaling via PAFR in mediating the pathogenesis of ischemic stroke by triggering a neuroinflammatory response.

Rats that received a PAFR antagonist before undergoing spinal cord injury had reduced expression of proinflammatory cytokine genes, such as \textit{Il1a}, \textit{Il1b}, and \textit{Il6} in their spinal cord tissue.\textsuperscript{109} Rats that received spared nerve injury had increased \textit{Lpcat1} mRNA and PAFAH levels in the spinal cord microglia.\textsuperscript{110} In this animal model of peripheral nerve injury, \textit{Lpcat1} and \textit{Lpcat2} mRNA were also constitutively expressed in spinal cord neurons.\textsuperscript{110} The development of mechanical allodynia was also inhibited in spared nerve injury-induced rats that were given an intrathecal PAFR antagonist.\textsuperscript{110} Therefore, following spinal cord injury, PAF/PAFR signaling, especially in glial cells and
neurons, causes inflammation and toxicity that may mediate the development of neuropathic pain.

After induced spinal cord injury, Ptafr knockout mice showed improved functional recovery, reduced inflammation, and inhibited astroglial activation and remodeling of the ECM, with improved axonal plasticity and regeneration. The administration of a PAFR antagonist to mice that underwent spinal cord injury also increased their functional recovery, especially during the chronic phase. Thus, the inhibition of PAF signaling via PAFR is also implicated in attenuating the inflammatory response and reactive gliosis, thus enhancing functional recovery after spinal cord injury.

PTAFR and IL17 gene expression were up-regulated in the peripheral blood of patients with relapsing multiple sclerosis, and the levels PTAFR expression were significantly correlated with multiple sclerosis disability scores. Organotypic cerebellar slices dissected from both wild-type mice that were treated with PAF after induced demyelination using LPC had fewer myelinated fibers, increased microglial activation, and down-regulated expression of the transforming growth factor beta (TGFβ) gene, signifying the role of PAF in deteriorating the LPC-induced demyelination. Similar effects were observed in the LPC-stimulated demyelination of cerebellar slices isolated from Ptafr knockout mice following the administration of PAF. However, the use of cerebellar slices dissected from Ptafr–/– mice and treated with both LPC and PAF showed the reduced expression of Il1b mRNA in these tissues compared to wild-type mice, implying that the role of PAF in increasing the severity of LPC-induced demyelination is PAFR-dependent and independent.

In the induced experimental allergic encephalomyelitis (EAE) mouse model of multiple sclerosis, genetic ablation of Ptafr resulted in milder disease with diminished inflammatory cell recruitment into the spinal cord, and reduced levels of IL17, (CCL2), CCL5, N-acetyl-beta-D-glucosaminidase (NAG) activity (an index of monocyte-derived macrophage infiltration), CD4+ leukocytes, and IL-17+ leukocytes in the brains of mice. Mice with induced EAE showed a higher expression of Ptafr mRNA and PAF levels in the spinal cord. In this EAE mouse model, the incidence of the disease and the severity of the symptoms were diminished in mice with genetic ablation of Ptafr. The grades of inflammation and demyelination in the spinal cord were diminished during the chronic phase, but not in the acute phase, of these Ptafr knockout EAE mice. This may be due to the observation that macrophages isolated from these Ptafr–/– EAE mice showed reduced phagocytic activity and TNF secretion when stimulated with PAF. Demyelination is known to occur when infiltrated macrophages phagocytose and digest myelin. The findings highlight the importance of PAF interaction with PAFR in the induction and development of EAE, especially during the chronic phase, by contributing to inflammation and demyelination. In a mouse model of severe meningoencephalitis, genetic ablation of Ptafr has also been shown to delay lethality and reduce the level of CXCL9 as well as the number of leukocytes, including CD4+ T cells, CD8+ T cells, and macrophages, in the brains of mice following the inoculation of herpes simplex virus-1 (HSV-1) intracranially. In meningoencephalitis, PAF/PAFR signaling is implicated in the neuroinflammatory response that leads to death.

