Biochemical Pharmacology, Vol. 41, No. 5, pp. 657–668, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

COMMENTARY

BIOCHEMICAL PHARMACOLOGY OF PLATELET-ACTIVATING FACTOR (AND PAF ANTAGONISTS) IN RELATION TO CLINICAL AND EXPERIMENTAL THROMBOCYTOPENIA

CHRISTOPHER J. MEADE,* HUBERT HEUER and RUDOLF KEMPE†
Departments of Pharmacology and †Medicine, Boehringer Ingelheim KG, D-6507 Ingelheim am Rhein, Federal Republic of Germany

The object of this review is to relate new developments in the field of platelet-activating factor (PAF) research to the problems of understanding the cause and perhaps improving the therapy of thrombocytopenia. Thrombocytopenia is a symptom associated with a wide variety of clinical conditions, and can have a variety of probable causes. No attempt will be made here to treat thrombocytopenia comprehensively. Instead, discussion will concentrate on those kinds of thrombocytopenia where current knowledge makes it worthwhile to look for involvement of PAF or the PAF receptor. Since much more is known about the role of PAF in platelet activation and adherence than in platelet production, this review will focus in particular on those kinds of clinical thrombocytopenia associated with an enhanced rate of platelet destruction.

HUMAN THROMBOCYTOPENIA SYNDROMES

Normal human blood contains between 150×10^9 and 600 ± 10^9 platelets/L. This number is dependent upon age, and variation within this range can occur quite normally, for example in women during the menstrual cycle. Transient and generally symptom-free thrombocytopenia (i.e. platelet count below the normal range) occurs relatively frequently in association with infections, especially viral infections, in association with surgical operations, especially major abdominal surgery and cardiac bypass operations, and during pregnancy. In a prospective study of over 2000 pregnancies in normal women in a Canadian hospital, 8.3% had platelet counts of less than 150×10^9/L at term, although the thrombocytopenia had no discernible clinical effect on either the women or their infants [1].

Infections, surgery or pregnancy are among the precipitating agents associated with more severe thrombocytopenia, which can also occur during liver disease, as a complication of therapy with various medicaments, in association with certain autoimmune diseases such as systemic lupus erythematosus and with no apparent external cause. A platelet count of below 50×10^9/L is associated with an increased risk of bleeding, although vascular accident rarely occurs unless the platelet count is below 20×10^9/L. Intracranial hemorrhage is a rare but particularly dangerous sequela of thrombocytopenia.

Thrombocytopenia may be caused by a failure of production of platelet precursors in the bone marrow, or by an enhanced destruction and shortened life-span of formed platelets. In the latter case, the number of megakaryocytes in the bone marrow is normal or may be increased, reflecting a compensatory increase in platelet production. The increased proportion of young platelets in the circulation can cause a right shift in the distribution curve of platelet size, while an enhanced rate of platelet destruction in the spleen may lead, in some cases, to splenomegaly.

The term idiopathic thrombocytopenic purpura (ITP) was originally applied to all those conditions in which thrombocytopenia was not accompanied by changes in leucocytes or erythrocytes (apart from those associated with bleeding) and where thrombocytopenia could not be explained by deficient megakaryocytosis or by known processes such as microangiopathy. The diagnosis was originally, and to a certain extent still is, one of exclusion, and the label "idiopathic" is appropriate to the lack of information, but in the light of modern knowledge the term, though widely used, is somewhat misleading. In most cases, there is at least an idea of what has caused the thrombocytopenia even if the final proof of a causative link is still lacking. The acute form of ITP, which occurs most commonly in children, is in more than 80% of cases sequel to a viral infection, and immune complexes may be important for pathogenesis. The chronic form occurs mostly in adults, generally is not associated with antecedent viral infection, and in about 80% of cases is associated with an IgG platelet autoantibody [2, 3]. In both forms of ITP, there appears to be an increased rate of clearance of platelets by the reticuloendothelial system principally in the spleen but also (in severe disease) in the liver. ITP is clinically the most common form of severe thrombocytopenia.

Thrombotic thrombocytopenic purpura (TTP) is a separate syndrome in which thrombocytopenia is associated with thrombi composed of fibrin and platelets which lodge in the arterioles and capillaries and which are probably responsible for most of the clinical manifestations (e.g. fever, fluctuating neurologic signs, and renal abnormalities). This

* Corresponding author.

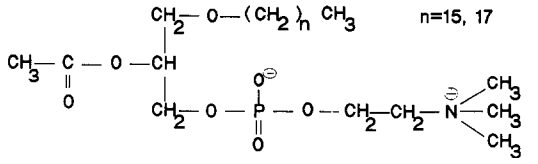

Fig. 1. Platelet-activating factor (PAF).

syndrome can also occur as a sequel of viral or other infections, or can be associated with platelet autoantibodies [4]. Hemolytic-uremic syndrome is similar to TTP, but platelet thrombi lodge predominantly in the kidney. Neurological symptoms are less prominent and renal function is impaired more severely. Both TTP and hemolytic-uremic syndrome are considerably rarer than ITP.

PLATELET-ACTIVATING FACTOR

Platelet-activating factor is a phospholipid which, in experimental animals, has been shown, even in minute quantities, to cause a pronounced thrombocytopenia [5–20]. Its structure is shown in Fig. 1. The presence of an acetyl group in the 2-position of the phospholipid confers a certain degree of water solubility so that the concentrations at which it can exert its biological effects are well below the critical micellar concentration, and the compound can behave like a soluble compound in its physiological context. *In vivo* the compound may also be bound extensively to serum proteins.*

In most cell types, the level of PAF in the resting cell is low, and PAF formation is dependent upon the presence of a membrane stimulus. There exist at least two pathways for PAF biosynthesis [21]. The most important during cell activation is via acetylation of 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso PAF), which can, in turn, be generated by the action of phospholipase A_2 upon 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine. A second mechanism is the transfer of the phosphocholine moiety from CDP-choline to 1-*O*-alkyl-2-acetyl-*sn*-glycerol. This second pathway may represent the biosynthetic pathway required to maintain steady-state levels of PAF.

Once synthesised PAF is readily degraded, and in healthy individuals, the level of PAF in the plasma is low (below 1 ng/mL). The kidney is probably the major source of the basal plasma PAF, since PAF cannot be found at all in the plasma of anephric patients [22]. Other tissue sources are liver [23], lung [24] and the phagocytic cells of blood and spleen [25, 26]. Many of the cells involved in inflammation or allergy (e.g. neutrophils, macrophages, lymphocytes, basophils, eosinophils) are good sources of PAF when activated [27–29]. Another good source of PAF (and perhaps of particular importance in certain types of thrombocytopenia) are human endothelial cells [29–33]. The extent to which human platelets themselves can produce PAF is unclear. The major product when platelets are stimulated appears to be lyso-PAF rather than PAF, possibly because platelets are rich in the enzymes responsible for PAF degradation [34]. However, when platelets are stimulated in the presence of cells such as neutrophils rich in the acetylation enzyme, then the amount of PAF produced by the cell mixture is greater than that of either cell alone [35].

Activity of PAF is controlled not only by synthesis, but also (with varying degrees of specificity) by natural inhibitors. C-reactive protein, and possibly other liver-derived proteins, form an important non-specific mechanism for limiting PAF-induced activation of platelets [36–38]. Another important controlling factor is the activity of the enzymes of PAF degradation, in particular alkylacetyl glycerophosphocholine acetylhydrolase (PAF acetylhydrolase). This enzyme occurs both within cells [39] and in human plasma [40, 41] where it is bound to lipoprotein [40]. The enzyme catalyzes the conversion of PAF to alkyl lysoglycero-phosphocholine.

The activity of PAF acetylhydrolase decreases as a pregnancy approaches term [42]. The fetus, and in particular fetal lung, produces significant quantities of PAF [43] which has also been detected in the amniotic fluid of women at term and in labor [44, 45]. It has been suggested that PAF may have a physiological role in the control of normal parturition [43]. The possible significance of this changed balance of PAF and its degradative enzyme for pregnancy-associated thrombocytopenia will be discussed later.

Not only can a fall in platelet count occur during late pregnancy and labor, but there is also a small decrease in platelet count which follows fertilization and implantation. In mice, desensitization to PAF prevents this "early pregnancy associated thrombocytopenia" (EPAT) [46]. Also, production of a thrombocytopenia-inducing factor by mouse and human embryos has been reported to proceed concomitantly with PAF synthesis [47, 48]. This suggests that the factor responsible for EPAT and PAF are one and the same. This conclusion has been challenged, however, in a subsequent study in which a factor, capable of inducing thrombocytopenia in splenectomized mice, was described in human embryo culture supernatants free of PAF [49].

