

## Platelets in the immune response: Revisiting platelet-activating factor in anaphylaxis

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Anaphylaxis is an acute, severe, life-threatening multisystem allergic reaction resulting from the sudden systemic release of biochemical mediators and chemotactic substances. Release of both preformed granule-associated mediators and newly generated lipid-derived mediators contributes to the amplification and prolongation of anaphylaxis. Platelet-activating factor (PAF) is a potent phospholipid-derived mediator the central role of which has been well established in experimental models of both immune-mediated and non-immune mediated anaphylaxis. It is produced and secreted by several types of cells, including mast cells, monocytes, tissue macrophages, platelets, eosinophils, endothelial cells, and neutrophils. PAF is implicated in platelet aggregation and activation through release of vasoactive amines in the inflammatory response, resulting in increased vascular permeability, circulatory collapse, decreased cardiac output, and various other biological effects. PAF is rapidly hydrolyzed and degraded to an inactive metabolite, lysoPAF, by the enzyme PAF acetylhydrolase, the activity of which has shown to correlate inversely with PAF levels and predispose to severe anaphylaxis. In addition to its role in anaphylaxis, PAF has also been implicated as a mediator in both allergic and nonallergic inflammatory diseases, including allergic rhinitis, sepsis, atherosclerotic disease, and malignancy, in which PAF signaling has an established role. The therapeutic role of PAF antagonism has been investigated for several diseases, with variable results thus far. Further investigation of its role in pathology and therapeutic modulation is highly anticipated because of the pressing need for more selective and targeted therapy for the management of severe anaphylaxis. (*J Allergy Clin Immunol* 2015;135:1424-32.)

**Key words:** Allergy, anaphylaxis, mast cells, platelet-activating factor, acetylhydrolase, signaling, receptor antagonists, platelets, sepsis, mediators

### Abbreviations used

LDL: Low-density lipoprotein  
Lp-PLA<sub>2</sub>: Lipoprotein-associated phospholipase A<sub>2</sub>  
LT: Leukotriene  
PAF: Platelet-activating factor  
PAF-AH: PAF acetylhydrolase  
PAFR: Platelet-activating factor receptor

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Platelet-activating factor (PAF), also known as 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, AGEPC, or PAF-acether, is a highly potent phospholipid that is thought to play a central role in the cause of numerous immune and inflammatory conditions.<sup>1</sup> From an evolutionary standpoint, the earliest fossil records indicate the presence of PAF in many protozoans, yeasts, and bacteria, perhaps playing a regulatory role. Thereafter, several studies identified the presence of platelet-activating factor receptors (PAFRs) in various cell types, especially those involved in host defense, including basophils, mast cells, macrophages, and monocytes, in addition to neutrophils, eosinophils, and endothelial cells (Table I).<sup>2</sup>

The role of PAF was first reported in the literature by a French immunologist, Jacques Benveniste, who sought to describe its relationship with histamine through an IgE-dependent process and as a mediator of anaphylaxis.<sup>3-5</sup> In the 1960s, rabbit models demonstrated the role of PAF in the release of histamine by antigen- and leukocyte-dependent mechanisms mediated through thermoregulation and calcium homeostasis.<sup>6</sup> Subsequently, the role of PAF in platelet aggregation and activation and the resultant increased vascular permeability was further elucidated.<sup>3-5</sup>

Since its discovery, our breadth of knowledge surrounding the biochemical function of PAF has been expanding, given the exponential growth in its research. PAF remains a lively topic of interest and holds promise for the future for its clinical implications and utility.<sup>7-9</sup>

### SOURCES OF PAF

PAF production in endothelial cells is triggered by thrombin, vasopressin, angiotensin II, anti-factor VIII, and IL-1.<sup>10</sup> Fig 1<sup>10,11</sup> summarizes both sources of PAF, as well as stimuli for its release. Both neutrophils and eosinophils can be stimulated to synthesize and release PAF through chemotaxis and by C5a, C5a-des-Arg, and formyl-methionyl-leucyl-phenylalanine, whereas only eosinophils respond to eosinophil chemotactic factor of anaphylaxis

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**TABLE I.** Cells and systems in which PAF has a signaling role<sup>2</sup>

Cells or system	Roles/responses
Platelets	Adherence, secretion of granules, aggregation
PMN leukocytes	Adherence, aggregation, oxygen production, chemotaxis, priming
Eosinophils	Chemotaxis, secretion of granules, LT production
Monocytes/macrophages	Aggregation, oxygen production, cytokine production
Lymphocytes	Inhibition of IL-2 production
Vascular endothelial cells	Adherence of neutrophils and monocytes, increased vascular permeability
Smooth muscle cells	Growth, contraction
Bronchus	Bronchoconstriction, accumulation of eosinophils
Heart	Alterations in rhythm and cardiac output
Systemic and pulmonary vessels	Altered vascular tone
Liver	Glycogenolysis
Kidney	Increased glomerular filtration
Neuronal systems	Excitatory synaptic transmission, release of neurotransmitters
Uterus	Contraction
Ovary	Ovulation
Amnion membrane	Prostaglandin E <sub>2</sub> production
Fetal lung	Maturation
Osteoclasts	Alterations in cytosolic free Ca <sup>2+</sup>

and the calcium ionophore A23187.<sup>10</sup> PAF is secreted in IgG-mediated reactions by basophils<sup>12</sup> and in IgE-mediated reactions by mast cells.<sup>13</sup> These cells can be stimulated to release histamine and PAF through a number of biochemical pathways that might play a role in mast cell activation.<sup>12,14,15</sup>

PAF released by endothelial cells is thought to play a vasoconstrictive role in vascular physiology through promotion of leukocyte adhesion at the cell surface, although it can also act as a potent vasodilator. The variability in its role is multifactorial, influenced not only by its concentration at the site in addition to the nature of the vasculature but also by its response to independent factors, such as circulating mediators (ie, leukotrienes [LTs] and TNF- $\alpha$ ) that might be present during a particular reaction.<sup>16</sup>

Although the focus of research on PAF has been in eukaryotic cells, PAF production has been shown in prokaryotic enterobacteria cells, specifically *Escherichia coli*.<sup>17</sup> The mechanism by which PAF exerts its effects through bacteria, such as *E coli*, might be associated with its potent endotoxin activity. PAF-acether produced by *E coli* has the same physicochemical and biological characteristics as synthetic PAF-acether and PAF derived from eukaryotic cells.<sup>17</sup>

Lymphocytes can be stimulated to produce PAF through at least 2 pathways: the calcium ionophore A23187 or synthesis or activation of acetyl transferases.<sup>10</sup> In the context of B-cell ontogeny, PAF can enhance the humoral response through antigen-stimulated B cells.<sup>18</sup>

## Synthesis of PAF

PAF is produced through rapid synthesis in response to stress, either through remodeling or a *de novo* pathway, and is not stored

intracellularly. In remodeling (Fig 2)<sup>19</sup> the pathway is primarily triggered by inflammatory mediators and results in the removal of a fatty acid from the phospholipid backbone by phospholipase A<sub>2</sub>. This results in production of an intermediate, lysophosphatidylcholine, which forms PAF with the addition of an acetyl group. The fatty acid most commonly acetylated in the sn-2 position is arachidonic acid.<sup>19</sup>

*De novo* synthesis of PAF involves the addition of phosphocholine from cytidinediphosphocholine to the sn-3 site of 1-0-alkyl-2-acetyl-sn-glycerol. This process is catalyzed by cholinephosphotransferase, an enzyme the activity of which is increased in patients with certain malignancies.<sup>19</sup>

The molecular structure of PAF can vary in terms of the length of the O-alkyl side chain. The alkyl component is connected at the C1 carbon to a 16-carbon chain through an ether linkage, and the phosphocholine group can be found at C3.<sup>1</sup> Because the half-life of PAF is quite short, approximately 5 minutes in plasma and whole blood,<sup>20</sup> it can act as a single messenger through a short acetate unit located at the C2 carbon. Importantly, the serum half-life is inversely correlated to the activity of PAF-acetylhydrolase, where higher circulating concentrations of this lipoprotein-associated phospholipase result in the rapid degradation of PAF.<sup>21-23</sup>

Each structural component of PAF plays an integral role in maintaining its biological activity, and even a slight structural modification can lead to loss of biological activity. However, structurally related biologically active analogues of PAF do exist, and although there might be differences in the degree of biological activity between PAF and its analogues, the similarities allow for investigation into shared mechanisms of action with the potential for novel therapeutic options in the future.<sup>24-26</sup>

The platelet-activating factor receptor (PAFR) gene has been mapped to chromosome 1. It is localized within the lipid bilayer of the cell membrane and is comprised of a 7-transmembrane rhodopsin-like G protein-coupled receptor.<sup>19</sup> It is thought that a single receptor mediates the effects of PAF,<sup>27</sup> although the effects of the receptor have been demonstrated in many cells of the immune system and can be found distributed throughout the body.