In vitro experiments revealed that PAF activated the cholesterol ester hydrolase (CEH) and the release of cholesterol from stores of cholesterol esters, leading to the stabilization and thus abnormal accumulation of the amyloid-42 (Aβ42) in lipid rafts of neurons. The treatment of neurons with a PAFR antagonist or CEH inhibitors resulted in an altered trafficking of Aβ42, and thus Aβ42 was found outside rafts and eventually targeted to lysosomes, where it was rapidly degraded. This study reflects that PAF interaction with PAFR contributes to the abnormal accumulation of Aβ42, which is thought to trigger a sequence of events leading to clinical dementia. In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson disease mouse model in which dopaminergic neurons in the substantia nigra were destroyed, PAF levels and Ptafr gene expression were shown to be elevated in the striatum. Thus, it can be deduced that PAF interaction with PAFR is responsible for the dopaminergic neurons degeneration involved in the pathogenesis of Parkinson disease.

Tumors

PAF has been shown to initiate the transformation of the immortalized nontumorigenic breast epithelial cell line (MCF10A cell line), observed as the development of abnormal acinar structures. The prolonged treatment of 3-dimensional cultures of the MCF10A cell line with PAF for 20 days induced the activation of PI3-K/AKT signaling leading to transformation of these cells. The incubation of human bladder cancer cells with cigarette smoke extract (CSE) promoted the synthesis of PAF and PAFR in vitro. Meanwhile, lung microvascular endothelial cells treated with CSE inhibited PAF/PAF activity and thus elevated PAF production. Exposure to CSE also stimulated the adherence of bladder cancer cells and breast cancer cells to bladder and lung microvascular endothelial cells, respectively. In bladder and breast cancers, the mechanism of enhanced tumor cell adherence was abrogated by pretreatment with a PAFR antagonist. These in vitro models of bladder and breast cancers reflect the role of PAF interaction with PAFR in promoting tumor metastasis, especially in smokers. Other than cigarette smoke, the synthesis of PAF by cancer cells can also be triggered by ultraviolet B radiation and air pollution as pro-oxidative stressors. The incubation of ovarian cancer cells with PAF also enhanced spheroid formation, the development of cancer stem cells, and the expression of stemness genes of...
ovarian cancer cells.\textsuperscript{123} These effects of PAF were abrogated by a PAFR antagonist,\textsuperscript{123} implying that PAF signaling via PAFR is crucial for the stemness of ovarian cancer. The stemness of cancer cells is known to be responsible for tumor recurrence, metastasis, and chemoresistance.

The silencing of the \textit{PLA2G7} gene encoding PAFAH1 enhanced the viability, proliferation, and migration of the human \textit{BRCA1} mutant ovarian cancer cell line, signifying the role of PAFAH in protecting against the growth and metastasis of ovarian cancer.\textsuperscript{81} The \textit{PAFAH1B3} mRNA expression was increased in human lung adenocarcinoma tissues in comparison to noncancerous lung tissues.\textsuperscript{82} A lung adenocarcinoma cell line deficient in \textit{PAFAH1B3} portrayed the inhibition of cell proliferation, probably by inducing G0-G1 phase arrest.\textsuperscript{82} The invasiveness of these cells was also abrogated, possibly by affecting the epithelial—mesenchymal transition process.\textsuperscript{82} Similarly, the knockdown of \textit{PAFAH1B3} reduced the proliferation, migration, and invasion of papillary thyroid carcinoma cells\textsuperscript{83} and hepatocellular carcinoma cells.\textsuperscript{125} These \textit{in vitro} results in lung, thyroid, and liver cancers emphasize the role of PAFAH1B3 in promoting tumor growth and metastasis.

