Production of platelet-activating factor by human vascular endothelial cells: evidence for a requirement for specific agonists and modulation by prostacyclin

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ABSTRACT  Primary cultures of confluent endothelial cells derived from human umbilical veins produce platelet-activating factor (PAF) (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine) when stimulated with appropriate agonists. Highly purified human thrombin and calcium ionophore A23187 stimulate the incorporation of [3H] acetate into a lipid product that has been identified as PAF by its behavior in thin-layer chromatographic and high-performance liquid chromatographic systems, the presence of characteristic biologic activity, and appropriate response to phospholipases. A number of other humoral mediators, examined because they directly influence the activity of vascular cells or because they may mediate endothelial injury, do not stimulate PAF production by endothelial cells. This indicates that the synthesis of PAF by cultured human endothelial cells is a response to specific agonists and is not an unregulated event that occurs as a result of nonspecific cellular perturbation. The PAF produced by thrombin-treated endothelial cells is a potent stimulus for platelet activation, as assayed by the aggregation of human platelets in autologous plasma. The production of PAF by endothelial monolayers is attenuated by prostacyclin, another product of stimulated endothelial cells. Conversely, PAF production is enhanced by treatment of the endothelial cells with indomethacin, an inhibitor of prostacyclin synthesis from arachidonic acid, indicating that endogenously generated prostacyclin may modulate PAF synthesis. The potential to synthesize PAF, a unique lipid autocoid that stimulates the activation of both platelets and polymorphonuclear leukocytes, suggests that endothelial cells can directly influence the activity of these circulating effector cells. This biologic potential may be important in the interaction of the endothelium with circulating blood cells in physiologic conditions and in syndromes of vascular injury.


PLATELETS AND VASCULAR OCCLUSION

PLATELET-ACTIVATING FACTOR (PAF), originally described as an albumin-bound material produced by rabbit basophils that stimulated the release of histamine from autologous platelets,¹ is a mediator that may be important in the pathogenesis of a variety of immunologic and inflammatory syndromes (reviewed in refs. 2 and 3). The biochemical and biologic qualities of the factor have been further characterized,² ⁴ and its chemical identity has been shown to be 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine³ ⁵ ⁶ (figure 1). PAF activates leukocytes, platelets, and other effector cells isolated from experimental animals² ³ and causes a syndrome with features suggestive of anaphylactic shock when injected into rabbits or baboons.² A mediator with biochemical properties and biologic activity characteristic of PAF is produced by macrophages, monocytes, polymorphonuclear leukocytes (PMNs), and platelets isolated from experimental animals² ³ in addition to basophils.¹ Several human cell types that are active in immunologic and inflammatory responses have been reported to synthesize PAF, including circulating monocytes,⁷ ⁸ ⁹ alveolar macrophages,¹⁰ PMNs,⁷ ¹¹ ¹² and platelets.¹³ Clark et al.⁷ reported that PAF synthesized by stimulated human monocytes and PMNs is indistinguishable from PAF released from rabbit basophils. These observations have suggested that PAF is an autocoid that may mediate inflammatory events in humans, including some syndromes of inflammatory vascular injury.¹⁴ ¹⁵ PAF causes vasoconstriction followed by inflammation and edema when injected into the skin of normal subjects,¹ consistent with this hypothesis. Furthermore, PAF is a potent
PLATELET--ACTIVATING FACTOR

FIGURE 1. The structure of PAF.

stimulus for the activation of human PMNs14, 16-20 and platelets21 in vitro.

In previous studies we found that human endothelial cells produce PAF when stimulated with thrombin, demonstrating an additional cellular source of this mediator.22 The polar lipid product was identified as PAF by its migration with authentic PAF in two chromatographic systems, incorporation of tritiated precursors of PAF into the molecule, the ability of the lipid to stimulate PMN aggregation, a biologic activity characteristic of PAF,14, 17-20 and the loss of labeled acetate and biologic activity when the material was treated with phospholipase A2.3, 4 Camussi et al.23 independently reported that human endothelial cells synthesize PAF.

