PAF-induced synthesis of tetraenoic and pentaenoic leukotrienes in the isolated rabbit lung

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Grimminger, Friedrich, Konstantin Mayer, Ladislau Kiss, Dieter Walmrath, and Werner Seeger. PAF-induced synthesis of tetraenoic and pentaenoic leukotrienes in the isolated rabbit lung. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L268–L275, 2000.—In an isolated rabbit lung model, we tested the hypothesis that platelet-activating factor (PAF)-induced leukotriene (LT) synthesis is critically dependent on the free precursor fatty acid supply and the possible substitution of arachidonic acid (AA) by eicosapentaenoic acid (EPA). To augment the intravascular polymorphonuclear neutrophils (PMNs) in the isolated lung, human PMNs were infused into the pulmonary artery. LTs and hydroxyeicosatetraenoic acid (total sum of 5-lipoxygenase products = 7 and =27 pmol/ml in lungs both with and without infused PMNs, respectively). Combined administration amplified 5-lipoxygenase product formation, with a predominance of cysteinyl-LT synthesis in lungs both without (total sum = 67 pmol/ml) and, much more strikingly, with (total sum = 308 pmol/ml) an infusion of neutrophils. EPA (10 µM) elicited exclusive generation of 5-series LTs and 5-hydroxyeicosatetraenoic acid (total sum = 82 pmol/ml). Dual stimulation with PAF and EPA provoked amplification of EPA-derived 5-lipoxygenase product formation, again with predominance of cysteinyl-LTs in lungs without (total sum = 224 pmol/ml) and, in particular, with (total sum = 545 pmol/ml) preceding microvascular PMN entrapment. Combined application of PAF, AA, and EPA resulted in the synthesis of LTs derived from both fatty acids, with a predominance of 5-series products. We conclude that the PAF-evoked 5-lipoxygenase product formation in the neutrophil-harboring lung capillary bed is critically dependent on intravascular precursor fatty acid supply, with EPA representing the preferred substrate compared with AA. PMN-related transcellular eicosanoid synthesis is suggested to underlie the predominant generation of cysteinyl-LTs. The supply of n-3 versus n-6 precursor fatty acid may thus have a major impact on inflammatory mediator generation.

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Since its recognition as a substance released from rabbit basophils after IgE stimulation capable of inducing platelet aggregation, platelet-activating factor (PAF) has gained growing interest as a potent mediator of inflammation (4, 43). It causes activation of a variety of cells including polymorphonuclear neutrophils (PMNs), eosinophils, mononuclear phagocytes, lymphocytes, and endothelial cells; it increases adherence of PMNs to the vascular endothelium (43); and it was found to be a “priming” agent of inflammatory cells. With respect to diseases of the lungs, PAF has been implicated in the pathogenesis of allergic airway diseases, acute and chronic pulmonary hypertension, and noncardiogenic pulmonary edema (2, 30, 38). In humans, PAF inhalation provoked immediate pulmonary microvascular PMN sequestration (37). The major portion of the PAF influence on lung tissue appears to be mediated via secondary induction of leukotriene (LT) generation (3, 6).

In vitro, natural inflammatory ligands such as formylmethionyl-leucyl-phenylalanine (fMLP), C5a, LTB4, and PAF are poor activators of PMN eicosanoid metabolism. This feature changes fundamentally on simultaneous application of free precursor fatty acid, and PAF was characterized as a particularly potent stimulus of neutrophil LT synthesis in the presence of exogenous arachidonic acid (AA) (11). This finding may be relevant in view of substantial levels of free AA known to arise at sites of inflammatory events (18, 39, 40) and in view of recently described neutrophil-endothelial cooperation in LT synthesis (5). The latter includes not only a shift of the unstable LT intermediate LTA4 from the PMN (feeder cell) to the endothelium, with subsequent use of the enzymatic equipment of this acceptor cell (5, 10), but also a release of free AA from activated endothelial cells, with uptake and processing of this precursor by adjacent PMNs (5, 8).