Systemic PAF administration was observed to augment the growth of cutaneous non melanoma skin cancer in mice induced by cutaneous chemical carcinogens including dimethylbenz[a]anthracene/phorbol 12-myristate 13-acetate.\textsuperscript{126} Mice inoculated subcutaneously with ovarian cancer cells demonstrated increased subcutaneous tumor growth and elevated stemness of the tumor.\textsuperscript{123} These effects were abrogated in mice treated with a PAFR antagonist, suggesting that PAFR signaling contributes to the growth and stemness of ovarian cancer cells.\textsuperscript{123} In mice inoculated with melanoma tumor cells into the lateral tail vein, a PAFR antagonist was shown to prevent the metastasis of tumor cells into the lungs, suggesting a PAFR-dependent mechanism of melanoma tumor metastasis.\textsuperscript{127} Mice inoculated subcutaneously with human lung adenocarcinoma cells that lacked \textit{PAFAH1B3} expression showed reduced tumorigenesis and epithelial—mesenchymal transition with infiltration of neutrophils into xenograft tumor tissues.\textsuperscript{82} These results highlight the \textit{in vivo} role of \textit{PAFAH1B3} in promoting the growth and metastasis of lung cancer, and in inducing inflammation in the tumor microenvironment.

The growth of subcutaneously implanted B16F10 melanoma or TC-1 carcinoma cells was reduced in \textit{Ptafr}\textsuperscript{−/−} mice compared with wild-type mice.\textsuperscript{128} Subsequently, \textit{Ptafr} knockout mice also had increased recruitment of neutrophils (Gr1\textsuperscript{+}) and CD8\textsuperscript{+} lymphocytes in B16F10 tumors and of CD4\textsuperscript{+} lymphocytes in TC-1 tumors.\textsuperscript{128} These \textit{Ptafr} knockout mice had enhanced infiltration by M1-like (CD11C\textsuperscript{+}) macrophages and a lower percentage of M2-like (CD206\textsuperscript{+}) macrophages in both tumors.\textsuperscript{125} Based on this evidence, PAFR signaling seems to be crucial in suppressing inflammation in the tumor microenvironment, thus promoting tumor growth.

\section*{Anaphylaxis, Asthma, and Food Allergy}

\textit{In vitro} experiments have demonstrated that PAF caused the activation of mast cells derived from human peripheral blood, thus releasing histamines.\textsuperscript{129} PAF stimulated the activation of eosinophils and neutrophils isolated from human peripheral blood.\textsuperscript{130} PAF also induced the degranulation of human eosinophil—releasing factors, including IL-13, eotaxin-1, a protein blocking receptor for IL-1, basic fibroblast growth factor, RANTES, IL-9, and platelet-derived growth factor.\textsuperscript{130} The release of PAF was demonstrated in the plasma of mice during neutrophil-dependent passive and active systemic anaphylaxis.\textsuperscript{131,132} PAF release by cardiac mast cells, eosinophils, and neutrophils has been implicated in several cardiovascular dysfunctions and is frequently associated with severe anaphylaxis.\textsuperscript{133} These findings suggest that PAF can potentially mediate the amplification loop activation of mast cells, eosinophils, and neutrophils to cause anaphylaxis.

Infiltration of the of the airway by neutrophils expressing macrophage-1 antigen and lymphocyte function-associated antigen-1 was observed in induced sputum 4 hours after patients with mild asthma inhaled PAF. This inflammatory cell recruitment is hypothesized to be mediated by leukotriene B4, as elevated synthesis of this neutrophil chemoattractant was also reported in the induced sputum of patients with asthma.\textsuperscript{134} Thus, macrophage-1 antigen and lymphocyte function-associated antigen-1 facilitate the PAF-stimulated neutrophil trafficking into the lungs of mild asthma patients.

\textit{In vitro} experiments demonstrated that PAF induced the activation and proliferation of human bronchial smooth muscle cells, as well as the synthesis of proinflammatory cytokines, such as IL-6, IL-1B, and TNF from these cells.\textsuperscript{135,137} In a mouse model of allergen-induced asthma sensitized by ovalbumin (OVA), followed by intranasal administration of methacholine as the allergen, rPAFAH inhibited late-phase pulmonary inflammation, consisting of eosinophil infiltration into the lungs, airway mucus hypersecretion, and airway hyperreactivity.\textsuperscript{136} These \textit{in vitro} and \textit{in vivo} experiments emphasize the role of PAF in mediating inflammatory pathways in asthma.