The production of PAF by vascular endothelial cells may mediate some of the responses of the endothelium to inflammation and injury and could potentially explain the increased adherence of leukocytes and platelets to the vascular surface under such conditions. Therefore we have further characterized the production of the lipid by stimulated endothelial cells. We report here: (1) that the synthesis of PAF by cultured human endothelium requires stimulation by specific agonists, (2) that PAF production is minimally altered by preincubation of endothelial cells with acetate, (3) that a variety of potential agonists that have vasoactive or proinflammatory properties do not stimulate its accumulation, (4) that the production of PAF by stimulated endothelial cells is enhanced by inhibition of cyclooxygenase and inhibited by treatment of the monolayers with prostacyclin (PGL2), and (5) that the endothelial cell-derived lipid can activate human platelets. These findings are correlated with our previous observations on the synthesis of PAF by human endothelium.

Methods

Materials. The sodium salt of [3H]acetic acid (2.8 Ci/mmol) was purchased from New England Nuclear. Lipid standards and unlabeled PAF were from Avanti Polar Lipids, Inc. Highly purified human thrombin and coagulation factor Xa, PGI2, and cyanobacterial peptide24 were the generous gifts of George Broze (Washington University, St. Louis), John Pike (Upjohn Diagnostics, Inc., Kalamazoo, MI), and Harold W. Siegelman (Brookhaven National Laboratory, Upton, NY), respectively. The biologic activity of thrombin was demonstrated by its ability to cause platelet aggregation and to stimulate the release of 14C-serotonin from isolated, prelabeled platelets. All other reagents were purchased from Sigma Chemical Co. except as noted and were of the highest grade available.

Human umbilical vein endothelial cell culture. Endothelial cells were isolated from umbilical veins and cultured in modified medium 199 with 20% pooled human serum as described.23 25 The production of PAF by the endothelial monolayers was studied after they became completely confluent,25 which usually occurred 4 to 9 days after their isolation and inoculation onto 35 mm gelatinized Petri dishes. The identity of endothelial cells grown in this fashion was verified by morphologic appearance,26 the presence of human factor VIII antigen,26 and the production of PGI2 in response to thrombin,27 bradykinin,28 and histamine.29 Contamination by cells that did not have morphologic characteristics absolutely typical of confluent endothelial cells was determined by phase-contrast microscopy to be 0.2% or less in all monolayers used. Only primary cultures were studied.

Incorporation of [3H]acete into [3H]PAF. PAF synthesis in response to agonist stimulation was assayed with a minor modification of our previously described technique.22 The reaction was initiated by replacing the growth medium with 1 ml of Hanks’ buffered salt solution containing 10 mM HEPES (N-2-hydroxyethyl-piperazine-N’-2-ethylsulfonic acid) buffer (pH 7.4), 2.5 μCi of carrier-free [3H]acetate, and the appropriate agonist (thrombin, calcium ionophore A23187, etc.) or control solution. In some experiments the monolayers were preincubated for various times (0 to 25 min) at 37°C with [3H]acetate and the reaction was initiated by exchanging the preincubation medium for medium containing labeled acetate with or without the agonist. The monolayers were incubated for 5 or 10 min at 25° C or the indicated temperature; experiments done at 37°C used a variable-powered, constant-temperature heating platform.

In experiments to test the effect of indomethacin, the indomethacin was first dissolved in dimethylsulfoxide and diluted to the final concentration (25 μM) in Hanks’ balanced salt solution. The final concentration of dimethylsulfoxide was 0.1% or less in each experiment and was included in the control buffer. The endothelial monolayers were preincubated with indomethacin or control solution for 15 min before addition of the agonist. In experiments with prostacyclin, it was dissolved in 50 mM Tris buffer (pH 9.5) and diluted to a final concentration of 2 mM immediately before addition to the endothelial cell monolayer, and was incubated with the monolayers for 2 to 10 min before addition of the agonist. Pharmacologic concentrations of PGI2 were used because the amount of active prostaglandin that entered the cells was unknown and because we did not use drugs that may potentiate the effects of PGI2, such as isobutylmethylxanthine.