Oral supplementation with n-3 fatty acids, in particular eicosapentaenoic acid (EPA), has been suggested as a therapeutic regimen for suppressing PMN-related inflammatory events (24). Recently, n-3 fatty acid-enriched lipid infusions have been developed for use under clinical conditions, intending to shift the AA-to-EPA ratio toward predominance of the latter lipid mediator precursor (14, 18, 41). Infusion of lipid emulsions is known to activate endothelial lipoprotein lipase, including a translocation of this enzyme from its cellular binding sites into the vascular compartment, with a resultant increase in plasma free fatty acids originating from the infused lipid preparation (26, 31). Concentrations of both nonesterified AA and EPA of >10 µM have been measured in the plasma free fatty
acid fraction under conditions of parenteral nutrition with n-6 and n-3 fatty acid-based lipid emulsions (15).

Against this background, it is of interest that in PAF-challenged neutrophils in vitro, exogenous EPA may fully substitute for AA to shift the LT profile to 5-series product formation (11). Pentaenoic LTs possess markedly reduced proinflammatory potencies compared with the tetraenoic compounds, and even competition with AA-derived metabolites for receptor occupancy has been suggested (23, 24). In the present study, we employed ex vivo perfused rabbit lungs in which we provoked microvascular leukostasis by infusion of freshly prepared ligand-responsive PMNs. We questioned whether PAF-induced LT synthesis might also be critically dependent on free precursor fatty acid supply under conditions of an intact organ with a complete vascular compartment and native extracellular matrix. We noted a dramatic amplification of exclusionary injection of PAF plus AA and PAF plus EPA, respectively; moreover, the metabolite profiles strongly suggested PMN-related transcellular LT synthesis. EPA was revealed to be the preferred substrate under these conditions. These findings support therapeutic attempts to suppress ligand- and PMN-related inflammatory events via an intravascular supply of n-3 fatty acids as alternate substrates for 5-lipoxygenase product formation.

METHODS

Materials. The leukotrienes LTC₄, LTD₄, LTE₄, and LTB₄ as well as the synthetic LTA₄ methyl ester were a generous gift from Dr. J. Rokach (Merck Frosst, Kirkland, Quebec, Canada). Additional LTs were graciously supplied by Dr. H. Bartmann (Hoechst, Frankfurt, Germany), 5-, 8-, 9-, 11-, 12-, and 15-hydroxyeicosatetraenoic acid (HETE), 55,125-diHETE, 5,15-diHETE, 12-hydroxyheptadecatrienoic acid, LTA₅ methyl ester, LTD₅, 5-hydroxyeicosapentaenoic acid (HEPE), and PAF (1-O-octadecyl-2-acetyl-sn-glycero-3-phosphocholine) were obtained from Paesel (Frankfurt, Germany). The nonenzymatic hydrolysis products of LTAs were prepared by acid hydrolysis of the synthetic LTAs. All LTs were checked for purity and quantified spectrophotometrically before use as previously described (10). Anti-LTB₄ antisera was obtained from Dr. J. Salmon (Wellcome Research Laboratories, Beckenham, UK). The cross-reactivity of the LTB₄ antibody with LTB₅ was 71%. The LTC₄ RIA kit was purchased from Amersham (Braunschweig, Germany). Reactivity of the antibody with LTC₄, LTD₄, and LTE₄ was 100, 82, and 23%, respectively; cross-reactivity with LTC₂ and LTD₂ was 66 and 5.3%, respectively; AA and EPA were purchased from Sigma (Munich, Germany). These fatty acids were initially divided into aliquots and stored at -20°C under nitrogen, and a fresh portion was used for each experiment. Chromatographic supplies included glass-distilled HPLC-grade solvents (Fluka, Heidelberg, Germany) and octadecylsilyl 5-µm (Hypersil) and silica gel 5-µm column packing (Machery-Nagel, Duren, Germany) as well as C-18 Sep-Pak cartridges (Waters Associates, Milford, MA). DL-Lysine-monoace-tylsalicylate-glycine (9:1) was obtained from Bayer (Leverkusen, Germany). RPMI 1640 medium, Hanks’ HEPES buffer, and fetal calf serum were obtained from Boehringer Mannheim (Mannheim, Germany), and Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). A photometric test for the quantification of lipopolysaccharide was received from Kabi Vitrum (Coatest endotoxin, Munich, Germany). All other biochemicals were obtained from Merck (Munich, Germany).