In \textit{Ptafr}\textsuperscript{−/−} mice sensitized prior to an oral challenge with OVA, reduced food allergic responses, including levels of serum anti-OVA IgE, intestinal eosinophil infiltration, mucus production in the small intestine, and clinical parameters of the disease, were reported in comparison to wild type.\textsuperscript{137} \textit{Ex vivo} experiments portrayed diminished synthesis of IL-4, IL-5, and IL-10 as T helper 2 response by the spleen cells obtained from sensitized \textit{Ptafr} knockout mice as compared with wild-type mice when incubated with OVA.\textsuperscript{137} This study indicates the important role of PAF.
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Table 1  Studies Involving PAF as a Marker in Inflammation-Mediated Diseases

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<td>Renal disorders such as idiopathic IgA nephropathy, E. coli O157-associated hemolytic uremic syndrome, and diabetic nephropathy</td>
<td>In vitro: PAF signaling via PAFR facilitates the mechanism of foam cell formation by macrophages. In vivo: In rats with induced angina, PAF contributes to the pathogenesis of myocardial ischemia.</td>
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<td>Central nervous system disorders such as cerebral ischemia &amp; infarction, spinal cord injury, multiple sclerosis, meningitis, Alzheimer disease and Parkinson disease</td>
<td>In vivo: In a mouse model of chronic kidney disease, PAF/PAFR signaling is implicated in the progression of the disease, by activating inflammatory response. In vivo: In a rat model of diabetes, PAF interaction with PAFR is demonstrated to be involved in the development of the disease. Ex vivo: PAF enhancement of the severity of LPC-induced demyelination of cerebellar slices isolated from mice is reported to be dependent and independent of PAFR. In vivo: In a mouse model of multiple sclerosis, PAF signaling via PAFR is involved in the activation and development of EAE, especially during the chronic phase, by stimulating inflammation and demyelination. In vivo: In a mouse model of severe meningencephalitis, PAF interaction with PAFR stimulates neuroinflammatory response causing death. In vitro: PAF interaction with PAFR in neurons facilitates the abnormal accumulation of Aβ42, for the development clinical dementia. In vivo: In a mouse model of MPTP-induced Parkinson disease, PAF signaling via PAFR is implicated in dopaminergic neuron degeneration that is crucial for the development of Parkinson disease.</td>
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<tr>
<td>Tumors such as human meningiomas, bladder cancers, cervical cancer, endometrial cancer, epithelial ovarian cancer, lung adenocarcinoma and thyroid carcinoma</td>
<td>In vitro: PAF triggers the transformation of the MCF10A cell line.118,119 In vitro: CSE promotes the synthesis of PAF that is implicated in tumor metastasis of bladder and breast cancer cells dependently of PAFR.120,121 In vitro: PAF/PAFR signaling is demonstrated to promote the stemness of the ovarian cancer cells.123 In vitro: PAFAH attenuates the growth and metastasis of the human BRCA1 mutant ovarian cancer cell line.124 In vitro: PAFAH1B3 enhances the growth and metastasis of lung, thyroid and liver cancer cells.125 In vivo: In mice, PAF enhances the growth of cutaneous nonmelanoma skin cancer induced by cutaneous chemical carcinogens.126 In vivo: In mice injected subcutaneously with ovarian cancer cells, PAFR signaling contributes to growth and stemness of ovarian cancer cells.127 In vivo: In mice inoculated with melanoma tumor cells into the lateral tail vein, the metastasis of tumor cells into the lungs occurs dependently on PAFR.128 In vivo: In mice inoculated subcutaneously with human lung adenocarcinoma cells, PAFAH1B3 stimulates the growth, metastasis, and inflammation of lung tumor.82 In vivo: In mice inoculated subcutaneously with B16F10 melanoma or TC-1 carcinoma cells, PAFR signaling suppresses the inflammation that subsequently enhances the growth of B16F10 and TC-1 tumors.129 In vitro: PAF stimulates the activation of mast cells, eosinophils, and neutrophils, causing their degranulation.129,130 PAF also facilitates the amplification loop activation of mast cells, eosinophils, and neutrophils, causing anaphylaxis.131-133 In vitro: PAF triggers the activation and proliferation of HBSMCs, as well as the secretion of the proinflammatory cytokines from these cells.86,135 In vivo: In a mouse model of allergen-induced asthma, PAF induces late-phase pulmonary inflammation.134 In vivo: In a mouse model of food allergy, PAF/PAFR signaling mediates both systemic and local inflammation in the small intestine.136 In vivo: In a mouse model of sepsis, PAF interaction with PAFR stimulates neutrophil dysfunction, causing a failure of the immune system to control the spread of bacteria leading to death.137 rPAFAH is also postulated to have the therapeutic potential for the treatment of sepsis, by enhancing bacterial clearance and stimulating the synthesis of anti-inflammatory mediators systemically.138</td>
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Anaphylaxis, asthma, and food allergy