After incubation of the endothelial cell monolayers with labeled acetate under the specific conditions described, the reaction was stopped by adding 50 mM acetic acid in methanol. The cells were then scraped from the plate and it was washed twice with 1 ml methanol. The cell fraction and the methanol washes were pooled, and 7 nmol labeled carrier PAF and 1.25 ml of chloroform were added. The resulting monophase was split with
chloroform and 0.1M sodium acetate and the lower phase was washed three times with preequilibrated upper phase. The lower phase was dried under N₂, and the lipids were resuspended in a known volume of chloroform/methanol (9:1). A portion was used to determine the total radioactivity and the lipids in the remainder were separated by thin layer chromatography (TLC) as described.²² Areas containing PAF were scraped from the plate and the radioactivity was determined by scintillation spectrophotometry. Duplicate assays showed that the mean variation of PAF production by monolayers from the same isolate studied on the same day was ±5%.

**High-performance liquid chromatography (HPLC) of fractions containing PAF.** In some experiments the extracts from endothelial monolayers were chromatographed on TLC plates, and the areas migrating with authentic PAF were scraped into a centrifuge tube. The lipids were eluted with chloroform/methanol (2:1, vol/vol) and then methanol. The lipids in this extract were then separated by HPLC as described.²²

**Recovery of platelet-activating activity from thrombin-stimulated endothelial cells.** Six dishes of endothelial cells were stimulated with thrombin (2 U/ml) for 5 min and extracted as previously outlined, with the exception that carrier PAF was not included. The lipids in the extract were separated by HPLC, 0.5 ml volumes of the effluent were collected, dried under nitrogen, and redissolved in 75 μl of 0.15M NaCl containing 1 mg/ml fatty acid–free bovine serum albumin. In addition, 5 nmol of authentic PAF was applied to the HPLC and fractions were collected and processed in the same way, except that the lipid in each fraction was redissolved in 0.5 ml of NaCl containing fatty acid–free bovine serum albumin.

The recovery of bioactivity was assayed by analyzing each fraction for the ability to stimulate the aggregation of cytochalasin B–treated human granulocytes as described.²² The HPLC fractions containing this activity were found to elute at the same time as those containing authentic PAF,²² which also retained proaggregatory activity for neutrophils (not shown).

Aliquots of the fractions that contained neutrophil proaggregatory activity were then pooled and evaluated for their ability to stimulate human platelet aggregation²² by means of a modification of a published method.³⁰ Venous blood from a normal man who had taken no drugs for 14 days was drawn with a "two syringe" technique and anticoagulated with 1 volume of 3.8% sodium citrate to 9 volumes of blood. Platelet-rich plasma was removed with a plastic transfer pipet after centrifugation at 250 g for 0 min at room temperature and kept at 37°C in an atmosphere of 5% CO₂ until use. Platelet-rich plasma was prepared by centrifuging the remaining blood at 750 g for 20 min; the platelet-poor plasma was then handled in the same way as the platelet-rich plasma. Platelet aggregometry was carried out in a Payton Model DB 2 channel aggregometer. Aliquots (0.45 ml) of the platelet-rich plasma were stirred at 900 rpm at 37°C for 2 min, 50 μl of sample (extract from endothelial cells or authentic PAF retrieved from HPLC, authentic PAF not subjected to HPLC, or control buffer) was added, and the resulting aggregation wave was recorded for 5 min. Light transmission was set at 0% with platelet-rich plasma and 100% with platelet-poor plasma, with 100% transmission causing a pen deflection of 21.5 cm on the recorder.

**Results**

**PAF production by human endothelial cells occurs in response to agonist stimulation.** Human thrombin reproducibly stimulated confluent endothelial monolayers to produce PAF. In experiments with primary endothelial cell cultures from 21 different isolates, thrombin caused a 15-fold increase in the incorporation of [³H] acetate into the polar lipid migrating with authentic PAF when compared with monolayers treated with buffer (figure 2). The amount of [³H] acetate that accumulated in the PAF fractions in these experiments was similar to the incorporation in studies with six other endothelial isolates that we previously reported.²² Endothelial monolayers treated for 5 min with 1 or 2 U/ml thrombin did not contract²¹ nor was there morphologic evidence of injury under the conditions of these experiments; they excluded trypan blue, and maintained unchanged monolayer morphology for up to 72 hr after treatment with thrombin (2 U/ml for 5 to 30 min). Calcium ionophore A23187 also stimulated endo-

![Figure 2](image-url)
other experiments were done at 25°. Preincubation of endothelial monolayers with [3H] acetate for up to 25 min before stimulation did not increase the incorporation of the label into PAF over that achieved when the acetate and the agonist were added simultaneously (figure 3B). This result indicates that exogenous acetate is rapidly assimilated into the cellular pool that is available for PAF synthesis, presumably as acetyl coenzyme A, and suggests that the intracellular distribution of acetate is uniform rather than compartmental.