THE PAF RECEPTOR

There is good evidence that most, if not all, effects of PAF are mediated through one or more specific receptors. This evidence includes the extreme potency of PAF (e.g. doses as small as 3 ng/kg/min will cause thrombocytopenia in guinea pigs [14]); the existence of high-affinity binding sites (K_D in the lower nanomolar range) on cell membranes, including membranes from human platelets [50–57] and endothelial cells [55–57]; the stereoselectivity of

* *Author's note:* Some confusion has been caused in the literature because the term PAF has also been used in a few publications to describe a factor, probably protein, present in the plasma of some TTP patients and capable of causing platelet aggregation [3]. Whether this "factor" represents a complex of 1-*O*-alkyl-2-acetyl-glycero-3-phosphocholine and a carrier protein, or is an entirely different material, has yet to be determined.

binding as evidenced using PAF stereoisomers [58]; the existence of homologous desensitization [59, 60], and the induction by PAF at least under certain conditions, and in many if not all cell types, of typical receptor-mediated signal transduction processes including GTPase activation [61], inositol phosphate production [60], and protein kinase mobilization [62].

The receptor on platelets has been particularly well investigated. In a study with human platelets, the high-affinity PAF receptors behaved as a single population, and there was no evidence that occupancy of one receptor site could alter the binding affinity of a second receptor [54].

The apparent number of platelet PAF-binding sites reported in the literature has varied widely, and this may be because the PAF receptor can also exist in a low-affinity state which is not detected in usual Scatchard analyses. Conversion between low- and high-affinity conformational states depends on the ionic environment (e.g. Mg^{2+}, Ca^{2+} concentration) [63, 64]. The effects of ionic environment on the affinity of the human platelet receptor are not the same as the effects on the human neutrophil leukocyte receptor. Thus, K^+ decreases Mg^{2+}-potentiated [^{3}H]PAF binding to human leukocyte membranes but shows no effect on binding to human platelet membranes. Also, sensitivity of the PAF-stimulated GTPase to cholera or pertussis toxins is different in platelet and neutrophil membranes. For these and other reasons it has been suggested that platelet and neutrophil PAF receptors belong to different subtypes [63].

In certain cell types (in relation to thrombocytopenia, the endothelial cell is particularly important) it is likely that not only extracellular PAF and PAF receptors on the cell surface, but also intracellular PAF and PAF receptors within the cell, play an important role. Vascular endothelial cells generate appreciable quantities of PAF on exposure to agents such as bradykinin, ATP or thrombin [65–67] or in response to oxidant-induced damage [68], but at least under normal tissue culture conditions, most of this PAF is not released.

Nevertheless, changes in endothelial cell function produced by some of these agents have been shown to correlate with PAF formation. Thus, in thrombin-stimulated or oxidant damaged human endothelial cells, there is a tight correlation of the concentration–response relationships and time–courses for PAF production and enhanced capability for neutrophil adherence [67, 68].

Complete and unequivocal identification of any PAF receptor has at the time of writing yet to be achieved. Most receptors for physiological mediators are proteins, and it is likely that PAF receptors are also of this type [55]. A number of PAF binding proteins have been described, for example in human platelet extracts [69–71], but it is not clear whether these proteins function as receptors or whether they are, for example, involved in the translocation of PAF between different compartments within the cell [72]. A critical test to differentiate between a protein which merely binds PAF and one that truly functions as a receptor will be possible when the PAF receptor has been cloned and it is possible to correlate functional changes in sensitivity to PAF effects with expression of the cloned gene. Sequencing and cloning will also provide the unequivocal answer to the question whether PAF receptor subtypes exist or whether the data which have been used to support the subtype concept must be otherwise explained.

PAF RECEPTOR ANTAGONISTS

Further evidence for the existence of a specific PAF receptor is the competitive inhibition of both PAF binding and PAF activities by a range of PAF antagonists. Not all are competitive receptor antagonists, but some of the most widely studied fall into this category, for example the PAF-analog CV-3988 [51], the hetrazepine WEB 2086 [54, 56, 57] and the ginkgolide BN 52021 [52]. The availability of radiolabeled antagonists such as [^{3}H]WEB 2086 has enabled direct measurement of binding kinetics of the drug to both human platelets [54, 73] and human endothelial cells [57]. Binding of radiolabeled WEB 2086 was inhibited competitively by non-labeled PAF and vice versa. The calculated number of WEB 2086 binding sites on the platelet (260) was found similar to the calculated number of PAF binding sites measured under similar conditions in the same study (240), and it therefore seems that this inhibitor (like several other antagonists, but in contrast to, for example, 52770 RP) binds at the same site as the PAF molecule.

Important effects of PAF on platelets include aggregation and the release of thromboxanes, serotonin and adenine nucleotides. These mediators, in turn, can alter the properties of the vascular endothelium such as its permeability, or ability to act as a site for cell adherence.

A number of studies have shown a correlation between the ability of PAF antagonists to block the high-affinity PAF binding site and their ability to block platelet aggregation [57, 74, 75] or release of mediators such as serotonin [57], suggesting that the high-affinity PAF binding site is functional. Not only surface membrane, but also intracellular PAF receptors can be blocked by appropriate PAF antagonists. This conclusion follows, for example, from the ability of WEB 2086 and CV-6209 to block bradykinin, A23187 and, to a lesser extent, ATP-induced prostaglandin I_2 (PGI_2) production, processes which are closely coupled to elevation of intracellular PAF levels [66].

Although some PAF antagonists, for example CV-3988 (Fig. 2c), are clearly related in structure to PAF, the structural relationships between PAF and either WEB 2086 (Fig. 2a) or BN 52021 (Fig. 2b) are less clear. Molecular modeling studies based on these compounds and their analogs, however, have attempted to show common structural features of the compounds and PAF itself [73, 74].

Several PAF antagonists (including WEB 2086, a ginkgolide mixture containing BN 52021, and CV-3988) are being, or have been, tested in the clinic [76–79].

PAF-INDUCED THROMBOCYTOPENIA IN ANIMALS

A reduction in blood platelet count after injection

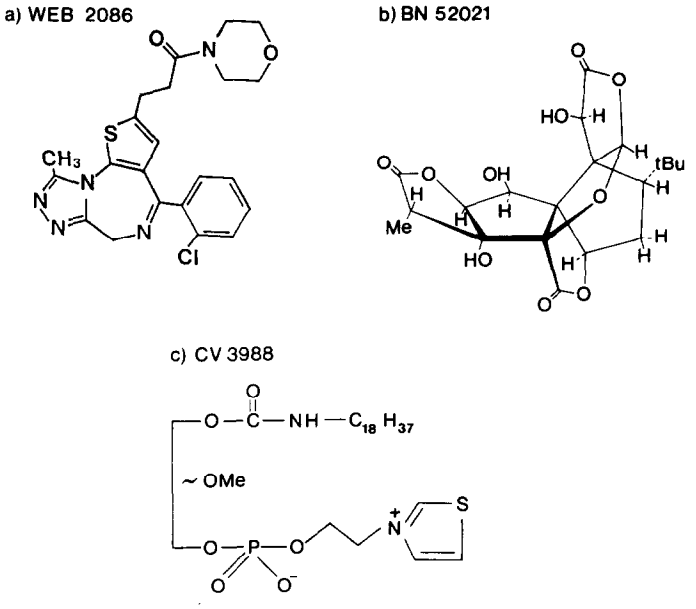

Fig. 2. Structures of some of the PAF antagonists cited in the text.

Table 1. Inhibition of PAF-induced thrombocytopenia by PAF receptor antagonists

Species	Antagonist	Reference
Rabbit	FR-900452	12
Guinea pig	BN 52021	10
	WEB 2086	14
	RO-19-3704	17
	Various pyrido[2,1-*b*] quinazolinecarboxamide derivatives	18
	CV-3988, L-652,731	19
	48740 RP	20
Sheep	L-652,731	13
Rat	BN 52021, 48740 RP	
	WEB 2086	16

or infusion of PAF has been demonstrated in, among other species, rabbits [5, 6, 12], hamsters [11], guinea pigs [7, 8, 14, 17–20], sheep [13] and rats [15, 16]. A preliminary study has shown a similar phenomenon after intratracheal PAF instillation in human subjects [80].