Sepsis

In a mouse model of polyomicrobial sepsis induced by cecal ligation and puncture, the level of serum cytokines, including TNF, IL-10, and IL-6, and nitrate levels were reduced and the bacterial clearance and survival rates were increased in Ptafr knockout mice, as well as in mice treated with a PAFR antagonist.138 Both groups of mice showed enhanced neutrophil recruitment to the peritoneal cavity due to the induction of polyomicrobial sepsis.138 PAF contributes to this innate immune dysfunction during sepsis, possibly by signaling via PAFR in inflammation induced by food allergens in mice.
inducing an altered physiological response of neutrophils including depolarization of membrane potential, intracellular alkalization, and cellular size, as demonstrated by in vitro experiments. These studies also highlight that during an ongoing infection, PAF/PAFR signaling may contribute to the failure of the immune system to control the spread of bacteria by inducing neutrophil dysfunction, leading to sepsis and thus death.

After lethal sepsis was induced by the CLP procedure, the administration of rPAFAH improved bacterial clearance and increased inflammatory mediators, including CCL2 and nitric oxide, in the peritoneal cavity, thus highlighting the therapeutic potential of rPAFAH treatment of sepsis. Previous human studies, as well as in vitro, ex vivo, and in vivo experiments demonstrating the role of PAF as a marker in inflammatory diseases are outlined in Table 1.

The Role of PAF in Parturition

PAF in the Amniotic Fluid and Fetal Membrane

PAF levels have been shown to increase in the amniotic fluid and amnion in humans during labour. It is hypothesized that PAF in the amniotic fluid is released from the fetal lungs to act as a signal for parturition and fetal lung maturation. This is consistent with a study that observed an elevation in PAF concentration and activity of lyso-PAFAT 6 days following the culture of human fetal lungs. Similarly, in mice, PAF levels were found to be elevated in the amniotic fluid and fetal lungs toward the end of gestation. Further, exposure of the human fetal membrane disc to PAF stimulated the expression of prostaglandin-endoperoxide synthase 2 (PTGS2) mRNA and the synthesis of prostaglandin.

Studies in mice have shown that steroid coactivator 1 (SRC1) and SRC2 play a role in activating Lpcat transcription, which catalyzes the synthesis of PAF from lysophosphatidylcholine. Double heterozygous Src1 and Src2 (Src1/2 dhet) female mice mated to the male mice of the same genotype were found to have delayed labor when compared with wild-type controls. The PAF level was also observed to be lower in the fetal lungs of Src1/2 dhet mice. Moreover, the intra-amniotic administration of PAF on gestational day (gd) 17.5 induced normal on-time parturition in Src1/2 dhet mice.

PAF in the Placenta

PAF and PTAFR mRNA expression was detected in placenta obtained from normal term delivery women. However, there has been no study in humans demonstrating the contribution of placental PAF to labor. The PAFAH level was shown to be elevated in the rat placenta, leading to a decrease in placental PAF as parturition approached its end. This decrease in PAF is hypothesized to be involved in fetoplacental circulation, causing a hypertensive response.