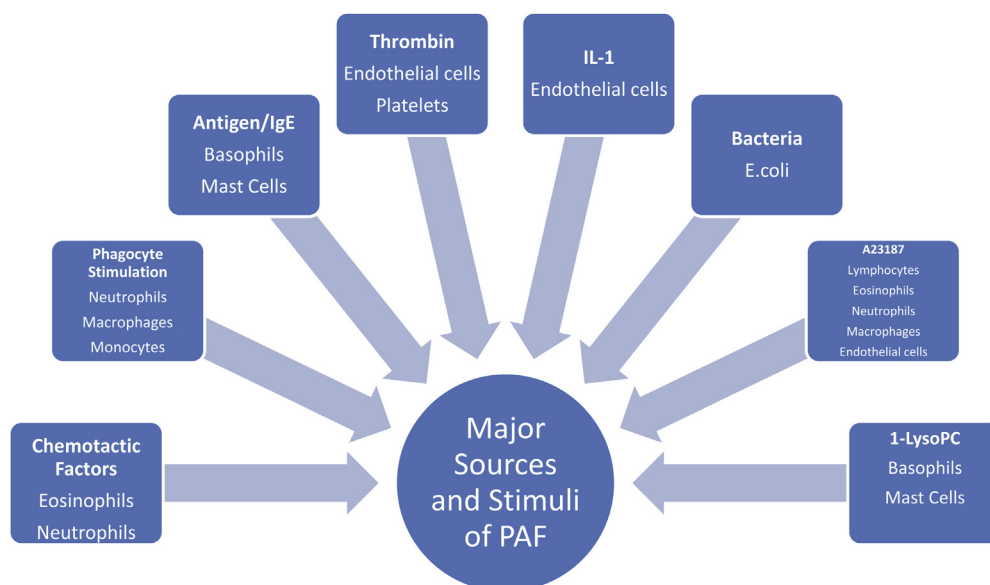
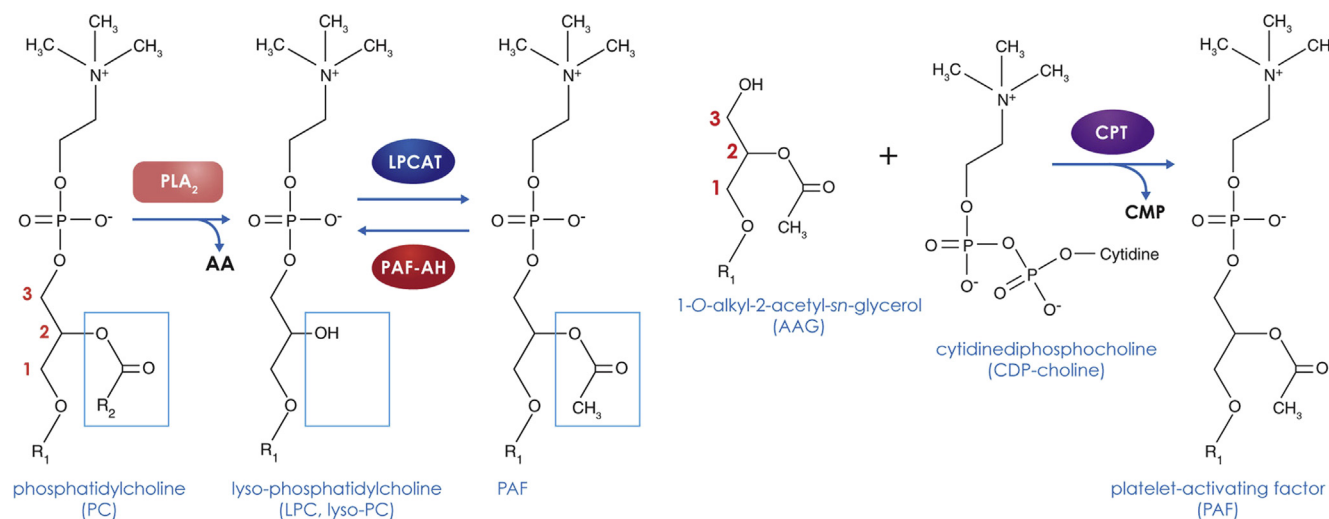
PAF can be produced by many cell types in response to various stimuli, although its generation by endothelial cells and platelets has been studied most extensively. This molecule can be distributed in many body fluids, including serum, saliva, and bronchial fluids, as well as in various cells, such as keratinocytes, although the exact location of synthesis has not been determined (Table II).<sup>11</sup> For PAF to be secreted, it must be transferred to the plasma membrane, a process thought to occur through transport proteins or fusion of lipid vesicles.<sup>1</sup>

Research has shown that the concentration of PAF within various cell types differs considerably and is dependent on the expression of PAF acetylhydrolases (PAF-AHs), the nature of its binding sites, and environmental factors, such as pH and temperature.<sup>1,17</sup>

## Regulation of PAF

PAF metabolism is tightly regulated by a family of enzymes called PAF-AHs, which have the ability to hydrolyze and inactivate the compound through enzymatic removal of acetyl groups at the sn-2 position (Fig 3).<sup>28,29</sup>

Some extracellular types of PAF-AHs circulate as a complex with low- and high-density lipoproteins and are termed

FIG 1. Sources of PAF.<sup>10,11</sup>FIG 2. Synthesis of PAF through remodeling (left) and *de novo* pathways (right).<sup>19</sup> Figure permission courtesy of Cayman Chemical, Ann Arbor, Michigan.

lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>). Levels of these Lp-PLA<sub>2</sub> isotypes are increased in conditions of oxidative stress and inflammation. Although the mechanisms of PAF regulation have not yet been fully delineated, its production can be controlled by extrinsic factors, such as pH, fatty acids, and metal ions.

### Biological effects of PAF

The biological actions of PAF are thought to be regulated by several mechanisms, including the expression and activity of the PAFR, through regulation of synthetic pathways and cell signaling, as well as degradation through PAF-AH.<sup>2</sup>

PAF has been shown to exert its effects at concentrations as low as 10<sup>-12</sup> mol/L,<sup>22,30</sup> with a half-life of approximately 3 to 13 minutes, depending on the time to inactivation by PAF-AH.<sup>22,30-32</sup> PAF and PAF-AH levels have been shown to be inversely

correlated in studies of anaphylaxis, demonstrating more severe clinical symptoms with higher PAF levels.<sup>33</sup>

Since its discovery, our knowledge surrounding the role of PAF has expanded to include its effects on platelet aggregation and its role as a vasodilator and bronchoconstrictor, its role in host defense, and its role as a mediator of the inflammatory response (Table III). PAF has been shown to play a pivotal role as a mediator of anaphylaxis<sup>33</sup> and might help elucidate potential therapeutic targets for the management of PAF-mediated anaphylaxis.