The effect of PAF on platelet count has been shown in animal studies to be blocked by PAF-receptor antagonists. Table 1 summarizes the principal studies to date using PAF-receptor antagonists in animal models of thrombocytopenia. While these studies make it clear that the effects of PAF are receptor mediated, the location of the PAF receptor whose interaction with PAF is essential for experimental thrombocytopenia has yet to be established. Clearly, the PAF receptor on platelets is likely to be important, but the ability of PAF (albeit at the relatively high dose of 6 μg/kg i.v.) to induce thrombocytopenia in rats, whose platelets neither bind nor respond to PAF when tested in the presence of autologous plasma, makes it unlikely that the thrombocytopenia observed in experimental animals is merely an *in vivo* correlate of the platelet aggregation observed *in vitro* [15, 16]. Other receptor-mediated actions of PAF may be involved, such as changes in endothelial cell adhesiveness or the production of platelet active mediators (e.g. thromboxane A_2 or certain lipoxygenase products) by non-platelet cell types such as endothelial cells, Kupffer cells or splenic macrophages. This latter possibility is supported by the observation that PAF-induced thrombocytopenia in rats can not only be blocked by the PAF-receptor antagonists BN 52021, 48740 RP and WEB 2086, but also by the lipoxygenase inhibitor nordihydroguaiaretic acid [16]. A role for arachidonic acid metabolites in PAF-induced thrombocytopenia is also suggested by studies in guinea pigs. In this species intravenous PAF can cause an increase in plasma thromboxane B_2, an effect blocked by the PAF-receptor antagonists CV-3988 and L-652,731 [19]. The thromboxane synthetase inhibitor FCE 22178 has been reported to inhibit PAF-induced thrombocytopenia in guinea pigs [9], but in another study the same species aspirin (a drug which can also block thromboxane generation) was ineffective at a dose at which it blocked the thrombocytopenia induced by arachidonic acid [7].

The histological picture in PAF-induced thrombocytopenia is of platelet micro-aggregates which lodge in the small vessels of the lung and other organs. Studies on the small blood vessels of guinea pigs have suggested that one of the first effects of PAF is to cause retraction and vacuolization of vascular endothelial cells. Sub-intimal tissue becomes exposed, and it is to the exposed areas between the endothelial cells that the platelets adhere with subsequent formation of a platelet thrombus. Treatment with the PAF-receptor antagonist BN 52021

concomitant with PAF challenge inhibits thrombus formation and endothelial cell injury. When exposure to BN 52021 occurs after formation of a thrombus, then embolization can be forced. However, a few minutes after such forced embolization, if perfusion with the PAF antagonist is not continued, then a new thrombus begins to grow at the same site. Formation of the new thrombus, though occurring in the absence of superfused PAF, is still inhibited by the PAF antagonist, suggesting that once the vascular endothelium is damaged endogenous production of PAF at the site of the lesion can drive thrombus formation [81].

The platelet aggregates formed in response to PAF perfusion are generally only loosely held together, and can be dissociated when blood from thrombocytopenic animals is diluted *in vitro* [16]. Reversibility of aggregation can also be shown *in vivo*. In guinea pigs, accumulation of ^{111}In-labeled platelets in the lung after PAF administration can be reversed by the PAF receptor antagonists WEB 2086, WEB 2170 or STY 2108 not only when these are given prophylactically but also when they are given therapeutically [82, 83].

Exposure to PAF *in vitro* causes platelets to reveal fibrinogen binding sites [84], and these may be important not only for *in vitro* aggregation but possibly also for *in vivo* platelet adherence and thrombocytopenia.

PAF is known also to be able to influence fibrinogen breakdown. In a study in rats, infusion of a high dose (8 μg/kg/hr) of PAF i.v. was shown to cause a fall in the level of fibrinogen, and a rise in the level of fibrin degradation products. These effects were blocked by the PAF-receptor antagonist CV-3988 [85].

COMPARISON OF THROMBOCYTOPENIA IN HUMANS WITH PAF-INDUCED THROMBOCYTOPENIA IN ANIMALS

The histopathology of thrombocytopenia following PAF infusion shows some resemblances to the human condition thrombotic thrombocytopenic purpura. In both conditions, the underlying feature is the lodging of platelet aggregates in small vessels at sites where the vascular endothelium has become damaged. In both conditions, fibrin can become associated with the platelet aggregates. However, in the animal model, the lung is the major site in which platelet aggregates lodge, whereas in human TTP although the lungs may be involved, aggregates occur at many other sites and indeed it is often the location of microthrombi in kidney or brain which are responsible for the clinical symptoms observed [86].

Resemblances can also be drawn between PAF-induced thrombocytopenia and thrombocytopenia associated with septicemic shock. Here again the human disease is characterized by formation of platelet aggregates at damaged vascular surfaces. In septicemic thrombocytopenia the spleen and, in severe disease, the lung and liver are the major sites at which platelet aggregates lodge [85, 86].

Clinically, thrombocytopenia can occur in septicemia without marked changes in fibrinogen levels in the plasma, but in severe cases, septicemia can be associated with a generalized formation of thrombi at multiple sites within the body and a massive activation of fibrinogen breakdown leading to disseminated intravascular coagulation. It is in patients with this syndrome that the risk of bleeding is greatest. A syndrome with some similarities to disseminated intravascular coagulation has also been described in rats after infusion of high doses of PAF [85]. The similarities between the pathology of endotoxin- and PAF-induced thrombocytopenia are even closer when animal models of endotoxin-induced thrombocytopenia are considered. In experimental animals, it is predominantly in the lung that i.v. injection of endotoxin (like i.v. injection of PAF) causes platelets to aggregate [87].

While similarities can be drawn between TTP or septicemic thrombocytopenia and the pathology of PAF-induced changes in experimental animals, the most common form of clinically important thrombocytopenia, ITP, shows a very different histopathology from that after PAF infusion. In ITP there is no obvious microangiopathy and no evidence for fibrinogen breakdown in the blood vessels. The mechanism of removal of platelets involves principally not the lung but the spleen, which may in a few ITP patients show a modest enlargement.

Even if, as discussed later, PAF were to be involved in the pathogenesis of ITP, it is not surprising that the histopathology of PAF-induced thrombocytopenia and ITP are so different, as it is likely that other factors (e.g. anti-platelet autoantibodies) are involved in the clinical disease but not represented in the PAF-induced thrombocytopenia animal models. There is considerable scope for devising models in which PAF-perfused animals are either first treated with sub-maximal amounts of anti-platelet antibody or immunized to produce such antibody. The effects of PAF in the presence of an anti-platelet antibody may be quite different from the effect of PAF alone.

POSSIBLE MECHANISMS FOR THROMBOCYTOPENIA

This section will deal in a speculative manner with possible ways in which PAF or PAF receptors may be involved in experimental or clinical syndromes of enhanced platelet destruction and thrombocytopenia. At least four kinds of interference can be suggested that may lead to abnormal platelet destruction or sequestration, viz:

(1) Excessive production of PAF
(2) Deficiency of endogenous PAF inhibitors
(3) Stimulation or enhancement of PAF-receptor triggering by mechanisms other than combination with the PAF molecule
(4) Stimulation, by PAF, of binding of an abnormal element (e.g. auto-antibody) capable of causing platelet clearance or destruction.

More than one of these mechanisms may operate together, and other mechanisms (e.g. immunological mechanisms, mechanisms involving PAF-induced activation of the reticuloendothelial system) may also contribute.

Table 2. Some diseases reported in the literature to be associated with increased levels of PAF in plasma or tissues

Disease	Reference
Disseminated intravascular coagulation	89
Septic shock	90, 91
Anaphylactic shock	91
Drug-induced allergic reaction	92
Cirrhosis of the liver	93
Asthma (patients with late phase)	94
Systemic lupus erythrematosis	95

All the above diseases have also been reported to be associated with thrombocytopenia or shortened platelet life span, though in the particular patients used for PAF measurements, platelet counts were not reported. In the asthma study [94], samples were acylated before assay so that both lyso-PAF and PAF were measured.