PAF in the Myometrium and Decidua

PAFAH was shown to be secreted from human decidual macrophages isolated at term pregnancy, indicating that these leukocytes could play an important role in PAF metabolism in normal term labor. cPAF and proinflammatory cytokines, including TNF, IL-1A, and IL-1B, were portrayed to reduce PAFAH production by the decidual macrophages in vitro, suggesting that PAF has an inhibitory effect on secretion of PAFAH by decidual macrophages. Similarly, CSE also impeded PAH-AH secretion by decidual macrophages and peripheral blood monocytes. This observation can possibly explain the mechanisms by which PAF could be elevated systemically and in gestational tissues in women who smoke during pregnancy, eliciting preterm delivery.

An increase in the contraction-associated genes and proteins, including gap junction protein alpha 1 and oxytocin receptor, in the myometrium of these Src1/2 dhet mice was portrayed following the administration of cPAF on gd 17.5 in comparison to phosphate-buffered saline buffer administration. Further, in the uterus of rats, a reduction in PAFAH activity was reported as gestation approached its end, potentially causing elevation of the PAF level. In a mouse model of dysmenorrhea, cPAF was also reported to elicit pain behavior, elevate uterine contractility, and impair perfusion, stimulating transient hypoxia in the uterus.

PAF in the Cervix

In human uterine cervical fibroblasts, inhibition of PAFR using WEB 2170 attenuated PAF-induced cytokine and MMP1 production. PAFAH protein expression was also higher in the cervical stroma of term-not-in-labor pregnant women than in nonpregnant and post-partum women. Intracervical administration of PAF stimulated the infiltration of polymorphonuclear leukocytes into the cervix and hence, cervical ripening in rats. Thus, in these gestational tissues, PAF may trigger inflammatory pathways, either dependent on or independent of PAFR, eventually activating the mechanisms of labor, including uterine contraction, cervical ripening, and fetal membrane rupture. PAFAH plays an important role in regulating the elevated level of PAF in the myometrium and cervix toward the end of gestation.

The Potential Positive Feedback of PAF on Inflammatory Pathways in Preterm Delivery

An increase in PAF has been found in the amniotic fluid of pPROM women with preterm delivery compared with pPROM women who delivered at term. Elevated levels
Elevated PAFR protein expression was documented in the cervix of term-not-in-labor women, compared with nonpregnant women. Adapted with modifications from Wahid et al.\textsuperscript{150} under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

Overall, in the gestational tissues of term and preterm delivery women, the expression of PAF, PAFR, and PAFAH at both gene and protein levels is summarized in Figure 3\textsuperscript{150}

Activated platelets are also known to express CD40L on their membrane.\textsuperscript{151} Once the CD40L is cleaved by the CD40 receptor expressed by professional antigen presenting cells and nonimmune cells during inflammation, the soluble form of CD40L is secreted, contributing to more than 90% of the detectable sCD40L in the plasma.\textsuperscript{151,152} Compared with nonpregnant women, elevated sCD40L levels were found in the plasma of normal pregnant women.\textsuperscript{153} An increased sCD40L concentration was also reported in women at term with labor compared with women at term without labor.\textsuperscript{153} These results suggest a physiological increase in sCD40L in both normal pregnancy and parturition. PTL women exhibited a higher plasma sCD40L concentration than women with normal pregnancies, which could not be accounted for by intra-amniotic infection and/or inflammation.\textsuperscript{153} Women with PTL were also depicted with a higher mean platelet factor 4 and beta-thromboglobulin plasma concentrations than women with term labor, signifying the increased platelet activation in these women.\textsuperscript{154}