Enhanced synthesis of PAF by stimulated endothelial cells requires specific agonists. We evaluated a variety of humoral mediators that are known to act directly on vascular endothelial cells, or are postulated to directly modify the biological or biochemical activity of these cells, for their ability to stimulate PAF production. Table 1 lists a group of these mediators that did not

![FIGURE 3A](image-url)

**FIGURE 3A.** Effect of temperature and prelabeling on the incorporation of [3H] acetate into PAF by stimulated endothelial cells. Duplicate monolayers of endothelial cells from the same culture were stimulated for various times at 25° or 37° C as described in Methods and figure 2. There was a small increase in [3H] acetate incorporation into PAF in monolayers incubated at 37° C compared with those incubated at 25° C. The incorporation of [3H] acetate into PAF was enhanced when the incubations were done at 37° C rather than 25° C (figure 3A), but the incremental increase at each time point was relatively small. For this reason all

![FIGURE 3B](image-url)

**FIGURE 3B.** Monolayers of endothelial cells from the same culture were preincubated with [3H] acetate for the times shown and then stimulated for 5 min as described. The incorporation of [3H] acetate into PAF was not significantly altered by preincubation of the endothelial cells with the labeled compound when compared with addition of the acetate and the agonist simultaneously (0 preincubation time).
TABLE 1
Potential mediators of vascular effects that did not induce the synthesis of PAF by cultured endothelial cells

<table>
<thead>
<tr>
<th>Potential Mediator</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Endotoxin (10 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Angiotensin II (1 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Zymosan-activated plasma (10%)</td>
<td></td>
</tr>
<tr>
<td>n-Formylmethionyl-leucyl-phenylalanine (1 μM)</td>
<td></td>
</tr>
<tr>
<td>Concanavalin A (1 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (10 μM)</td>
<td></td>
</tr>
<tr>
<td>Substance P (1 μM)</td>
<td></td>
</tr>
<tr>
<td>Homocysteine (100 μM)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (10 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Fibrin and fibrin peptidesD</td>
<td></td>
</tr>
<tr>
<td>Urokinase (250 U/ml)</td>
<td></td>
</tr>
<tr>
<td>Serotonin (1 μM)</td>
<td></td>
</tr>
<tr>
<td>Epinephrine (1 μM)</td>
<td></td>
</tr>
<tr>
<td>Collagen (100 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Coagulation factor X (2 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Vasooactive intestinal peptide (3 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Insulin (25 mM)</td>
<td></td>
</tr>
<tr>
<td>Gastrin (3 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Trypsin (0.0025%)</td>
<td></td>
</tr>
<tr>
<td>Phorbol myristate acetate (1 μM)</td>
<td></td>
</tr>
<tr>
<td>Cyanobacterial peptide (0.1 μg/ml)</td>
<td></td>
</tr>
<tr>
<td>Streptokinase (250 U/ml)</td>
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</tbody>
</table>

*These compounds were tested in the standard assay, which measures the incorporation of [3H] acetate into PAF. (Methods and figure 2) at the concentrations shown in parentheses. In each experiment, positive controls of thrombin (2 U/ml) or the calcium ionophore A23187 (5 μM) were included and gave values of 10,000 to 20,000 cpm of [3H] acetate incorporation into PAF. The compounds listed did not result in incorporation over control (buffer-only) incubations.  
†Lipopolysaccharide from Escherichia coli serotype 055:B5.  
‡Used as a source of C5a desarg and prepared as described.  
§Human fibrinogen (10 μg) was treated with 0.20 U thrombin in 0.1 ml of buffer for 120 min at 37°C and chilled, and an aliquot of the fluid phase (1:1000 dilution) was added with labeled acetate to the endothelial cell monolayer.  
¶A 10-fold higher concentration gave a small increase over background (<10% of the thrombin response).

enhance PAF production. These findings indicate that the synthesis of PAF by human endothelial cells in response to thrombin (figure 2) is not a response that occurs when the cells are perturbed in a non-specific fashion. Furthermore, the results are important because several of the humoral mediators that we tested (table 1) also enhance the adherence of inflammatory cells to endothelium, an interaction that could be explained by production of PAF by the endothelial cells. We have previously shown that PAF itself does not stimulate endothelial cells to make additional PAF.