EVIDENCE OF EXCESSIVE PRODUCTION OF PAF

Only limited information is available on the production of PAF *in vivo*, as a result on the one hand of the very rapid hydrolysis of PAF in plasma and tissue fluids [40, 41] and on the other hand on the limitations of the techniques for PAF extraction and assay [88]. Table 2 lists papers in the literature showing elevated plasma PAF levels associated with selected clinical conditions. Plasma levels give an indication of systemic PAF release, but because of the rapid degradation of PAF by plasma PAF acetylhydrolase, local release is also likely to play an important role in disease processes. Unfortunately measurement of local PAF levels in clinical disease is relatively difficult. All of the clinical conditions listed in Table 2 can be associated with thrombocytopenia but only in one study [89] has an attempt been made to correlate in the same study the two parameters thrombocytopenia and plasma PAF measurements. In this study, C16-PAF levels were monitored in a patient with a pre-disseminated intravascular coagulation state (as established with the aid of the protamine sulfate test). As long as the C16-PAF levels remained low (below 15 pg/mL blood), then the thrombocyte count remained in the normal range. A sudden increase in plasma C16-PAF (up to 204 pg/mL blood) was accompanied by a dramatic fall in thrombocyte count. Some of the other conditions in Table 2 (e.g. asthma) are included for completeness, and not because the thrombocytopenia is by itself clinically important.

Extensive studies of PAF plasma levels in ITP and TTP, the two most important clinical conditions in which it is the thrombocytopenia and attendant risk of bleeding which is the clinically relevant parameter have not, as yet, been published. However, some measurements of PAF levels were made during treatment of a single ITP patient with a PAF antagonist [91]. This patient, who appeared to have an endogenous inhibitor of PAF present in his plasma, did, at one stage in treatment with a PAF antagonist, develop a relatively high (13 ng PAF/mL) level of plasma PAF, although at the commencement of treatment PAF could not be detected.

The increased plasma levels of PAF in conditions such as systemic lupus erythematosus or anaphylactic shock may be the result of cell activation by immune complexes. Cell types reported in the literature as being activated to PAF production by immune complexes include leucocytes, mast cells, basophils, lymphocytes, macrophages and renal mesangial cells [26–28, 96, 97]. Stimulation via the immunoglobulin Fc receptor is a possible mechanism for this effect, as it is known that activation of lymphocyte Fc receptors via a specific monoclonal antibody can cause PAF release [98].

Systemic lupus erythematosus is characterized by immune complexes containing nucleic acids and antinucleic acid antibody. In the acute phase of the disease, the basophils are degranulated and the amount of PAF releasable from the leucocytes upon DNA challenge is decreased markedly in comparison with basophils either from normal subjects or from patients in remission [99]. During the quiescent phase of the disease, the blood basophils degranulate and release PAF on challenge with DNA (but not other, unrelated antigens). When platelets are also present, electron microscopy shows formation of loose associations between basophil and platelets, and the platelets show aspects of degranulation, even in the presence of inhibitors of the ADP and arachidonic acid dependent aggregation pathways. One interpretation of these results is that basophil stimulation, with release of PAF, has already occurred in the acute phase of the disease whereas in the quiescent phase the cells are primed by anti-DNA antibody for this event.

Immune complexes may not only be important as stimulators of PAF release, but they may also modulate the action of PAF on platelets, via interactions between the PAF receptor and the platelet Fc receptor. It is known that interaction of human platelets with aggregated IgG (which also occurs via the Fc receptor) can enhance the ability of PAF to stimulate platelet aggregation [100].

Some of the effects of immune complexes (or IgG aggregates) in animal models can be abrogated with PAF antagonists, but an attempt to block IgG aggregate-induced thrombocytopenia in guinea pigs with such an antagonist was unsuccessful [101].

The studies in septicemia show a high proportion of patients with elevated plasma PAF levels, particularly in the group having positive blood cultures [90, 91]. Where available, measurements of platelet sensitivity to PAF have shown specific desensitization to this mediator, an observation not surprising in view of the readiness with which the PAF receptor is specifically desensitized in the presence of PAF [60].

Recent work has suggested an important role for the monokines, tumor necrosis factor (TNF), and interleukin 1 in shock [102]. This together with the known ability of these peptides to stimulate production of PAF by endothelial cells [29, 31] and various blood components [29] may indicate one source of the PAF in shock plasma. In addition, PAF itself can stimulate both TNF and IL-1 formation [103], and pre-exposure to PAF can enhance both *in vitro* and *in vivo* responses to PAF [104], providing the possibility of positive feedback.

In the clinical studies, it remains to be established

whether the increased plasma PAF activity in septicemia is an epiphenomenon, or whether PAF contributes to the symptoms of septicemic shock, including septicemia-associated thrombocytopenia. However, data are available using specific PAF antagonists in experimental endotoxemia in laboratory animals, a condition in which increased PAF levels can also be demonstrated [105]. Attempts to block endotoxin-induced thrombocytopenia in experimental animals with PAF antagonists have met with varying degrees of success, perhaps reflecting the complexity of effects which endotoxin exerts. In one study in rabbits [12], pretreatment with the PAF antagonist FR-900452 was reported to reduce significantly the thrombocytopenia, but not the leucopenia, induced by *Escherichia coli* endotoxin. In another study in rats, another PAF antagonist, CV-3988, had a modest effect on the endotoxin-induced fall in platelet counts, as well as on various parameters associated with intravascular coagulation such as levels of fibrin degradation products in the plasma [85]. Accumulation of ^{111}In-labeled platelets in the lung was reduced by PAF antagonists in one study in the guinea pig, but in this study the endotoxin was administered by aerosol, not i.v. [87].

DEFICIENCY OF PAF INHIBITORS

Deficiencies of both specific (e.g. PAF acetylhydrolase) and non-specific (e.g. C-reactive protein) mechanisms for controlling PAF-induced platelet activity could, in theory, lead to enhanced PAF-induced platelet activation.

PAF is degraded very rapidly in plasma by PAF acetylhydrolase so that a deficiency of this enzyme is likely to enhance markedly the effects of PAF release. Deficiency of PAF acetylhydrolase may not be uncommon. In a study of 816 Japanese adults, 32 showed a deficiency of this enzyme [106]. Family studies suggested deficiency was inherited as an autosomal recessive trait. Enzyme-deficient individuals were apparently healthy, though predisposed to asthmatic symptoms. An association has also been reported between acetylhydrolase deficiency and active systemic lupus erythematosus, but it is not clear whether the association occurs because the enzyme deficiency results from the disease, or because the deficiency itself predisposes to systemic lupus [95]. Similarly, in a limited study of patients with thrombocytopenia associated with septicemia lower serum PAF-acetylhydrolase levels were recorded (Meade CJ and Heuer H, unpublished results). Again, it is difficult to distinguish between cause and effect of the clinical syndrome. By contrast, in 12 patients with adult-type ITP, serum PAF-acetylhydrolase levels were not significantly different from a normal control group (Meade CJ, unpublished results).

If PAF has a role in platelet removal, then it might be expected that individuals with acetylhydrolase deficiency would be predisposed to thrombocytopenia, but no studies have as yet been published addressing this question. Two groups of patients may be particularly worth investigating for an enzyme deficiency. The first group is those patients with TTP, whose disease can be alleviated by transfusion of fresh normal plasma (which presumably contains the acetylhydrolase enzyme). The second group is women whose thrombocytopenia was precipitated by pregnancy, since acetylhydrolase in any case normally decreases at the approach of term [42]. A case has been published in which a severe thrombocytopenia was both precipitated by pregnancy and alleviated by uterine evacuation [107]. Cases such as this would be of particular interest for investigation because the main source of PAF in the plasma of pregnant women is probably the fetus, not the mother [43].

STIMULATION OF PAF RECEPTOR ACTIVITY BY MECHANISMS OTHER THAN COMBINATION WITH A PAF MOLECULE

Since it is likely that PAF acts through a specific protein receptor on the platelet surface, there presumably exists a possibility of auto-antibodies being formed against this protein. Examples are known of diseases caused by stimulation of a receptor by combination with an auto-antibody, for example certain types of thyrotoxicosis in which the thyroid cells are stimulated by an auto-antibody directed against the thyroid-stimulating hormone (TSH) receptor. Examples also exist of diseases where a receptor activity is influenced by an auto-antibody which combines not with the receptor itself but with a protein at a site close by, e.g. myasthenia gravis.

Approximately 80% of cases of chronic ITP [2, 3], and a small proportion of TTP cases (108) appear to be associated with an increased level of platelet-associated immunoglobulin (usually IgG). The significance of this platelet-associated immunoglobulin has been much debated. One view is that it represents an artifact, due to the presence of platelet fragments, or differences in size distribution between platelets from ITP patients and normals. However, flow cytometric analysis of immunoglobulin binding as a function of platelet size does not support this idea [109]. The alternative view is that immunoglobulin molecules bound to the platelet represent genuine auto-antibodies to platelet antigens. The target for these antibodies is unknown, although some appear to be directed against major platelet glycoproteins of the GP Ib or GP IIb/IIIa complex [109–112]. The GP IIb/IIIa complex is thought to play an important role in platelet adhesion. The complex is the site of attachment of von Willebrand factor as well as fibrinogen, but requires the prior presence of GP Ib for appropriate spatial orientation. Two models might explain how the auto-antibodies and the PAF receptor could interact. In the first model, the auto-antibodies react directly with epitopes of the PAF receptor, causing platelet activation and adherence. This is a possibility, but no evidence exists to support this idea. In the second model, PAF increases expression of the antigens against which auto-antibodies are directed.