### REVIEW OF THE SPECIFIC ROLE OF PAF IN ANAPHYLAXIS

Anaphylaxis is an acute, potentially life-threatening multisystem allergic reaction resulting from the sudden systemic release of preformed and lipid-derived mediators from basophils

**TABLE II.** Distribution of PAFRs in various tissues and cell types<sup>11</sup>

Cell and tissue	K <sub>c</sub> (nmol/L)	B <sub>max</sub> (sites or cells as indicated)
Human platelets	37 ± 13	1399 ± 498
Human platelet	1.58 ± 0.36	1983 ± 391
Human platelet	0.05	242 ± 64
Rabbit platelet	0.5	400
Rabbit platelet membrane	1.36	150-300
Rat platelet	No specific PAF binding	
Neutrophil	0.11 ± 0.02	5 × 10 <sup>6</sup>
Neutrophil	45	2.8 × 10 <sup>4</sup>
Neutrophil membrane	0.2	1100
Macrophage	0.08	7872
	0.25	117 fmol/mg protein
Mononuclear leukocyte	5.7	1.11 × 10 <sup>4</sup>
Lung membrane	0.49	140 fmol/mg protein
Liver membrane	0.5 ± 0.14	140 ± 18 fmol/mg protein
Gerbil brain membrane	3.66 ± 0.92 (high)	0.83 pmol/mg protein
	20.4 ± 0.5 (low)	1.1 pmol/mg protein
Synaptic plasma membrane	0.023 (high)	8.75 fmol/mg protein
	25	0.96 pmol/mg protein
Rat retina	2.9	0.85 pmol/mg protein
Eosinophil	1.6	3.5 × 10 <sup>4</sup>
Kupffer cells	0.12-0.14	1.06 × 10 <sup>4</sup>

**TABLE III.** Summary of pathophysiologic and physiologic effects of PAF<sup>11</sup>

Targets	Effects
Hematologic	Platelet aggregation and secretion, thrombosis
Microbial	Chemotaxis of eosinophils, neutrophils and macrophages, endotoxin release
Immunologic	Anaphylaxis, inflammation through increased vascular permeability and edema
Gastrointestinal	Pancreatitis, ulceration, glycogenolysis
Respiratory	Asthma through smooth muscle constriction and acute lung injury
Hormonal	Pregnancy, ovulation, ovoidimplantation
Cardiac	Negative inotropic results, positive chronotropic results, vasodilator
Renal	Decreases renal blood flow, sodium excretion and diuresis

mast cells and basophils leads to exocytosis of preformed or granule-associated mediators, such as histamine, tryptase, chymase, heparin, and carboxypeptidase A. Production of newly generated lipid-derived mediators, including LTC<sub>4</sub>, LTB<sub>4</sub>, LTE<sub>4</sub>, prostaglandin D<sub>2</sub>, and PAF, occurs within minutes,<sup>6,21,38</sup> contributing to the amplification and prolongation of anaphylaxis.

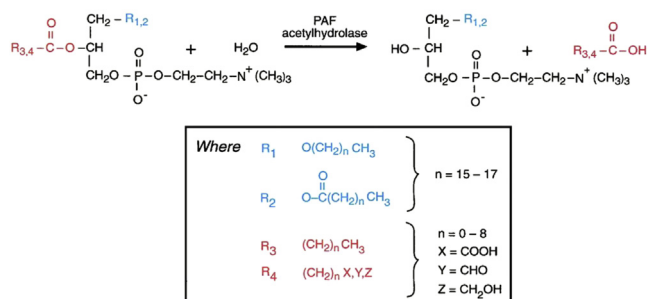
PAF is a potent phospholipid-derived biological mediator with messenger function, the role of which as a central mediator has been well established in experimental models of anaphylaxis, particularly active systemic anaphylaxis.<sup>28,31,39,40</sup> Serum PAF levels correlate with the severity of anaphylaxis in human subjects and mouse models.<sup>31,41</sup> It is produced and secreted by several types of cells, including mast cells, monocytes, tissue macrophages, platelets, eosinophils, Kupffer cells, endothelial cells, neutrophils, and spermatozoa,<sup>31,39,42,43</sup> and has been found to be active at concentrations as low as 10<sup>-12</sup> mol/L,<sup>5</sup> despite its short half-life.

G protein-linked receptors expressed on the surfaces of a variety of cells, such as platelets, monocytes, macrophages, and neutrophils, mediate the intracellular actions of PAF,<sup>21,28</sup> producing various biological effects, including circulatory collapse, decreased cardiac output, increased vascular permeability, portal hypertension, and smooth muscle contraction in the airways, gut, and uterus.<sup>39,41,42</sup> In mice PAF has been shown to activate and induce degranulation of mouse eosinophils.<sup>44</sup> PAF signaling results from its binding to the PAFR rather than to direct physicochemical effects on the target cell.<sup>28,45</sup> Initiation of intracellular signaling events requires binding of PAF to its receptor to produce biological effects.<sup>28</sup> Many of the cells producing PAF are also targets for its bioactivity.<sup>42</sup>

### PAFR-mediated responses

PAF has been shown to be produced in both IgE-dependent and IgE-independent anaphylaxis in murine models through cross-linking on FcεRI on mast cells and FcγRIII on stimulated macrophages, respectively.<sup>21</sup> PAF acts through a specific PAFR on the plasma membranes of target cells by binding to a transmembrane G protein-coupled receptor, leading to mobilization of intracellular calcium and activation of kinases.<sup>11,42,45</sup> Expression of the PAFR is regulated by intracellular cyclic AMP, which can downregulate PAFR mRNA expression and reduce PAF-induced arachidonic acid release.<sup>42,45</sup> Arachidonic acid

Reaction catalyzed by PAF acetylhydrolases.



**FIG 3.** PAF degradation is catalyzed through PAF-AH and occurs at the sn-2 position through hydrolysis of an acetyl residue. Therefore the remnants are lysoPAF and acetate molecules.<sup>28,29</sup> Reprinted with permission from Stafforini et al.<sup>29</sup>

and mast cells.<sup>34,35</sup> Clinical presentations vary from mild and self-limited reactions to rapid and fatal outcomes. Cutaneous symptoms of urticaria and angioedema are the most common manifestations of anaphylaxis but might be absent or delayed, particularly in rapidly progressive reactions. Severe and life-threatening reactions are generally related to a rapid onset of symptoms. Death from anaphylaxis is usually the result of cardiovascular collapse or respiratory obstruction involving the lower airway, upper airway, or both.<sup>34,36</sup>

The pathogenesis of anaphylaxis can be immunologic or nonimmunologic in nature,<sup>35,37</sup> leading to the release of biochemical mediators and chemotactic substances. Degranulation of



metabolites have been shown to reduce cardiac contractility in rat models.<sup>11</sup> After binding of PAF to its receptor on platelets, monocytes, macrophages, and neutrophils, the activation of several kinases, including protein kinase C and mitogen-activated protein kinase, results in release of arachidonic acid and prostaglandins, including prostaglandin E<sub>2</sub>, from vascular smooth muscle cells,<sup>11,42,45</sup> exerting biological activity and manifestations of anaphylaxis. Downregulation of signaling through PAFR occurs through epinephrine and other vasoactive agents that activate adenylate cyclase, leading to increased intracellular calcium levels.<sup>36,42</sup>

Systemic mast cell activation after antigen exposure in patients with anaphylaxis has been attributed to hematogenous dissemination of allergen, and although plausible, the rapid onset of events after an oral exposure suggests other factors might be important<sup>13</sup> and that additional mechanisms might lead to amplification of anaphylaxis.

Increases in mast cell-specific  $\beta$ -tryptase support the central role of mast cells in patients with anaphylaxis,<sup>38</sup> with 2 common mast cell phenotypes recognized in human subjects, based on their protease content. One population containing only tryptase (MCt cells) is found at mucosal surfaces, such as bronchial epithelium and the small bowel, whereas the other population containing chymase, tryptase, carboxypeptidase A, and cathepsin G (known as MCtc cells) is typically located in skin and connective tissue.<sup>13,36</sup> Distribution of mast cell phenotypes will influence the clinical events of anaphylaxis.