Analytic procedures. LTs of the 4- and 5-series, HETEs, and HEPEs, were extracted from 20-ml samples of the recirculating buffer medium by octadecysilyl solid-phase extraction columns as described by Grimminger et al. (10). Conversion into the methyl ester was performed by the addition of freshly prepared diazomethane in ice-cold diethyl ether. Reverse-phase HPLC of nonmethylated compounds was carried out on octadecysilyl columns (Hypersil; 5-µm particles) with a mobile phase of methanol-water-acetic acid (72:28:0.16, pH 4.9). In addition to the conventional ultraviolet (UV) detection at 270 (LTs) and 237 (HETEs and HEPEs) nm, a photodiode array detector (Waters model 990) that provided full UV spectra (190–600 nm) of eluting compounds was used and allowed checking for peak purity and subtraction of possible coeluting material. For additional verification, samples were collected in fractions in selected experiments and subjected to post-HPLC RIA with anti-LTB₄ and anti-LTC₄ antibodies as described by Schulz and Seeger (34). Straight-phase HPLC of methylated compounds was carried out with a mobile phase consisting of hexane-isopropanol-acetate (86:14:0.1), and the column was eluted isocratically at a flow rate of 1.0 ml/min. All data obtained by the different analytic procedures were corrected for the respective recoveries of the overall analytic procedure and are given in picomoles per milliliter of perfusate. Recovery was determined by separate experiments with different quantities of the individual compounds in the appropriate concentration range. For quantification of LTs, 5-HETE, and 5-HEPE, correspondence of the values calculated from UV absorbance in the two different chromatographic procedures and in selected experiments post-HPLC RIA was required (deviation < 15%). The detection limits of the chromatographic procedures for the different lipoxigenase products in the recirculating perfusate were 0.8 pmol/ml for LTC₄/5, 0.5 pmol/ml for LTD₄/5, 0.6 pmol/ml for LTE₄/5, 0.2 pmol/ml for LTB₄/5, and 0.8 pmol/ml for 5-hydroxyeicosatetra-(penta)enoic acid (HET(P)E).

Preparation of PMNs. Heparinized human donor blood was centrifuged in a discontinuous Percoll gradient (22) to yield a PMN fraction of ~97% purity. The granulocytes were kept in RPMI 1640 medium with 20% calf serum for 60–90 min. Immediately before experimental use, the cells were washed twice and suspended in Hanks’ HEPES buffer (with Ca, without Mg). Cell viability as assessed by trypan blue exclusion ranged >96% throughout, and lactate dehydrogenase release was consistently <3%.

Ex vivo lung perfusion. The model has been previously described (35). Briefly, rabbits of either sex (body weight 2.2–2.6 kg) were deeply anesthetized and anticoagulated with heparin. The lungs were excised during perfusion with Krebs-Henseleit buffer through cannulas in the pulmonary artery and the left atrium. Formaldehyde-sterilized and endotoxin-free perfusion circuit tubing was used throughout. The buffer contained 132.8 mM NaCl, 5.2 mM KCl, 1.1 mM KH₂PO₄, 24.1 mM NaHCO₃, 2.4 mM CaCl₂, 1.3 mM MgPO₄ and 240 mg of glucose per 100 ml. The lungs were freely suspended from a force transducer and placed in a temperature-equilibrated chamber at 37°C. They were ventilated with 5% CO₂, 17% O₂, and 79% N₂ (tidal volume 30 ml; frequency 30 breaths/min; end-expiratory pressure 1 mmHg); pH of the perfusion fluid ranged between 7.35 and 7.45. After extensive rinsing of the vascular bed, the lungs were perfused with a recirculating pulsatile flow of 100 ml/min. The left atrial pressure was set at 2 mmHg under baseline conditions (zero referenced at the hilum) to guarantee zone III conditions at end expiration throughout the lung. The alternate
use of two separate perfusion circuits, each containing 150 ml, allowed exchange of perfusion fluid. Perfusion pressure, ventilation pressure, and weight of the isolated organ were registered continuously. The lungs selected for the study fulfilled the following criteria: 1) homogeneous white appearance without signs of hemostasis or edema formation, 2) pulmonary arterial and ventilation pressures in the normal range, and 3) isogravimetric during a steady-state period of 40 min. The endotoxin content of the recirculating perfusate was repeatedly measured and ranged <10 pg/ml (detection limit).