PAF synthesized by endothelial cells activates human platelets. Endothelial monolayers were stimulated with thrombin (2 U/ml) and extracted, and the pooled extract was separated by HPLC. Fractions of the effluent that migrated with authentic PAF were tested for their ability to aggregate human PMNs, as described, and were found to potently induce aggregation of the isolated leukocytes (Methods). Aliquots of the endothelial cell fractions that contained PMN proaggregatory activity were tested for their ability to stimulate platelet aggregation, along with fractions that contained authentic PAF that had been passed over the HPLC column in an identical manner. The results of this experiment are shown in figure 4A and document that the lipid product from thrombin-stimulated endothelial cells causes the aggregation of human platelets. The concentration curve for authentic PAF indicated a threshold between 10^-8 M and 10^-10 M (figure 4B), suggesting that the amounts of PAF synthesized by human endothelial cells could potentially result in the activation of platelets in plasma or blood.

Effect of treatment of endothelial cells with indomethacin and prostacyclin on the synthesis of PAF. When endothelial monolayers were incubated with the cyclooxygenase inhibitor indomethacin before stimulation, the synthesis of PAF was enhanced (table 2). The magnitude of the increased response was highly variable, although the qualitative response itself was consistent. The increase that followed indomethacin occurred after stimulation with different agonists and at sub maximal as well as maximal responses. Indomethacin pretreatment reduced the release of PGI2 from stimulated endothelial cells by 95% or greater (not shown). These experiments suggested that PGI2, the major cyclooxygenase product of stimulated human macrovascular endothelium, inhibits the production of PAF. Consistent with this hypothesis, we found that the addition of exogenous PGI2 caused a small, but reproducible, attenuation of PAF accumulation in stimulated endothelial cells (table 2).

Discussion

In previous studies, we found that primary cultures of human umbilical vein endothelial cells synthesize PAF when stimulated with thrombin. Thrombin treatment of cultured umbilical vein endothelial cells caused a dose-related increase in incorporation of triitated acetate into a polar lipid that migrated with authentic PAF when the lipids products were separated by TLC; the concentrations of thrombin that stimulated
production of the lipid were similar to those that stimulate other endothelial cell responses, such as PGI₂ release. The accumulation of the product was maximal 5 min after treatment of the endothelial monolayers with thrombin, did not occur when the thrombin was inactivated, and required divalent cations. When the [³H] acetate-containing lipid was eluted from the thin-layer chromatogram and treated with phospholipase A₂, greater than 90% of the radiolabel was lost from the molecule, confirming that the [³H] acetate was located at the sn-2 position of the glycerol chain.

The radiolabeled polar lipid produced by thrombin-stimulated endothelial cells also eluted with authentic [³H] PAF when extracts were analyzed by HPLC. Fractions of the lipid extract from thrombin-stimulated cells that eluted at the same time as authentic PAF caused the aggregation of isolated human PMNs. The ability of the lipid to activate the PMNs, as measured

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>% Response compared with buffer pretreatment</th>
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<tbody>
<tr>
<td>Indomethacin (n = 14)</td>
<td>185 ± 102 (p &lt; .01)³</td>
</tr>
<tr>
<td>PGI₂ (n = 9)</td>
<td>78 ± 15 (p &lt; .01)</td>
</tr>
</tbody>
</table>

³The results were analyzed for statistical significance with a two-tailed, unpaired Student’s t test.