It has been shown that PAF releases histamine through PAFR on human lung mast cells and peripheral blood-derived mast cells but not skin mast cells. This is supported by the histochemical findings of preferential expression of PAFR in the mast cell population containing only the tryptase phenotype.<sup>13,39</sup> Activation of the PAFR-coupled Gai leads to degranulation through PLCg1 and PLCb2 activation in human mast cells,<sup>13</sup> providing insight into the specific role of PAF and its receptor's interaction in amplification of the allergic response.

In human subjects serum PAF levels were significantly increased in patients with acute allergic reactions, with higher levels noted in patients with severe anaphylaxis.<sup>46</sup> Patients with severe anaphylaxis had respiratory or cardiovascular compromise, in which both organ systems are targets of PAF bioactivity. High PAF levels better correlated with severe anaphylaxis compared with serum tryptase or histamine levels.<sup>21,23,31,47</sup> Conversely, subjects with the lowest PAF levels had the least severe reactions.<sup>31</sup>

### Role of PAF-AH and PAFR antagonists in patients with anaphylaxis

PAF is rapidly hydrolyzed and degraded to an inactive metabolite, lysoPAF, by the enzyme PAF-AH. PAF-AH is a calcium-independent enzyme belonging to group 7 of the family of phospholipases A<sub>2</sub>.<sup>16</sup> Circulating levels of PAF are controlled by the activity of PAF-AH,<sup>31,48</sup> which is an important determinant of PAF's short half-life.<sup>9,10,31</sup>

Patients with the lowest PAF-AH levels were at highest risk of severe anaphylaxis, whereby PAF-AH activity correlated inversely with PAF levels.<sup>21,31,42</sup> Several studies have independently confirmed that PAF-AH deficiency predisposes to severe anaphylaxis, and more rapid rates of inactivation of PAF result in milder allergic manifestations.<sup>21,23,39</sup>

Basal PAF-AH levels studied in patients with Hymenoptera venom allergy showed that patients with grade III or IV anaphylaxis had the lowest basal enzyme activity,<sup>49</sup> confirming earlier published findings<sup>21,30</sup> showing that low PAF-AH levels are a risk factor for severe anaphylaxis. Factors affecting PAF-AH levels have been studied, including stability of precursor molecules,<sup>50</sup> susceptibility to oxidant attack<sup>51</sup> and clearance rate from plasma.<sup>47</sup> PAF-AH in plasma forms circulating complexes with lipoproteins; in particular, low-density lipoprotein (LDL) and removal of LDL from the circulation might increase the clearance rate of PAF-AH and modify the activity of PAF-AH in blood. Decreasing LDL levels pharmacologically might unintentionally lead to an increased risk of anaphylaxis.<sup>47</sup>

There has been much interest around the development of drugs that selectively block the actions of PAF as both long-term prophylaxis and emergency treatment and for those at high risk for fatal anaphylactic reactions. In animal studies PAF antagonists have shown protection against airway hyperreactivity, shock, allergic disorders, ischemic tissue damage, and other inflammatory disorders.<sup>52,53</sup> In animal models treatment with PAFR antagonists significantly reduced the severity of peanut-induced anaphylaxis and accelerated recovery from anaphylactic reactions.<sup>53</sup> Pretreatment with a combination of antihistamine and PAFR antagonist has shown synergistic and dramatic benefit.<sup>53</sup> However, there continues to be a pressing need for more selective and targeted therapy for the management of severe anaphylaxis.

### PAF analogues

Although structurally related, the biochemical activity of PAF analogues might be reduced in comparison. Triggiani et al<sup>54</sup> investigated the preferential production of an acyl analogue of PAF (1-O-acyl-2-acetyl-sn-glycero-3-phosphocholine) by basophils, mast cells, and endothelial cells in comparison with inflammatory cells that produce PAF. Recent research has investigated the role of analogues containing lysoPAF, acetylPAF, and butanoylPAF in assessing PAFR function and as possible therapeutic targets.<sup>55</sup> McIntyre<sup>24</sup> investigated the properties of oxidized analogues of PAF and found their functional activities to be similar to those of PAF itself.

Similar to PAF analogues, structural homologues of PAFR have been investigated as possible therapeutic targets in damage caused by environmental stressors and microbes.<sup>25</sup> Both PAF and PAFR, as well as their analogues, have been implicated in atherosclerosis, reproduction, tumorigenesis, and inflammation.<sup>26</sup>

### PAF IN OTHER DISEASES

In addition to its role in anaphylaxis, PAF has also been implicated as a mediator in both allergic and nonallergic inflammatory diseases. The roles of PAF and PAF-AH are still emerging in a number of conditions, and precise pathogenic roles have yet to be elucidated. The therapeutic role of PAF antagonism has been investigated for several diseases, with variable results thus far. PAF and PAF-AH have been reviewed for allergic rhinitis, asthma, sepsis, atherosclerotic disease, and malignancy, disease entities in which PAF signaling has an established role.

### Allergic rhinitis and asthma

PAF is thought to be an important mediator in the pathogenesis of allergic rhinitis. PAF has been demonstrated in nasal lavage

fluid from patients with allergic rhinitis after allergen challenge.<sup>56</sup> Human PAF nasal provocation leads to increased nasal airway resistance, reduced nasal volume, and symptoms of nasal obstruction.<sup>57-59</sup> Biochemically, PAF challenge results in nasal hyperresponsiveness to histamine and kinins and recruitment of neutrophils and eosinophils to the nasal mucosa.<sup>58,60,61</sup> PAFRs are expressed in nasal mucosal epithelial cells, submucosal glands, vascular endothelial cells, mast cells, leukocytes, and lymphocytes.<sup>62</sup> In animal models of allergic rhinitis, PAFR antagonists reduce nasal airway resistance and vascular permeability after allergen challenge.<sup>63,64</sup> Rupatadine is a dual antagonist of histamine and PAFRs.<sup>65</sup> A recent meta-analysis of 10 trials of rupatadine in patients with allergic rhinoconjunctivitis, comprising 2573 patients, found a favorable risk-benefit profile and efficacy for relief of overall allergy symptoms (ie, both nasal and ocular symptoms).<sup>66</sup> Rupatadine is licensed for use in patients with allergic rhinoconjunctivitis in several countries outside North America.

PAF is one of many mediators involved in the pathogenesis of asthma. PAF inhalation resulted in bronchoconstriction, bronchial reactivity, impaired gas exchange, mucus hypersecretion, and infiltration of inflammatory cells.<sup>67-71</sup> LTs, mediators with an established role in asthma, can act downstream of PAF in asthmatic patients.<sup>70-72</sup> The 5-lipoxygenase inhibitor zileuton prevented the effects of inhaled PAF in patients with mild asthma, suggesting that the respiratory effects of PAF might be mediated by thromboxane and LTs.<sup>70,71,73,74</sup> In addition, PAF-AH levels were significantly lower in adults with asthma compared with those in healthy control subjects, supporting an association between PAF-AH deficiency and asthma.<sup>70,71,75</sup> Despite this, trials of PAF antagonists and recombinant PAF-AH have not shown clinical benefit in asthma, likely reflecting the multitude of proinflammatory mediators underlying asthma pathogenesis.<sup>70,71</sup>