STIMULATION, BY PAF, OF BINDING OF AN AUTO-ANTIBODY CAPABLE OF CAUSING PLATELET CLEARANCE OR DESTRUCTION

The concept that PAF and auto-antibodies may interact via a PAF-induced expression of the epitopes against which the auto-antibody is directed is an

attractive one. Binding of PAF to its receptor on the platelet unmasks the glycoprotein IIb/IIIa complex [84], and this complex is one of the targets against which platelet-associated auto-antibodies appear to be directed [109–112].

The concept that one of the factors contributing to ITP is the synergistic interaction between platelet auto-antibody and PAF is also attractive because it explains why, on the one hand, platelet auto-antibody is found in a very high proportion of chronic ITP cases, while on the other hand it has not always been possible to correlate levels of either platelet-associated immunoglobulin or plasma anti-platelet auto-antibody with disease severity. The effectiveness of splenectomy in the treatment of many cases of ITP and the importance of the spleen for the clearance of antibody-coated platelets from the circulation in this disease [113, 114] can be explained on the basis of a PAF/auto-antibody synergism model in terms of the spleen being a major site of both auto-antibody [115, 116] and PAF [25] production.

Anti-platelet antibodies (from sensitization as a result of blood transfusion), at concentrations at which they themselves have little or no direct effect, have been shown to synergize with PAF to produce enhanced platelet aggregation, serotonin release, β-thromboglobulin release and thromboxane synthesis [117]. Such synergy may not necessarily be at the receptor level, since PAF also synergizes with many other platelet-activating agents such as ADP, adrenalin, collagen or thrombin. Nor are the anti-platelet antibodies produced by allogeneic sensitization likely to have the same specificities for antigens on the platelet surface as spontaneous auto-antibodies. Nevertheless, this observed synergy does provide a valuable model for studying the mechanism of interaction between PAF and an anti-platelet antibody during platelet activation.

THERAPEUTIC IMPLICATION OF AN INVOLVEMENT OF PAF IN THROMBOCYTOPENIA

At present the most widely used treatment for ITP is high-dose corticosteroids. In cases refractory to steroids, therapy using splenectomy or other immunosuppressive agents such as azathioprine may be tried. Intravenous immunoglobulin has also been used with some success. High-dose steroids also have a place in the therapy of TTP and thrombocytopenia associated with systemic lupus erythematosus. Corticosteroids are known to be able both to block PAF synthesis [118] and to inhibit PAF-induced platelet aggregation [119].

Treatment with 6-methyl prednisolone is also able to block PAF-induced thrombocytopenia in the rabbit [119]. Whether effects on PAF synthesis or action play any role in the beneficial effects of steroids in clinical thrombocytopenia remains, however, to be established.

A patient diagnosed as having ITP apparently responded to treatment with the PAF-receptor antagonist WEB 2086 by a marked rise in platelet count. When the therapy was withdrawn, platelet count fell, only to rise again when therapy was reintroduced [120]. The PAF antagonist, even at higher doses than those used in the study on the ITP patient, had no effect on platelet count in normal individuals [79]. These findings led to a clinical trial of WEB 2086 in adult-type ITP, which, however, yielded negative results [121].

No trials have, at the time of writing, been carried out using PAF antagonists and either TTP, hemolytic uremic syndrome, or thrombocytopenia associated with disseminated intravascular coagulation.

Clinical thrombocytopenia is a heterologous collection of diseases. It is likely that several different pathological mechanisms are involved, and the involvement of PAF in these mechanisms is still unclear.

The importance of understanding a disease process prior to attempting novel therapies is well illustrated by thrombocytopenia where, for example platelet transfusion has in some cases been helpful in ITP, but may produce a worsening of TTP or hemolytic-uremic syndrome.

In the case of possible therapy with PAF antagonists, caution must be exercised, for example because, even if a PAF antagonist has no effect on bleeding time in normal individuals, it may have an effect on bleeding time in individuals whose hemostatic potential is already impaired.

There is a need for a greater understanding of the biochemical pharmacology of PAF in relation to thrombocytopenia and hopefully such understanding will help decide if there is a place for a PAF antagonist in the therapy of any of the types of clinical thrombocytopenia.

REFERENCES

1. Burrows RF and Kelton JG, Incidentally detected thrombocytopenia in healthy mothers and their infants. *N Engl J Med* **319**: 142–145, 1988.
2. Scharrer I, Idiopathische Thrombozytopenie und andere thrombozytäre Blutungsübel. *Med Welt* **37**: 42–46, 1986.
3. Tullis JL, Idiopathic thrombocytopenic purpura: An update. *Compre Ther* **13**: 26–33, 1987.
4. Lian EC-Y, Pathogenesis of thrombotic thrombocytopenic purpura. *Semin Hematol* **24**: 82–100, 1987.
5. Pinckard RN, Halonen M, Palmer JD, Butler C, Shaw JO and Henson PM, Intravascular aggregation and pulmonary sequestration of platelets during IgE-induced systemic anaphylaxis in the rabbit: Abrogation of lethal anaphylactic shock by platelet depletion. *J Immunol* **119**: 2185–2193, 1977.
6. Bussolino F and Camussi G, Effect of prostacyclin on platelet-activating factor induced rabbit platelet aggregation. *Prostaglandins* **20**: 781–791, 1980.
7. Vargaftig BB, Lefort J, Chignard M and Benveniste J, Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur J Pharmacol* **65**: 185–192, 1980.
8. Coëffier E, Borrel M-C, Lefort J, Chignard M, Broquet C, Heymans F, Godfroid J-J and Vargaftig BB, Effects of PAF-acether and structural analogues on platelet activation and bronchoconstriction in guinea-pigs. *Eur J Pharmacol* **131**: 179–188, 1986.
9. Giorgetti C, Cadelli GC, Ferti C, Baroldi P and Fabbri LM, A thromboxane-synthetase inhibitor reduces bronchoconstriction and thrombocytopenia induced

by platelet activating factor in guinea-pigs. *J Allergy Clin Immunol* **77**: 122, 1986.