FIGURE 4B. Authentic PAF (that had not been subjected to HPLC) caused a concentration-dependent increase in platelet aggregation. The aggregation wave stimulated by 10⁻⁸M PAF reversed and returned to baseline spontaneously; aggregation curves stimulated by higher concentrations of PAF did not reverse during the 5 min recording period.
by aggregation, was lost when the fractions were incubated with phospholipase A2 but not when they were incubated with buffer. Thus the polar lipid produced by human endothelial cells in response to thrombin was characterized as PAF by its migration in two chromatographic systems, the presence of biological activity characteristic of PAF, and loss of the biologic activity and radiolabeled acetate when treated with phospholipase A2. Under the conditions of these experiments the PAF remained associated with the endothelial cells and was not released into the overlying buffer. Additionally, we found that stimulated human endothelial cells can convert 1-[3H]alkyl-sn-glycero-3-phosphocholine to [3H]alkyl-2-acyl-GPC and to [3H] PAF, indicating that the cells have the appropriate enzymes to acylate the labeled alkyl lipid to a potential precursor molecule (1-alkyl-2-acyl-GPC) or to PAF itself.

The experiments reported here confirm that thrombin reproducibly stimulates primary cultures of human endothelium to incorporate labeled acetate into the lipid product and that calcium ionophore A23187 can also act as an agonist (figure 2). Thrombin and ionophore A23187 stimulate other biochemical activities of human endothelial cells, including the release of arachidonate and the synthesis of PGI2. Although thrombin was a potent agonist for PAF production by the endothelial cells (figure 2), a number of other humoral agents did not stimulate PAF accumulation (table 1). Each of these agents was studied because it has been reported to enhance the adherence of platelets or PMNs to endothelium or to mediate endothelial injury, is locally generated during thrombosis, or directly stimulates a biologic response of endothelial cells, and thus was a potential agonist for PAF production. The fact that these agents did not stimulate PAF accumulation under the conditions of these experiments indicates that the production of PAF by endothelial monolayers is a regulated event that requires the action of specific agonists. This conclusion is also supported by additional observations from our laboratories that demonstrate that histamine, bradykinin, and ATP are agonists for PAF synthesis by human endothelial cells and that they appear to stimulate PAF production by interacting with specific receptors.

Camussi et al. have reported that umbilical vein endothelial cells synthesize and release PAF in response to ionophore A23187, rabbit antihuman factor VIII, angiotensin II, and vasopressin. Their studies differ from our findings in that they did not observe PAF synthesis in response to thrombin (in the absence of indomethacin; see below) unless the endothelial cells were repetitively stimulated with the agonist, and they reported that PAF is released from stimulated endothelial monolayers whereas we have found it to remain cell associated. The first discrepancy may be explained, in part, by differences in the time of incubation of endothelial cells with thrombin, since we have found that the maximal accumulation of PAF in response to thrombin occurs at 5 to 10 min after stimulation and Camussi et al. assayed supernates from endothelial monolayers after 45 min of stimulation with thrombin. The explanation for different findings with regard to release of PAF from the endothelial monolayers is unclear at this time. Camussi et al. have also reported that PAF is released from rabbit aortic endothelial cells injured by treatment with antibody directed against angiotensin-converting enzyme, a plasma membrane component of endothelium. In our experiments, endothelial cells maintained normal morphology and monolayer architecture after stimulation with thrombin, suggesting that there was no significant injury.

Figures 4A and 4B illustrate that the lipid isolated from thrombin-stimulated human endothelial cells is a potent activator of human platelets. The phospholipid product in the fractions containing this biologic activity migrated with authentic PAF in the HPLC separation and also stimulated the aggregation of human granulocytes (Results and ref. 22). The ability to activate both PMNs and platelets directly is an unusual biologic property of PAF. This lipid product from thrombin-stimulated endothelial cells is PAF. Furthermore, the ability of thrombin-stimulated endothelial cells to produce a mediator that activates platelets may contribute to the accumulation of thrombocytes on the endothelial surface in response to procoagulants or when the endothelium is injured. Thus the production of PAF by endothelium may be one mechanism by which thromboresistance is lost under these conditions.