## Sepsis

PAF is one of the multiple inflammatory mediators involved in the systemic inflammatory response that results in clinical sepsis.<sup>76</sup> PAF activates key cells of the innate immune system, including neutrophils, monocytes, and platelets, inducing platelet aggregation, cytokine synthesis, and release of neutrophil extracellular traps for extracellular microbial destruction.<sup>46,77,78</sup> PAF also interacts with other inflammatory mediators central to sepsis, including TNF- $\alpha$  and nitric oxide.<sup>76</sup> In animal studies increased serum PAF levels have been documented during sepsis, and infusion of PAF resulted in hypotension, shock, and death.<sup>76</sup> In human sepsis studies findings have been conflicting.<sup>76</sup> Some studies have found increased serum PAF levels in septic patients,<sup>79,80</sup> but others have found no significant increase compared with levels in healthy control subjects.<sup>81,82</sup> Plasma PAF-AH activity has also been found to be decreased in some patients with sepsis, raising the possibility of a pathogenic imbalance between increased PAF generation and decreased degradation.<sup>46</sup> A short-term analysis of patients with acute lung injury and acute respiratory distress syndrome predominantly caused by sepsis found that nonsurvivors had significantly lower plasma PAF-AH activity than survivors.<sup>83</sup>

Different approaches to pharmacologic termination of PAF activity in septic patients have been examined in clinical trials with limited success. One approach involved the use of direct

antagonists of PAFR. Collective results from studies totaling 1279 patients revealed a nonsignificant (3.1%) reduction in mortality with use of PAFR antagonists,<sup>84-89</sup> although subgroup effects were seen in some of the studies (reviewed by Marshall<sup>90</sup>). Animal studies have suggested that combination treatment with receptor antagonists for LTB<sub>4</sub> or other proinflammatory mediators with PAFR antagonists might be of benefit.<sup>91</sup> A second approach used recombinant PAF-AH to hydrolyze PAF.<sup>46</sup> This approach showed initial success in a multicenter phase II trial of 127 patients with severe sepsis, resulting in significant reduction in 28-day all-cause mortality (21% vs 44%,  $P = .03$ ).<sup>92</sup> A subsequent phase III randomized placebo-controlled trial of patients with severe sepsis examined administration of recombinant PAF-AH daily for 5 consecutive days. This study was halted after an interim analysis revealed no significant reduction in 28-day all-cause mortality or secondary end points, such as acute respiratory distress syndrome or days free from coagulopathy, respiratory, renal, and cardiovascular failure.<sup>93</sup> Patients in this study were not stratified according to endogenous PAF-AH levels, and it has been suggested that this might have contributed to the apparent lack of efficacy.<sup>46</sup>

## Atherosclerosis

PAF-AH, also known as Lp-PLA<sub>2</sub>, has been identified as a marker to stratify patients at risk of coronary atherosclerotic disease. PAF-AH is released from macrophages in atherosclerotic plaques, and plasma PAF-AH forms complexes with lipoproteins in LDL cholesterol.<sup>94,95</sup> Increased PAF-AH expression has been demonstrated in human atherosclerotic plaques,<sup>96</sup> and an animal study demonstrated increased PAF-AH activity in atherosclerotic vessels compared with nonatherosclerotic vessels.<sup>94,96</sup> Whether PAF-AH has a direct pathogenic role in atherosclerosis or rather is a biomarker reflective of other factors that increase atherosclerotic risk has not yet been established.<sup>94</sup> It has been postulated that PAF-AH might play a direct role in atherogenesis by liberating arachidonic acid from LDL cholesterol particles, providing the precursor for generation of prostaglandins and LTs.<sup>95</sup> Increased PAF-AH expression has been demonstrated in ruptured and rupture-prone atherosclerotic plaques, suggesting a potential role in plaque progression or instability.<sup>97</sup> Plasma PAF-AH activity is associated with the presence of angiographic coronary artery disease independent of conventional risk factors, such as LDL cholesterol.<sup>95,98</sup> Consensus guidelines recommend measurement of PAF-AH (Lp-PLA<sub>2</sub>) levels in moderate- to high-risk patients as an adjunct to traditional cardiovascular risk assessment.<sup>99</sup> Therapeutic use of PAF-AH inhibition has been met with disappointing results thus far. Darapladib, an oral PAF-AH inhibitor, was initially demonstrated to prevent expansion of necrotic cores in coronary atheroma compared with standard-of-care treatment.<sup>100</sup> However, 2 recent large double-blind, placebo-controlled trials found no reduction in major coronary events with addition of darapladib to optimal medical therapy in patients with acute coronary syndromes or stable coronary disease.<sup>101,102</sup> A smaller multicenter placebo-controlled study also found no significant improvement in radiographically imaged vascular inflammation with short-term use of rilapladib, another oral PAF-AH inhibitor.<sup>103</sup> A recent population-based study among Han Chinese patients suggests that select polymorphisms in the PAF-AH gene correlate

with higher circulating PAF-AH levels and higher coronary risk.<sup>104</sup>

## Malignancy

A key role for PAF is developing in the field of oncology. PAF has been identified as one of the mediators present in the tumor microenvironment, and PAF signaling has been implicated in tumor growth, angiogenesis, and metastasis in a number of *in vitro* and animal models.<sup>9,105,106</sup> PAF and PAFR agonists are generated after exposure to oxidative stress and inhibit the antitumor immune response.<sup>107</sup> In an animal model of lung cancer, administration of PAFR agonists led to increased tumor growth and lung metastasis.<sup>106</sup> In a recent animal study the oxidative stress of traditional chemotherapy promoted PAF production and subsequent treatment failure, suggesting a role for PAF signaling in treatment outcomes.<sup>108</sup> Interestingly, these untoward effects were inhibited by antioxidants, COX-2 inhibitors, or depletion of regulatory T cells.<sup>108</sup> Further studies with these strategies and PAF antagonists are anticipated.

## PAF: EMERGING ROLES AND FUTURE DIRECTIONS

In addition to its involvement in allergy, atherosclerosis, malignancy, and sepsis, a role in a number of diverse conditions from asthma to pre-eclampsia is emerging for PAF signaling. The importance of PAF-AH as an antioxidant is being increasingly recognized. A study of patients with adult-onset asthma correlated higher PAF-AH activity with decreased development of asthma.<sup>109</sup> The effect of dietary antioxidants on PAF signaling is also being explored. In healthy volunteers healthy dietary patterns and dietary antioxidant intake were inversely correlated with serum PAF and PAF synthetic enzyme levels, suggesting the potential for dietary influences on PAF levels.<sup>110</sup> Along these lines, another group looked at lipid profiles and PAF-AH activity in neonates born to women with pre-eclampsia compared with healthy neonates.<sup>111</sup> The neonates of women with severe pre-eclampsia had significantly higher PAF-AH activity and lipid profile changes suggestive of chronic inflammation.<sup>111</sup> It is not yet known whether this inflammatory profile at birth is associated with long-term sequelae.<sup>111</sup> Finally, PAF-AH expression is not limited to humans and other mammals. *Leishmania* species, an intracellular protozoan, expresses PAF-AH, which appears to play a key role in virulence of the organism.<sup>112</sup>

It is clear that the PAF signaling pathway is fundamental to a number of pathologic processes, and therefore control of this pathway should hold great promise in the therapeutic arena. We are still in the early stages of understanding PAF signaling, and further investigation of its role in pathology and therapeutic modulation is highly anticipated.