In mice, the intrauterine administration of 10 to 40 μg of cPAF on gd 15 triggered preterm delivery in four of nine CD-1 mice.\textsuperscript{29} The C57BL/6 and C3HeB/FeJ strains of mice died within just a few hours of the intrauterine administration of 15 μg to 100 μg of cPAF.\textsuperscript{155} A lower dose of intrauterine administration of cPAF resulted in either death or no apparent effect in C57BL/6 and C3HeB/FeJ strains of mice.\textsuperscript{156,157} Preterm birth was induced in BALB/c mice by both intraperitoneal (2 μg) or intrauterine (35 μg) injection of cPAF on gd 16.5.\textsuperscript{29} The viability and survival of the pups from the cPAF- induced mice were significantly reduced.\textsuperscript{29} An increased expression of genes coding for proinflammatory cytokines, such as Il1b and Il6 was reported in the decidua, myometrium, and placenta of mice given cPAF.\textsuperscript{29} In rats, intravenous infusion of cPAF for 7 days from gd 14 to gd 21 resulted in a decrease in fetal and placental weights on gd 21.\textsuperscript{156,157} Thus, it is clear from these studies that the use of different strains of mice results in different outcomes, especially when challenged locally with cPAF. Overall, cPAF is efficient in stimulating preterm delivery and poor neonatal outcomes by stimulating inflammation in gestational tissues. In these animal studies, synthetic cPAF is used to mimic continuously elevated endogenous PAF. This is achieved through a chemical modification that incorporates a carbamyl group to confer resistance to the degradation by PAFAH, thus ensuring continued bioavailability. Naturally, this form of PAF certainly does not exist either systemically or locally in gestational tissues.

Mice with genetic ablation of Tlr4 were also less susceptible to preterm delivery and poor neonatal outcomes.\textsuperscript{29} Similarly, the use of (+)-naltrexone to inhibit TLR4 signaling also seemed to be effective in preventing preterm delivery and poor neonatal outcomes induced by cPAF.\textsuperscript{29}

In the decidua, myometrium, and placenta of Tlr4−/− mice, or mice with inhibited TLR4 signaling, the...
expression of II1b and II6 was down-regulated after the administration of cPAF. Following treatment with cPAF, lower concentrations of proinflammatory mediators, including TNF and CCL5, were secreted from peritoneal macrophages obtained from Ptafr<sup>-/-</sup>, Tlr2<sup>-/-</sup>, and Tlr4<sup>-/-</sup> mice than in wild-type mice. Thus, it can be deduced from this evidence that other than PAFR, PAF induction of inflammation in the gestational tissues and systemically causing preterm delivery is also dependent on TLR2 and TLR4.

**Pafah<sup>-/-</sup>** mice were shown to be more susceptible to Escherichia coli--induced preterm delivery than wild-type mice. To support this, elevated expression of proinflammatory cytokines and chemokines, including Tnf, II1b, and Ccl5 mRNA, was detected in the uterus, decidua, and placenta of the Pafah knockout mice administered with killed E. coli as compared with wild-type mice. The administration of a PAF antagonist CV-6209 intraperitoneally before intrauterine LPS administration elicited a lower percentage of preterm birth, accompanied by a higher percentage of viable pups among the undelivered preterm pups, compared with mice that were administered LPS alone.

Similarly, rats intraperitoneally administered with both LPS and a PAF antagonist, WEB-2170, on gd 16 were hampered from LPS-induced premature cervical ripening, assessed by the measurements of the cervical light-induced fluorescence and cervical resistance to stretch. From these results, it can be interpreted that the endogenous PAF production augments infection-induced inflammation in maternal and fetal tissues, to cause preterm delivery in mice. Another possibility is that the infection could induce the synthesis of higher levels of PAF endogenously eliciting preterm delivery.

**Conclusion**

Overall, PAF is implicated in stimulating inflammation in chronic diseases and disorders. Evidence also suggests that PAF is important in mediating inflammation in gestational tissues elicited in term and preterm delivery. However, more studies are warranted in different populations of humans to investigate the role of PAF in the activation of inflammatory pathways, both systemically and in gestational tissues, in triggering preterm delivery. Elevated PAF levels in high-risk pregnant women who are smokers and/or exposed to environmental pollutants, women with infections, multiple pregnancies, and polyhydramnios. This renders PAF a potential candidate to be targeted for the prediction, intervention, and prevention of preterm delivery. Anti-inflammatory drugs should also be supplemented with relevant antibiotics given to women at risk of preterm delivery caused by intrauterine infection. Targeting PAF-mediated inflammatory pathways using pharmacological agents promises tremendous potential in addition to other interventional modalities for the prevention and treatment of diseases associated with inflammation-induced immunopathology.

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**Disclosure Statement**

None declared.

**References**