Indomethacin, an inhibitor of cyclooxygenase and of the production of PGI2 and other metabolites of arachidonic acid via the cyclooxygenase pathway, enhanced the accumulation of PAF by endothelial monolayers (table 2). This finding suggests an interaction between arachidonic acid metabolism and PAF production in endothelial cells. The experiments in which exogenous PGI2 suppressed PAF production (table 2) support this possibility. Camussi et al. also reported that exogenous PGI2 inhibited PAF release by stimulated endothelial cells and that indomethacin had
a variable effect on PAF release. Although pharmacologic concentrations of prostacyclin were used (Methods), the findings in table 2 additionally suggest that endogenously generated PG12 may influence the synthesis or degradation of PAF. Modulation of synthesis seems more likely, since an equivalent effect of indomethacin was seen at a variety of concentrations of agonist (i.e., the concentration-response curve was shifted upward). PG12 inhibits arachidonate release and increases intracellular cyclic AMP in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine in cultured endothelium,42,43 indicating that endogenous PG12 can influence biochemical events in vascular cells.43 The data in table 2 suggest that the inhibition of endothelial cell prostacyclin synthesis with indomethacin, or other cyclooxygenase blockers, will enhance PAF accumulation in response to stimulation with appropriate agonists, such as thrombin. Because PAF stimulates enhanced platelet adheriveness (ref. 21 and figure 4), increased PAF synthesis may favor platelet adherence to the endothelial surface. Czervionke et al.40 have observed augmented platelet adherence to aspirin-treated endothelial monolayers that were stimulated with thrombin, an effect that they attributed to decreased PG12 release and a resultant abrogation of the direct inhibitory effect of PG12 on the platelets. Our studies suggest that increased PAF production is an alternative mechanism for the enhanced platelet adherence under these conditions.

In additional studies we have found that several agonists that stimulate PG12 release by endothelial cells (thrombin, bradykinin, histamine, ATP) also stimulate PAF synthesis, although the durations of production of the two autocoids are quite different.22a These observations suggest that a common phospholipid precursor may provide arachidonate and 1-alkyl-sn-glycero-3-phosphocholine for metabolism to PG12 and PAF, respectively; 1-alkyl-2-arachidonoyl-sn-glycerol-3-phosphocholine has been reported to provide both arachidonate and 1-alkyl-GPC for subsequent metabolism in stimulated neutrophils.44

The observations that we have described indicate that the production of PAF can be added to the list of biosynthetic potentials of the human vascular endothelial cell.46 The ability of PAF to activate platelets and neutrophils suggests that this biosynthetic capability may be important in the interaction of endothelium with circulating blood cells and perhaps with other adjacent cells as well. The ability of stimulated endothelial cells to produce PAF, a platelet activator, and PG12, a potent inhibitor of platelet activity,21,45 indicates the potential for the endothelium to regulate the interaction between the two cells directly (figure 5). This may be important in vascular homeostasis, since platelets are required to maintain endothelial integrity.22 The production of the two autocoids may similarly affect the interaction of polymorphonuclear leukocytes and endothelial cells.22,25 The potential to produce PAF also suggests, however, that this response of stimulated endothelial cells may contribute to pathologic thrombosis and vascular injury. Products released from activated platelets36,47 and granulocytes34,36,48-50 can directly injure endothelium, and both of these effector cells adhere to sites of vascular damage under the appropriate conditions.34,39,41,51,52 The unregulated production of PAF by endothelial cells, or an uncontrolled response of the PMNs and platelets to this inflammatory mediator, may thus influence the local activation of the blood cells and pro-

**FIGURE 5.** A proposed schema for the role of lipid metabolites of endothelial cells in regulating interactions with circulating blood cells. In response to appropriate agonist stimulation (thrombin, others), arachidonic acid (AA) and 1-alkyl-GPC are released from membrane phospholipids (PL) and are metabolized to PG12 and PAF, respectively. 1-alkyl-GPC is converted to PAF after addition of acetate at the sn-2 position, a reaction catalyzed by an acetyltransferase, with acetyl coenzyme A (AcoA) as the acetyl donor. PAF may then promote the adherence and activation of circulating effector cells, such as platelets or PMNs. PG12 is synthesized from AA via subsequent steps catalyzed by cyclooxygenase and prostacyclin synthetase; it is a potent inhibitor of platelet activation stimulated by PAF21 and other agonists and may modulate some PMN responses as well. In addition, PG12 may modulate the synthesis of PAF from 1-alkyl-GPC by inhibiting its release from phospholipid precursors (as with arachidonate; ref. 42) or by other mechanisms.
mote the initiation or amplification of vascular injury. Although there is currently no direct evidence for the participation of PAF in human vascular disease, the observations that it causes pulmonary hypertension and edema, coronary vasoconstriction, and increased dermal capillary permeability in animal preparations suggest this possibility.

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