## REFERENCES

- Venable ME, Zimmerman GA, McIntyre TM, Prescott SM. Platelet-activating factor: a phospholipid autacoid with diverse actions. *J Lipid Res* 1993;34:691-702.
- Imaizumi TA, Stafforini DM, Yamada Y, McIntyre TM, Prescott SM, Zimmerman GA. Platelet-activating factor: a mediator for clinicians. *J Intern Med* 1995;238:5-20.
- Benveniste J. Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils. *Nature* 1974;249:581-2.
- Benveniste J, Henson PM, Cochrane CG. Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils, and a platelet-activating factor. *J Exp Med* 1972;136:1356-77.
- Benveniste J, Le Couedic JP, Polonsky J, Tence M. Structural analysis of purified platelet-activating factor by lipases. *Nature* 1977;269:170-1.
- Barbaro JF, Zvaifler NJ. Antigen induced histamine release from platelets of rabbits producing homologous PCA antibody. *Proc Soc Exp Biol Med* 1966;122:1245-7.
- Singh P, Singh IN, Mondal SC, Singh L, Garg VK. Platelet-activating factor (PAF)-antagonists of natural origin. *Fitoterapia* 2013;84:180-201.
- Siraganian RP, Osler AG. Destruction of rabbit platelets in the allergic response of sensitized leukocytes. I. demonstration of a fluid phase intermediate. *J Immunol* 1971;106:1244-51.
- Tsoupras AB, Iatrou C, Frangia C, Demopoulos CA. The implication of platelet activating factor in cancer growth and metastasis: Potent beneficial role of PAF-inhibitors and antioxidants. *Infect Disord Drug Targets* 2009;9:390-9.
- Braquet P, Rola-Pleszczynski M. Platelet-activating factor and cellular immune responses. *Immunol Today* 1987;8:345-51.
- Chao W, Olson MS. Platelet-activating factor: receptors and signal transduction. *Biochem J* 1993;292:617-29.
- Pinckard RN, Farr RS, Hanahan DJ. Physicochemical and functional identity of rabbit platelet-activating factor (PAF) released in vivo during IgE anaphylaxis with PAF released in vitro from IgE sensitized basophils. *J Immunol* 1979;123:1847-57.
- Kajiwaru N, Sasaki T, Bradding P, Cruse G, Sagara H, Ohmori K, et al. Activation of human mast cells through the platelet-activating factor receptor. *J Allergy Clin Immunol* 2010;125:1137-45.e6.
- Karasuyama H, Tsujimura Y, Obata K, Mukai K. Role for basophils in systemic anaphylaxis. *Chem Immunol Allergy* 2010;95:85-97.
- Siraganian R. Histamine secretion from mast cells and basophils. *TIPS Rev* 1983;4:432-7.
- Cines DB, Pollak ES, Buck CA. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998;91:3527-61.
- Thomas Y, Denizot Y, Dassa E, Boullet C, Benveniste J. Synthesis of paf-acether by *E. coli* K12 [in French]. *C R Acad Sci III* 1986;303:699-702.
- Bastien Y, Toledano BJ, Mehio N, Cameron L, Lamoukhaid B, Renzi P, et al. Detection of functional platelet-activating factor receptors on human tonsillar B lymphocytes. *J Immunol* 1999;162:5498-505.
- Brock T. Platelet-activating factor: activator of inflammation, angiogenesis, and metastasis. Available at: <https://www.caymanchem.com/app/template/Article.vm/article/2135>. Accessed May 1, 2015.
- Yoshida H, Satoh K, Koyama M, Hiramoto M, Takamatsu S. Deficiency of plasma platelet-activating factor acetylhydrolase: roles of blood cells. *Am J Hematol* 1996;53:158-64.
- Vadas P, Gold M, Perelman B, Liss GM, Lack G, Blyth T, et al. Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008;358:28-35.
- Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor, a pleiotropic mediator of physiological and pathological processes. *Crit Rev Clin Lab Sci* 2003;40:643-72.
- Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2013. *J Allergy Clin Immunol* 2014;133:324-34.
- McIntyre TM. Bioactive oxidatively truncated phospholipids in inflammation and apoptosis: formation, targets, and inactivation. *Biochim Biophys Acta* 2012;1818:2456-64.
- Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. *Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. *Nature* 1995;377:435-8.
- Ninio E, Jancar S, Rios F, McIntyre T, O'Neill C, Travers J. Platelet-activating factor receptor, introduction. IUPHAR database (IUPHAR-DB). Activated at: <http://www.iupharb.org/DATABASE/FamilyIntroductionForward?familyId=55>. Accessed July 13, 2014.
- Ishii S, Kuwaki T, Nagase T, Maki K, Tashiro F, Sunaga S, et al. Impaired anaphylactic responses with intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. *J Exp Med* 1998;187:1779-88.
- Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. *Annu Rev Biochem* 2000;69:419-45.
- Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor acetylhydrolases. *J Biol Chem* 1997;272:17895-8.
- Stafforini DM. Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2). *Cardiovasc Drugs Ther* 2009;23:73-83.
- Cao Y, Stafforini DM, Zimmerman GA, McIntyre TM, Prescott SM. Expression of plasma platelet-activating factor acetylhydrolase is transcriptionally regulated by mediators of inflammation. *J Biol Chem* 1998;273:4012-20.
- Castro Faria Neto HC, Stafforini DM, Prescott SM, Zimmerman GA. Regulating inflammation through the anti-inflammatory enzyme platelet-activating factor-acetylhydrolase. *Mem Inst Oswaldo Cruz* 2005;100(suppl 1):83-91.