10. Desquand S, Touvay C, Randon J, Lagente V, Vilain B, Maridonneau-Parini I, Etienne A, Lefort J, Braquet P and Vargaftig BB, Interference of BN 52021 (ginkgolide B) with the bronchopulmonary effects of PAF-acether in the guinea-pig. *Eur J Pharmacol* **127**: 83–95, 1986.
11. Björk J and Smedegard G, Acute microvascular effects of paf-acether as studied by intravital microscopy. *Eur J Pharmacol* **96**: 87–94, 1983.
12. Okamoto M, Yoshida K, Nishikawa M, Kohsaka M and Aoki H, Platelet activating factor (PAF) involvement in endotoxin induced thrombocytopenia in rabbits: Studies with FR-900452, a specific inhibitor of PAF. *Thromb Res* **42**: 661–671, 1986.
13. Smallbone, BW, Taylor NE and McDonald JWD, Effects of L-652,731 a platelet-activating factor (PAF) receptor antagonist on PAF and complement-induced pulmonary hypertension in sheep. *J Pharmacol Exp Ther* **242**: 1035–1040, 1987.
14. Pretolani M, Lefort J, Malanchère E and Vargaftig BB, Interference by the novel PAF-acether antagonist WEB 2086 with the bronchopulmonary responses to PAF-acether and to active and passive anaphylactic shock in guinea-pigs. *Eur J Pharmacol* **140**: 311–321, 1987.
15. Martins MA, Silva PMRE, Castro HC, Neto F, Lima MCR, Cordeirò RSB and Vargaftig BB, Interactions between local inflammatory and systemic haematological effects of PAF-acether in the rat. *Eur J Pharmacol* **136**: 353–360, 1987.
16. Martins MA, Martins PMRS, Castro Faria Neto HC, Bozza PT, Dias PMFL, Cordeiro RSB and Vargaftig BB, Intravenous injections of PAF-acether induce platelet aggregation in rats. *Eur J Pharmacol* **149**: 89–96, 1988.
17. Lagente V, Desquand S, Hadvary P, Cirino M, Lellouch-Tubiana A, Lefort J and Vargaftig BB, Interference of the paf antagonist Ro 19-3704 with paf and antigen-induced bronchoconstriction in the guinea-pig. *Br J Pharmacol* **94**: 27–36, 1988.
18. Tilley JW, Burghardt B, Burghardt C, Mowles TF, Leinweber F-J, Klevans L, Young R, Hirkaler G, Fahrenholtz K, Zawoiski S and Todaro LJ, Pyrido[2,1-*b*]quinazolinecarboxamide derivatives as platelet activating factor antagonists. *J Med Chem* **31**: 466–472, 1988.
19. Danko G, Sherwood JE, Grissom B, Kreutner W and Chapman RW, Effect of the PAF antagonists CV-3988 and L-652,731 on the pulmonary and hematological responses to guinea pig anaphylaxis. *Pharmacol Res Commun* **20**: 785–798, 1988.
20. Lefort J, Sedivy P, Desquand S, Randon J, Coëffier E, Maridonneau-Parini I, Floch A, Benveniste J and Vargaftig BB, Pharmacological profile of 48740 R.P., a PAF-acether antagonist. *Eur J Pharmacol* **150**: 257–268, 1988.
21. Snyder F, Enzymatic pathways for platelet-activating factor, related alkyl glycerolipids and their precursors. In: *Platelet-Activating Factor and Related Lipid Mediators* (Ed. Snyder F), pp. 89–114. Plenum Press, London, 1987.
22. Caramelo C, Fernandez-Gallardo S, Marin-Cao D, Inarrea P, Santos JC, Lopez-Novoa JM and Sanchez Crespo M, Presence of platelet activating factor in blood from humans and experimental animals. Its absence in anephric individuals. *Biochem Biophys Res Commun* **120**: 789–796, 1984.
23. Buxton DB, Hanahan DJ and Olson MS, Stimulation of glycogenolysis and platelet-activating factor production by heat aggregated immunoglobulin perfused liver. *J Biol Chem* **259**: 13758–13761, 1984.
24. Camussi G, Pawlowski I, Bussolino F, Caldwell PRB, Brentjens J and Andres G, Release of platelet activating factor in rabbits with antibody-mediated injury of the lung: The role of leukocytes and of pulmonary endothelial cells. *J Immunol* **131**: 1802–1807, 1983.
25. Sanchez-Crespo M, Inarrea P and Gomez-Cambronero J, Platelet-activating factor: a secretory product from phagocytes. In: *Platelet-Activating Factor and Related Lipid Mediators* (Ed. Snyder F), pp. 355–374, Plenum Press, London, 1987.
26. Arnoux B, Jouvin-Marche E, Arnoux A and Benveniste J, Release of PAF-acether from human blood monocytes. *Agents Actions* **12**: 713–716, 1982.
27. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W and Tetta C, Release of platelet activating factor (PAF) and histamine. II. The cellular origin of human PAF: Monocytes, polymorphonuclear neutrophils and basophils. *Immunology* **42**: 191–199, 1981.
28. Virella G, Lopes-Virella MF, Schuler C, Sherwood T, Espinoza GA, Winocour P and Colwell JA, Release of PAF by human polymorphonuclear leucocytes stimulated by immune complexes bound to Sepharose particles and human erythrocytes. *Immunology* **50**: 43–51, 1981.
29. Camussi G, Bussolino F, Salvidio G and Baglioni C, Tumour necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. *J Exp Med* **166**: 1390–1404, 1987.
30. Camussi G, Aglietta M, Malavasi F, Tetta C, Piacibello W, Sanavio F and Bussolino F, The release of platelet-activating factor from human endothelial cells in culture. *J Immunol* **131**: 2397–2403, 1983.
31. Bussolino F, Brevario F, Tetta C, Aglietta M, Mantovani A and Dejana E, Interleukin-1 stimulates platelet-activating factor production in cultured human endothelial cells. *J Clin Invest* **77**: 2027–2033, 1986.
32. Camussi G, Bussolino F, Tetta C and Caldwell PR, Role of platelet activating factor of endothelial origin in the pathophysiology of the inflammatory reaction. In: *Proceedings of the First Sandoz Research Symposium* "New Horizons in Platelet-Activating Factor Research" (Eds. Winslow CM and Lee ML), pp. 245–254. Wiley, London, 1987.
33. Prescott SM, Zimmerman GA and McIntyre TM, The production of platelet-activating factor by cultured human endothelial cells: Regulation and function. In: *Platelet-Activating Factor and Related Lipid Mediators* (Ed. Snyder F) pp. 323–340. Plenum Press, London, 1987.
34. Touqui L, Hatmi M and Vargaftig BB, Human platelets stimulated by thrombin produce platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) when the degrading enzyme acetyl hydrolase is blocked. *Biochem J* **229**: 811–816, 1985.
35. Coeffier E, Joseph D, Prevost MC and Vargaftig BB, Platelet–leucocyte interaction: Activation of rabbit platelets by FMLP-stimulated neutrophils. *Br J Pharmacol* **92**: 393–406, 1987.
36. Miwa M, Hill C, Kumar R, Sugatani J, Olson MS and Hanahan DJ, Occurrence of an endogenous inhibitor of platelet-activating factor in rat liver. *J Biol Chem* **262**: 527–530, 1987.
37. Vigo C, Effect of C-reactive protein on platelet activating factor-induced platelet aggregation and membrane stabilization. *J Biol Chem* **260**: 3418–3422, 1985.
38. Nagpurkar A, Randell E, Choudhury S and Mookerjea S, Effect of rat phosphorylcholine-binding pro-