33. Vadas P, Gold M, Liss G, Smith C, Yeung J, Perelman B. PAF acetylhydrolase deficiency predisposes to fatal anaphylaxis. *J Allergy Clin Immunol* 2003;111(suppl):S206.
34. Lieberman P, Nicklas RA, Oppenheimer J, Kemp SF, Lang DM, Bernstein DI, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. *J Allergy Clin Immunol* 2010;126:477-80, e1-42.
35. Ring J, Grosber M, Brockow K, Bergmann KC. Anaphylaxis. *Chem Immunol Allergy* 2014;100:54-61.
36. Adkinson NF Jr. *Middleton's allergy principles and practice*. 8th ed. Philadelphia (PA): Saunders Elsevier; 2014.
37. Simons FE, Arduoso LR, Bilo MB, Dimov V, Ebisawa M, El-Gamal YM, et al. 2012 update: World Allergy Organization guidelines for the assessment and management of anaphylaxis. *Curr Opin Allergy Clin Immunol* 2012;12:389-99.
38. Kemp SF, Lockey RF. Anaphylaxis: a review of causes and mechanisms. *J Allergy Clin Immunol* 2002;110:341-8.
39. Nazonale I. Role of platelet-activating factor in cardiovascular pathophysiology. *Physiol Rev* 2000;80:1669-700.
40. Jönsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al. Mouse and human neutrophils induce anaphylaxis. *J Clin Invest* 2011;121:1484-96.
41. Shibamoto T, Liu W, Cui S, Zhang W, Takano H, Kurata Y. PAF, rather than histamine, participates in mouse anaphylactic hypotension. *Pharmacology* 2008;82:114-20.
42. Vadas P, Perelman B. Effect of epinephrine on platelet-activating factor-stimulated human vascular smooth muscle cells. *J Allergy Clin Immunol* 2012;129:1329-33.
43. Tjoelker LW, Stafforini DM. Platelet-activating factor acetylhydrolases in health and disease. *Biochim Biophys Acta* 2000;1488:102-23.
44. Dyer KD, Percopo CM, Xie Z, Yang Z, Kim JD, Davoine F, et al. Mouse and human eosinophils degranulate in response to platelet-activating factor (PAF) and lysoPAF via a PAF-receptor-independent mechanism: evidence for a novel receptor. *J Immunol* 2010;184:6327-34.
45. Thivierge M, Parent JL, Stankova J, Rola-Pleszczynski M. Modulation of human platelet-activating factor receptor gene expression by protein kinase C activation. *J Immunol* 1996;157:4681-7.
46. Yost CC, Weyrich AS, Zimmerman GA. The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. *Biochimie* 2010;92:692-7.
47. Perelman B, Adil A, Vadas P. Relationship between platelet activating factor acetylhydrolase activity and apolipoprotein B levels in patients with peanut allergy. *Allergy Asthma Clin Immunol* 2014;10:20.
48. Karasawa K, Harada A, Satoh N, Inoue K, Setaka M. Plasma platelet activating factor-acetylhydrolase (PAF-AH). *Prog Lipid Res* 2003;42:93-114.
49. Pravettoni V, Piantanida M, Primavesi L, Forti S, Pastorello EA. Basal platelet-activating factor acetylhydrolase: Prognostic marker of severe hymenoptera venom anaphylaxis. *J Allergy Clin Immunol* 2014;133:1218-20.
50. Gardner AA, Reichert EC, Alexander TS, Topham MK, Stafforini DM. Novel mechanism for regulation of plasma platelet-activating factor acetylhydrolase expression in mammalian cells. *Biochem J* 2010;428:269-79.
51. Marathe GK, Pandit C, Lakshmikanth CL, Chaithra VH, Jacob SP, D'Souza CJ. To hydrolyze or not to hydrolyze: the dilemma of platelet-activating factor acetylhydrolase. *J Lipid Res* 2014;55:1847-54.
52. Sanz MJ, Weg VB, Walsh DT, Williams TJ, Nourshargh S. Differential effects of the PAF receptor antagonist UK-74,505 on neutrophil and eosinophil accumulation in guinea-pig skin. *Br J Pharmacol* 1994;113:513-21.
53. Arias K, Baig M, Colangelo M, Chu D, Walker T, Goncharova S, et al. Concurrent blockade of platelet-activating factor and histamine prevents life-threatening peanut-induced anaphylactic reactions. *J Allergy Clin Immunol* 2009;124:307-14, e1-2.
54. Triggiani M, Schleimer RP, Warner JA, Chilton FH. Differential synthesis of 1-acyl-2-acetyl-sn-glycero-3-phosphocholine and platelet-activating factor by human inflammatory cells. *J Immunol* 1991;147:660-6.
55. Nankar SA, Bajaj P, Sravanthi R, Pande AH. Differential interaction of peptides derived from C-terminal domain of human apolipoprotein E with platelet activating factor analogs. *Biochimie* 2013;95:1196-207.
56. Miadonna A, Tedeschi A, Arnoux B, Sala A, Zanussi C, Benveniste J. Evidence of PAF-acether metabolic pathway activation in antigen challenge of upper respiratory airways. *Am Rev Respir Dis* 1989;140:142-7.
57. Leggieri E, Tedeschi A, Lorini M, Bianco A, Miadonna A. Study of the effects of PAF-acether on human nasal airways. *Allergy* 1991;46:466-71.
58. Austin CE, Foreman JC. The effect of platelet-activating factor on the responsiveness of the human nasal airway. *Br J Pharmacol* 1993;110:113-8.
59. Muñoz-Cano R, Valero A, Roca-Ferrer J, Bartra J, Sanchez-Lopez J, Mullol J, et al. Platelet-activating factor nasal challenge induces nasal congestion and reduces nasal volume in both healthy volunteers and allergic rhinitis patients. *Am J Rhinol Allergy* 2013;27:e48-52.
60. Turner PJ, Dear JW, Foreman JC. Involvement of kinins in hyperresponsiveness induced by platelet activating factor in the human nasal airway. *Br J Pharmacol* 2000;129:525-32.
61. Tedeschi A, Milazzo N, Miadonna A. Nasal eosinophilia induced by PAF-acether is accompanied by the release of eosinophil cationic protein. *Eur Respir J* 1994;7:1445-51.
62. Shirasaki H, Seki N, Kikuchi M, Kanaizumi E, Watanabe K, Konno N, et al. Expression and localization of platelet-activating factor receptor in human nasal mucosa. *Ann Allergy Asthma Immunol* 2005;95:190-6.
63. Narita S, Asakura K. The effects of anti-PAF and other agents on the nasal symptoms in sensitized guinea pigs. *Auris Nasus Larynx* 1993;20:175-83.
64. Albert DH, Malo PE, Tapang P, Shaughnessy TK, Morgan DW, Wegner CD, et al. The role of platelet-activating factor (PAF) and the efficacy of ABT-491, a highly potent and selective PAF antagonist, in experimental allergic rhinitis. *J Pharmacol Exp Ther* 1998;284:83-8.
65. Merlos M, Giral M, Balsa D, Ferrando R, Queralt M, Puigdemont A, et al. Rupatadine, a new potent, orally active dual antagonist of histamine and platelet-activating factor (PAF). *J Pharmacol Exp Ther* 1997;280:114-21.
66. Compalati E, Canonica GW. Efficacy and safety of rupatadine for allergic rhinoconjunctivitis: a systematic review of randomized, double-blind, placebo-controlled studies with meta-analysis. *Curr Med Res Opin* 2013;29:1539-51.
67. Cuss FM, Dixon CM, Barnes PJ. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet* 1986;2:189-92.
68. Félez MA, Roca J, Barberà JA, Santos C, Rotger M, Chung KF, et al. Inhaled platelet-activating factor worsens gas exchange in mild asthma. *Am J Respir Crit Care Med* 1994;150:369-73.
69. Wardlaw AJ, Chung KF, Moqbel R, MacDonald AJ, Hartnell A, McCusker M, et al. Effects of inhaled PAF in humans on circulating and bronchoalveolar lavage fluid neutrophils. Relationship to bronchoconstriction and changes in airway responsiveness. *Am Rev Respir Dis* 1990;141:386-92.
70. Kasperska-Zajac A, Brzoza Z, Rogala B. Platelet activating factor as a mediator and therapeutic approach in bronchial asthma. *Inflammation* 2008;31:112-20.
71. Kasperska-Zajac A, Brzoza Z, Rogala B. Platelet-activating factor (PAF): a review of its role in asthma and clinical efficacy of PAF antagonists in the disease therapy. *Recent Pat Inflamm Allergy Drug Discov* 2008;2:72-6.
72. Spencer DA, Evans JM, Green SE, Piper PJ, Costello JF. Participation of the cysteinyl leukotrienes in the acute bronchoconstrictor response to inhaled platelet activating factor in man. *Thorax* 1991;46:441-5.
73. Gómez FP, Iglesia R, Roca J, Barberà JA, Chung KF, Rodríguez-Roisin R. The effects of 5-lipoxygenase inhibition by zileuton on platelet-activating-factor-induced pulmonary abnormalities in mild asthma. *Am J Respir Crit Care Med* 1998;157:1559-64.
74. Chung KF, Aizawa H, Leikauf GD, Ueki IF, Evans TW, Nadel JA. Airway hyperresponsiveness induced by platelet-activating factor: role of thromboxane generation. *J Pharmacol Exp Ther* 1986;236:580-4.
75. Triggiani M, De Marino V, Sofia M, Faraone S, Ambrosio G, Carratù L, et al. Characterization of platelet-activating factor acetylhydrolase in human bronchoalveolar lavage. *Am J Respir Crit Care Med* 1997;156:94-100.
76. Mathiak G, Szweczyk D, Abdullah F, Ovadia P, Rabinovici R. Platelet-activating factor (PAF) in experimental and clinical sepsis. *Shock* 1997;7:391-404.
77. Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. *Crit Care Med* 2002;30(suppl):S294-301.
78. Yost CC, Cody MJ, Harris ES, Thornton NL, McInturf AM, Martinez ML, et al. Impaired neutrophil extracellular trap (NET) formation: a novel innate immune deficiency of human neonates. *Blood* 2009;113:6419-27.
79. Sörensen J, Kald B, Tagesson C, Lindahl M. Platelet-activating factor and phospholipase A2 in patients with septic shock and trauma. *Intensive Care Med* 1994;20:555-61.
80. Lopez Diez F, Nieto ML, Fernandez-Gallardo S, Gijon MA, Sanchez Crespo M. Occupancy of platelet receptors for platelet-activating factor in patients with septicemia. *J Clin Invest* 1989;83:1733-40.
81. Shinozaki K, Kawasaki T, Kambayashi J, Sakon M, Shiba E, Uemura Y, et al. A new method of purification and sensitive bioassay of platelet-activating factor (PAF) in human whole blood. *Life Sci* 1994;54:429-37.
82. Graham RM, Strahan ME, Norman KW, Watkins DN, Sturm MJ, Taylor RR. Platelet and plasma platelet-activating factor in sepsis and myocardial infarction. *J Lipid Mediat Cell Signal* 1994;9:167-82.
83. Li S, Stuart L, Zhang Y, Meduri GU, Umberger R, Yates CR. Inter-individual variability of plasma PAF-acetylhydrolase activity in ARDS patients and PAFAH genotype. *J Clin Pharm Ther* 2009;34:447-55.
84. Dhainaut JF, Tenaillon A, Le Tulzo Y, Schlemmer B, Solet JP, Wolff M, et al. Platelet-activating factor receptor antagonist BN 52021 in the treatment of severe



- sepsis: A randomized, double-blind, placebo-controlled, multicenter clinical trial. BN 52021 sepsis study group. *Crit Care Med* 1994;22:1720-8.
85. Dhainaut JF, Tenailon A, Hemmer M, Damas P, Le Tulzo Y, Radermacher P, et al. Confirmatory platelet-activating factor receptor antagonist trial in patients with severe gram-negative bacterial sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. BN 52021 sepsis investigator group. *Crit Care Med* 1998;26:1963-71.
  86. Froom AM, Greve JW, Buurman WA, van der Linden CJ, Langemeijer HJ, Ulrich C, et al. Treatment with the platelet-activating factor antagonist TCV-309 in patients with severe systemic inflammatory response syndrome: a prospective, multi-center, double-blind, randomized phase II trial. *Shock* 1996;5:313-9.
  87. Poeze M, Froom AH, Ramsay G, Buurman WA, Greve JW. Decreased organ failure in patients with severe SIRS and septic shock treated with the platelet-activating factor antagonist TCV-309: a prospective, multicenter, double-blind, randomized phase II trial. TCV-309 septic shock study group. *Shock* 2000;14:421-8.
  88. Suputtamongkol Y, Intaranongpai S, Smith MD, Angus B, Chaowagul W, Permikul C, et al. A double-blind placebo-controlled study of an infusion of lexipafant (platelet-activating factor receptor antagonist) in patients with severe sepsis. *Antimicrob Agents Chemother* 2000;44:693-6.
  89. Vincent JL, Spapen H, Bakker J, Webster NR, Curtis L. Phase II multicenter clinical study of the platelet-activating factor receptor antagonist BB-882 in the treatment of sepsis. *Crit Care Med* 2000;28:638-42.
  90. Marshall JC. Such stuff as dreams are made on: mediator-directed therapy in sepsis. *Nat Rev Drug Discov* 2003;2:391-405.
  91. Bélanger C, Elimam H, Lefebvre J, Borgeat P, Marleau S. Involvement of endogenous leukotriene B4 and platelet-activating factor in polymorphonuclear leukocyte recruitment to dermal inflammatory sites in rats. *Immunology* 2008;124:295-303.
  92. Schuster DP, Metzler M, Opal S, Lowry S, Balk R, Abraham E, et al. Recombinant platelet-activating factor acetylhydrolase to prevent acute respiratory distress syndrome and mortality in severe sepsis: phase IIb, multicenter, randomized, placebo-controlled, clinical trial. *Crit Care Med* 2003;31:1612-9.
  93. Opal S, Laterre PF, Abraham E, Francois B, Wittebole X, Lowry S, et al. Recombinant human platelet-activating factor acetylhydrolase for treatment of severe sepsis: results of a phase III, multicenter, randomized, double-blind, placebo-controlled, clinical trial. *Crit Care Med* 2004;32:332-41.
  94. Samsamshariat S, Basati G, Movahedian A, Pourfarzam M, Sarrafzadegan N. Elevated plasma platelet-activating factor acetylhydrolase activity and its relationship to the presence of coronary artery disease. *J Res Med Sci* 2011;16:674-9.
  95. Winkler K, Winkelmann BR, Scharnagl H, Hoffmann MM, Grawitz AB, Nauck M, et al. Platelet-activating factor acetylhydrolase activity indicates angiographic coronary artery disease independently of systemic inflammation and other risk factors: the Ludwigshafen Risk and Cardiovascular Health Study. *Circulation* 2005;111:980-7.
  96. Häkkinen T, Luoma JS, Hiltunen MO, Macphee CH, Milliner KJ, Patel L, et al. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 1999;19:2909-17.
  97. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;26:2523-9.
  98. Khakpour H, Frishman WH. Lipoprotein-associated phospholipase A2: an independent predictor of cardiovascular risk and a novel target for immunomodulation therapy. *Cardiol Rev* 2009;17:222-9.
  99. Davidson MH, Corson MA, Alberts MJ, Anderson JL, Gorelick PB, Jones PH, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A2 testing into cardiovascular disease risk assessment guidelines. *Am J Cardiol* 2008;101:51F-7F.
  100. Serruys PW, García-García HM, Buszman P, Erne P, Verheye S, Aschermann M, et al. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation* 2008;118:1172-82.
  101. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, et al. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. *JAMA* 2014;312:1006-15.
  102. STABILITY Investigators, White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, et al. Darapladib for preventing ischemic events in stable coronary heart disease. *N Engl J Med* 2014;370:1702-11.
  103. Tawakol A, Singh P, Rudd JH, Soffer J, Cai G, Vucic E, et al. Effect of treatment for 12 weeks with rilapladib, a lipoprotein-associated phospholipase A2 inhibitor, on arterial inflammation as assessed with 18F-fluorodeoxyglucose-positron emission tomography imaging. *J Am Coll Cardiol* 2014;63:86-8.
  104. Zheng GH, Xiong SQ, Chen HY, Mei LJ, Wang T. Associations of platelet-activating factor acetylhydrolase (PAF-AH) gene polymorphisms with circulating PAF-AH levels and risk of coronary heart disease or blood stasis syndrome in the Chinese Han population. *Mol Biol Rep* 2014;41:7141-51.
  105. Kispert SE, Marentette JO, McHowat J. Enhanced breast cancer cell adherence to the lung endothelium via PAF acetylhydrolase inhibition: a potential mechanism for enhanced metastasis in smokers. *Am J Physiol Cell Physiol* 2014;307:C951-6.
  106. Hackler PC, Reuss S, Konger RL, Travers JB, Sahu RP. Systemic platelet-activating factor receptor activation augments experimental lung tumor growth and metastasis. *Cancer Growth Metastasis* 2014;19:27-32.
  107. Sahu RP, Turner MJ, DaSilva SC, Rashid BM, Ocana JA, Perkins SM, et al. The environmental stressor ultraviolet B radiation inhibits murine antitumor immunity through its ability to generate platelet-activating factor agonists. *Carcinogenesis* 2012;33:1360-7.
  108. Sahu RP, Ocana JA, Harrison KA, Ferracini M, Touloukian CE, Al-Hassani M, et al. Chemotherapeutic agents subvert tumor immunity by generating agonists of platelet-activating factor. *Cancer Res* 2014;74:7069-78.
  109. Larkin EK, Gao YT, Gebretsadik T, Hartman TJ, Wu P, Wen W, et al. New risk factors for adult onset incident asthma: a nested case control study of host antioxidant defense. *Am J Respir Crit Care Med* 2015;191:45-53.
  110. Detopoulou P, Fragopoulou E, Nomikos T, Yannakoulia M, Stamatakis G, Panagiotakos DB, et al. The relation of diet with PAF and its metabolic enzymes in healthy volunteers. *Eur J Nutr* 2015;54:25-34.
  111. Fan P, Liu XH, He GL, Zhang S, Zhang JX, Bai H. Maternal and fetal plasma platelet-activating factor acetylhydrolase activity and distribution in pre-eclampsia. *Pediatr Res* 2012;72:426-31.
  112. Pawlowic MC, Zhang K. *Leishmania* parasites possess a platelet-activating factor acetylhydrolase important for virulence. *Mol Biochem Parasitol* 2012;186:11-20.