tein on platelet aggregation. *Biochim Biophys Acta* **967**: 76–81, 1988.
39. Blank ML, Lee T-C, Fitzgerald V and Snyder F, A specific acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J Biol Chem* **256**: 175–178, 1981.
40. Farr RS, Cox CP, Wardlow ML and Jorgensen R, Preliminary studies of an acid-labile factor (ALF) in human sera that inactivates platelet-activating factor (PAF). *Clin Immunol Immunopathol* **15**: 318–330, 1980.
41. Farr RS, Wardlow ML, Cox CP, Meng KE and Greene DE, Human serum acid-labile factor is an acylhydrolase that inactivates platelet-activating factor. *Fed Proc* **42**: 3120–3122, 1983.
42. Maki N, Hoffman DR and Johnston JJ, Platelet activating factor acetylhydrolase activity in maternal fetal and newborn rabbit plasma during pregnancy and lactation. *Proc Natl Acad Sci USA* **85**: 728–732, 1988.
43. Johnston JM, Bleasdale JE and Hoffman DR, Functions of PAF in reproduction and development: Involvement of PAF in fetal lung maturation and parturition. In: *Platelet-Activating Factor and Related Lipid Mediators* (Ed. Snyder F), pp. 375–402. Plenum Press, London, 1987.
44. Billah MM and Johnston JM, Identification of phospholipid platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in human amniotic fluid and urine. *Biochem Biophys Res Commun* **113**: 51–58, 1983.
45. Nishihira J, Ishibashi T, Imai Y and Muramatsu T, Mass spectrometric evidence for the presence of platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in human amniotic fluid during labor. *Lipids* **19**: 907–910, 1984.
46. Adamson LM, Smart YC, Stanger JD, Murdoch RN and Roberts TK, Mechanistic studies of early pregnancy associated thrombocytopenia (EPAT) in the mouse. *Am J Reprod Immunol Microbiol* **13**: 117–120, 1987.
47. O'Neill C, Examination of the causes of early pregnancy-associated thrombocytopenia in mice. *J Reprod Fertil* **73**: 567–577, 1985.
48. O'Neill C, Gidley-Baird AA, Pike IL and Saunders DM, Use of a bioassay for embryo-derived platelet-activating factor as a means of assessing quality and pregnancy potential of human embryos. *Fertil Steril* **47**: 969–975, 1987.
49. Amiel ML, Duquenne C, Benveniste J and Testart J, Platelet aggregating activity in human embryo culture media free of PAF-acether. *Hum Reprod* **4**: 327–330, 1989.
50. Valone FH, Coles E, Reinhold VR and Goetzl EJ, Specific binding of phospholipid platelet-activating factor by human platelets. *J Immunol* **129**: 1637–1641, 1982.
51. Terashita Z, Imura Y and Nishikawa K, Inhibition by CV-3988 of the binding of [^{3}H]-platelet activating factor (PAF) to the platelet. *Biochem Pharmacol* **34**: 1491–1495, 1985.
52. Korth R and Benveniste J, BN 52021 displaces [^{3}H]paf-acether from, and inhibits its binding to intact human platelets. *Eur J Pharmacol* **142**: 331–341, 1987.
53. Tahraoui L, Floch A and Cavero I, Optimization for a viable [^{3}H]PAF binding to human platelet membranes. *Prostaglandins* **35**: 794, 1988.
54. Ukena D, Dent G, Robaut C, Birke FW, Sybrecht GW and Barnes PJ, Radioligand binding of antagonists of platelet-activating factor to intact human platelets. *FEBS Lett* **228**: 285–289, 1988.
55. Parnham MJ, PAF receptors. *Prog Biochem Pharmacol* **22**: 17–24, 1988.
56. Korth R, Hirafuji M and Benveniste J, Comparison of human endothelial cells in culture and platelets for ^{3}H-paf-acether binding. *Fed Proc* **46**: 444, 1987.
57. Korth R, Hirafuji H, Keraly CL, Delautier D, Bidault J and Benveniste J, Interaction of the paf-acether antagonist WEB 2086 and its hetrazepine analogues with human platelets and endothelial cells. *Br J Pharmacol* **98**: 653–661, 1989.
58. Heymans F, Michel E, Borrel M-C, Wichrowski B, Godfroid J-J, Convert O, Coeffier E, Tence M and Benveniste J, New total synthesis and high resolution ^{1}H NMR spectrum of platelet-activating factor, its enantiomer and racemic mixtures. *Biochim Biophys Acta* **666**: 230–237, 1981.
59. Hallam TJ, Scrutton MC and Wallis RB, The role of 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (AcGEPC) and palmitoyl-lysophosphatidate in the responses of human blood platelets to collagen and thrombin. *FEBS Lett* **162**: 142–146, 1983.
60. Morrison WJ and Shukla SD, Desensitization of receptor-coupled activating of phosphoinositide-specific phospholipase C in platelets: Evidence for distinct mechanisms for platelet-activating factor and thrombin. *Mol Pharmacol* **33**: 58–63, 1988.
61. Avdonin PV, Svitina-Ulitina IV and Kulikov VI, Stimulation of high-affinity hormone-sensitive GTPase of human platelets by 1-*O*-alkyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphocholine (platelet activating factor). *Biochem Biophys Res Commun* **131**: 307–313, 1985.
62. O'Flaherty JT and Nishihira J, Arachidonate metabolites, platelet-activating factor, and the mobilization of protein kinase C in human polymorphonuclear neutrophils. *J Immunol* **138**: 1889–1895, 1987.
63. Hwang S-B, Identification of a second putative receptor of platelet-activating factor from human polymorphonuclear leukocytes. *J Biol Chem* **263**: 3225–3233, 1988.
64. Hwang S-B, Lam M-H and Hsu AH-M, Characterization of platelet-activating factor (PAF) receptor by specific binding of [^{3}H]L-659,989, a PAF receptor antagonist, to rabbit platelet membranes: possible multiple conformational states of a single type of PAF receptors. *Mol Pharmacol* **35**: 48–58, 1989.
65. McIntyre TM, Zimmerman GA, Satoh K and Prescott SM, Cultured endothelial cells synthesize both platelet-activating factor and prostacyclin in response to histamine, bradykinin and adenosine triphosphate. *J Clin Invest* **76**: 271–280, 1985.
66. Stewart AG, Dubbin PN, Harris T and Dusting GJ, Evidence for an intracellular action of platelet-activating factor in bovine cultured aortic endothelial cells. *Br J Pharmacol* **96**: 503–505, 1989.
67. Zimmerman GA, McIntyre TM and Prescott SM, Thrombin stimulates neutrophil adherence by an endothelial cell-dependent mechanism: Characterization of the response and relationship to platelet-activating factor synthesis. *Ann NY Acad Sci* **485**: 349–368, 1986.
68. Lewis MS, Whatley RE, Cain P, McIntyre TM, Prescott SM and Zimmerman GA, Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. *J Clin Invest* **82**: 2045–2055, 1988.
69. Valone FH, Isolation of a platelet membrane protein which binds the platelet-activating factor 1-*O*-hexadecyl - 2 - acetyl - SN - glycero - 3 - phosphorycholine. *Immunology* **52**: 169–174, 1984.
70. Nishihira J, Ishibashi T, Imai Y and Muramatsu T, Purification and characterization of the specific binding protein for platelet activating factor (1-*O*-alkyl-2-

acetyl-*sn*-glycero 3 phosphocholine) from human platelets. *Tohoku J Exp Med* **147**: 145–152, 1985.
71. Chau L-Y and Jii Y-J, Characterisation of ^{3}H-labelled platelet activating factor receptor complex solubilised from rabbit platelet membranes. *Biochim Biophys Acta* **970**: 103–112, 1988.
72. Banks JB, Wykle RL, O'Flaherty JT and Lumb RH, Evidence for protein-catalyzed transfer of platelet activating factor by macrophage cytosol. *Biochim Biophys Acta* **961**: 48–52, 1988.
73. Braquet P and Godfroid JJ, Conformational properties of the PAF-acether receptor on platelets based on structure–activity series. In: *Platelet Activating Factor and Related Lipid Mediators* (Ed. Snyder F), pp. 191–225. Plenum Press, New York, 1987.
74. Weber KH and Heuer H, Structure–activity relationships and effects of platelet-activating factor antagonists in the hetrazepine series. *Int Arch Allergy Appl Immunol* **88**: 82–87, 1989.
75. Tahraoui L, Floch A, Mondot S and Cavero I, High affinity specific binding sites for tritiated platelet-activating factor in canine platelet membranes: counterparts of platelet-activating factor receptors mediating platelet aggregation. *Mol Pharmacol* **34**: 145–151, 1988.
76. Chung KF, Dent G, McCusker M, Guinot PH, Page CP and Barnes PJ, Effect of ginkgolide mixture (BN 52063) in antagonising skin and platelet responses to platelet activating factor in man. *Lancet* **1**: 248–251, 1987.
77. Arnout J, Van Hecken A, Delepeleire I, Miyamoto Y, Holmes I, Deschepper P and Vermylen J, Effectiveness and tolerability of CV-3988, a selective PAF antagonist, after intravenous administration to man. *Br J Clin Pharmacol* **25**: 445–451, 1988.
78. Adamus WS, Heuer H, Meade CJ, Frey G and Brecht HM, Inhibitory effect of oral WEB 2086, a novel selective PAF-acether antagonist, on *ex-vivo* platelet aggregation. *Eur J Clin Pharmacol* **35**: 237–240, 1988.
79. Adamus WS, Heuer H, Meade CJ and Brecht HM, Safety, tolerability, and pharmacologic activity of multiple doses of the new platelet activating factor antagonist WEB 2086 in human subjects. *Clin Pharmacol Ther* **45**: 270–276, 1989.
80. Gateau O, Arnoux B, Deriaz H, Viars P and Benveniste J, Acute effects of intratracheal administration of PAF-acether (platelet activating factor) in humans. *Am Rev Respir Dis* **129**: A3, 1984.
81. Bourgain RH, Maes L, Braquet P, Andries R, Touqui L and Braquet M, The effect of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (paf-acether) on the arterial wall. *Prostaglandins* **30**: 185–197, 1985.
82. Casals-Stenzel J and Heuer H, Pharmacology of PAF antagonists. *Prog Biochem Pharmacol* **22**: 58–65, 1988.
83. Heuer H, Casals-Stenzel J, Muacevic G, Stransky W and Weber KH, Activity of the new and specific paf-antagonists WEB 2170 and STY 2108 on paf-induced bronchoconstriction and intrathoracic accumulation of platelets in the guinea-pig. *Prostaglandins* **35**: 798, 1988.
84. Kloprogge E, Mommersteeg M and Akkerman J-WN, Kinetics of platelet-activating factor 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine-induced fibrinogen binding to human platelets. *J Biol Chem* **261**: 11071–11076, 1986.
85. Imura Y, Terashita ZI and Nishikawa K, Possible role of platelet-activating factor (PAF) in disseminated intravascular coagulation evidenced by use of a platelet-activating factor antagonist CV-3988. *Life Sci* **39**: 111–117, 1986.
86. Gore I, Disseminated arteriolar and capillary platelet thrombosis a morphologic study of its histogenesis. *Am J Pathol* **26**: 155–167, 1950.
87. Beijer L, Botting J, Crook P, Oyekan AO, Page CP and Rylander R, The involvement of platelet activating factor in endotoxin-induced pulmonary platelet recruitment in the guinea pig. *Br J Pharmacol* **92**: 803–808, 1987.
88. Hanahan DJ and Weintraub ST, Platelet-activating factor. Isolation, identification and assay. *Meth Biochem Anal* **31**: 195–219, 1985.
89. Sakaguchi K, Masugi F, Chen Y-H, Inoue M, Ogihara T, Yamada K and Yamatsu I, Direct evidence of elevated levels of circulating platelet-activating factor in a patient with disseminated intravascular coagulation syndrome. *J Lipid Mediators* **1**: 171–173, 1989.
90. Bussolino F, Porcellini MG, Varese L and Bosia A, Intravascular release of platelet activating factor in children with sepsis. *Thromb Res* **48**: 619–620, 1987.
91. Schierenberg M, Darius H, Treese N, Lohman HF, Meyer J and Heuer HO, PAF-like activity in the plasma from patients with septicemia and other diseases. *Lipids*, in press.
92. Lazanas M, Demopoulos CA, Tournis S, Koussissis S, Labrakis-Lazanas K and Tsarouhas X, PAF of biological fluids in disease: IV. Levels in blood in allergic reactions induced by drugs. *Arch Dermatol Res* **280**: 124–126, 1988.
93. Caramelo C, Fernandez-Gallardo S, Santos JC, Inarrea P, Sanchez-Crespo M, Lopez-Novoa JM and Hernando L, Increased levels of platelet-activating factor in blood from patients with cirrhosis of the liver. *Eur J Clin Invest* **17**: 7–11, 1987.
94. Nakamura T, Morita Y, Kuriyama M, Ishihara K, Ito K and Miyamoto T, Platelet-activating factor in late asthmatic response. *Int Arch Allergy Appl Immunol* **82**: 57–61, 1987.
95. Tetta C, Bussolino F, Stratta P, Modena V, Ragni R, Bosco F and Camussi G, Unbalance between production and catabolism of platelet-activating factor (PAF) in human pathology. Studies of PAF acetylhydrolase (AH) in systemic lupus erythematosus (SLE). *Prostaglandins* **34**: 190, 1987.
96. Benveniste J, Henson PM and Cochrane CG, Leucocyte dependent histamine release from rabbit platelets. The role of IgE, basophils and platelet activating factor, *J Exp Med* **136**: 1356–1377, 1972.
97. Neuwirth R, Singhal P, Diamond B, Hays RM, Lobmeyer L, Clay K and Schlondorff D, Evidence for immunoglobin Fc receptor-mediated prostaglandins and platelet-activating factor formation by cultured rat mesangial cells. *J Cell Invest* **82**: 936–944, 1988.
98. Malavasi F, Tetta C, Funaro A, Bellone G, Ferrero E, Colli Franzone A, Dellabona P, Rusci R, Matera L, Camussi G and Caligaris-Cappio F, Fc receptor triggering induces expression of surface activation antigens and release of platelet-activating factor in large granular lymphocytes. *Proc Natl Acad Sci USA* **83**: 2443–2447, 1986.
99. Camussi G, Tetta C, Coda R and Benveniste J, Release of platelet-activating factor in human pathology. I. Evidence for the occurrence of basophil degranulation and release of platelet-activating factor in systemic lupus erythematosus. *Lab Invest* **44**: 241–251, 1981.
100. Valone FH, Synergistic platelet activation by aggregates of IgG and the phospholipid platelet-activating factor 1-*O*-alkyl-2-acetyl-*SN*-glycero-3-phosphorylcholine. *J Clin Immunol* **6**: 57–64, 1986.
101. Fernandez-Gallardo S, Gijon MA, Cano E and Sanchez-Crespo M, Platelet-activating factor mediates

thrombolysis but not thrombocytopenia in response to soluble aggregates of immunoglobulin G. In: *New Trends in Lipid Mediator Research* (Ed. Braquet P), Vol. 2, pp. 199–203. Karger, Basel, 1988.

102. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF and Cerami A, Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* **330**: 662–664, 1987.
103. Pignol B, Hénane S, Mencia-Huerta J-M, Rola-Pleszczynski M and Braquet P, Effect of platelet-activating factor (PAF-acether) and its specific receptor antagonist, BN-52021, on interleukin 1 (IL1) release and synthesis by rat spleen adherent monocytes. *Prostaglandins* **33**: 931–939, 1987.
104. Heuer HO, Letts G and Meade CJ, Tumour necrosis factor (TNF) and endotoxin prime effects of paf *in vivo*. *J Lipid Mediators* **2**: S101–S108, 1990.
105. Doebber TW, Wu MS, Robbins JC, Ma Choy B, Chang MN and Shen TY, Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist kadsurenone. *Biochem Biophys Res Commun* **127**: 799–808, 1985.
106. Miwa M, Miyake T, Yamanaka T, Sugatani J, Suzuki Y, Sakata S, Araki Y and Matsumoto M, Characterisation of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J Clin Invest* **82**: 1983–1991, 1988.
107. Natelson EA and White D, Recurrent thrombotic thrombocytopenic purpura in early pregnancy: Effect of uterine evacuation. *Obstet Gynecol* **66**: 54S–56S, 1985.
108. Lazarchick J, Graddick SL, Hall SA and Genco PV, Platelet directed antibody in the serum of patients with primary thrombotic thrombocytopenic purpura. *Ann Clin Lab Sci* **17**: 339–344, 1987.
109. Holme S, Heaton A, Kunchuba A and Hartman P, Increased levels of platelet associated IgG in patients with thrombocytopenia are not confined to any particular size class of platelets. *Br J Haematol* **68**: 431–436, 1988.
110. van Leeuwen EF, van der Ven JThM, Engelfriet CP and von dem Borne AEGKr, Specificity of autoantibodies in autoimmune thrombocytopenia. *Blood* **59**: 23–26, 1982.
111. Woods VL, Oh EH, Mason D and McMillan R, Autoantibodies against the platelet glycoproteins IIb/IIIa complex in patients with chronic ITP. *Blood* **63**: 368–375, 1984.
112. Nomura S, Nagata H, Sone N, Oda K, Kokawa T and Yasunaga K, Analysis of platelet antigen for antiplatelet antibodies in idiopathic thrombocytopenic purpura using flow cytometry. *Acta Haematol Jpn* **51**: 118–129, 1988.
113. Aster RH and Keene WR, Sites of platelet destruction in idiopathic thrombocytopenic purpura. *Br J Haematol* **16**: 61–73, 1969.
114. Ries CA and Price DC, [^{51}Cr]Platelet kinetics in thrombocytopenia: Correlation between splenic sequestration of platelets and response to splenectomy. *Ann Intern Med* **80**: 702–707, 1974.
115. McMillan R, Longmire RL, Yelenosky R, Donnell RL and Armstrong S, Quantitation of platelet-binding IgG produced *in vitro* by spleens from patients with idiopathic thrombocytopenic purpura. *N Engl J Med* **291**: 812–817, 1974.
116. Karpatkin S, Strick N and Siskind GW, Detection of splenic anti-platelet antibody synthesis in idiopathic autoimmune thrombocytopenic purpura (ATP). *Br J Haematol* **23**: 167–176, 1972.
117. Beer J and von Felten A, Synergismus zwischen unterschwelligen Dosen von antithrombozytären Antikörpern und "platelet activating factor": Ein möglicher Eliminationsmechanismus für humane Thrombozyten *in vivo*. *Schweiz Med Wochenschr* **114**: 1400–1401, 1984.
118. Chignard M, Le Couedic JP, Andersson P and Brange C, Use of steroidal antiinflammatory drug provides further evidence for a potential role of PAF-acether in bronchial anaphylaxis. *Int Arch Allergy Appl Immunol* **81**: 184–185, 1986.
119. Camussi G, Bussolino F, Tetta C, Deregibus MC, Segoloni G, Ragni R and Vercellone A, Platelet-activating factor (PAF), a mediator of platelet aggregation. Effect of corticosteroids on PAF-induced platelet aggregation. *Minerva Nefrol* **27**: 589–592, 1980.
120. Lohmann HF, Adamus WS and Meade CJ, Idiopathic thrombocytopenia treated with PAF-acether antagonist WEB 2096. *Lancet* **ii**: 1147, 1988.
121. Giers G, Janzarik H, Kempe ER and Mueller-Eckhardt C, Failure of the platelet activating factor antagonist WEB 2086 BS in treatment of chronic autoimmune thrombocytopenia. *Blut* **61**: 21–24, 1990.