Experimental protocol. After termination of the steady-state period, the recirculating perfusate was exchanged with fresh buffer medium containing 250 μM acetylsalicylic acid. PMNs (2 × 10⁶ in a total volume of 2 ml of Hanks’ HEPES buffer or vehicle only) were infused into the pulmonary artery. Random counts of the recirculating medium 3 and 15 min after PMN application documented a nearly quantitative sequestration of the PMNs in the lung vasculature (<3% circulating cells). Five minutes after PMN or vehicle injection, AA (dissolved in 50 μl of ethanol), EPA (dissolved in 50 μl of ethanol), PAF (dissolved in 50 μl of ethanol), or a combination of fatty acid and PAF or vehicle only was injected into the pulmonary artery to achieve the intended final buffer concentrations (10 μM AA or EPA and 5 μM PAF); and time was set at zero. For analysis of LT and HET(P)E generation, 20-ml perfusate samples were taken directly before and 5, 10, 20, and 30 min after stimulus application. In total, there were 10 study groups, each with 4 independent lung experiments: control (vehicle application only), AA, EPA, PAF, PMNs, PAF plus AA, PAF plus EPA, PAF plus AA plus EPA, PAF plus PMNs, PAF plus PMNs plus AA, and PAF plus PMNs plus EPA.

Statistical analysis. The data are given as means ± SE. Analysis of variance was used to test for differences between the different groups; a P value of <0.05 was considered to indicate significance.

RESULTS

In control lungs and lungs with preapplication of PMNs only, chromatographic analysis did not detect any release of 4- and 5-series LTs or HET(P)Es into the recirculating buffer medium. Ten micromolar AA provoked the generation of moderate amounts of LTB₄ (maximum perfusate concentration 5.7 ± 2.1 pmol/ml), LTC₄ (1.4 ± 0.4 pmol/ml), LTE₄ (3.3 ± 0.7 pmol/ml), and 5-HETE (16.3 ± 5.9 pmol/ml) in the absence of detectable quantities of LTD₄, 5-series LTs, and 5-HEPE (Figs. 1–3). PAF-alone stimulation (5 μM) elicited even lower quantities of LTB₄ (≈2–3 pmol/ml) and LTE₄ (≈3–4 pmol/ml) in the absence of detectable LTC₄, LTD₄, and 5-HETE formation in lungs both with and without human PMN application. Combined application of PAF and AA caused an amplification of 4-series LT and 5-HETE synthesis. Compared with the summed quantities of AA-alone and PAF-alone stimulation, LTB₄ levels increased 1.7-fold, LTC₄ 2.5-fold, LTE₄ 3-fold, and 5-HETE 1.6-fold. In addition, significant quantities of LTC₄ became detectable in response to this dual stimulation. An even more striking potentiation of 4-series LT and 5-HETE synthesis was noted when PAF plus AA was administered into the lungs after an infusion of PMNs. Under these conditions, the total sum of AA-derived 5-lipoxygenase products increased nearly 10-fold compared with the summed quantities of AA-alone and PAF-alone stimulation, with a marked predominance of 4-series cysteinyl-LT generation. In none of these experiments with AA application were any 5-series LTs or 5-HEPEdetectable.

In contrast to AA, the administration of 10 μM EPA elicited exclusive liberation of 5-series LTs and 5-HEPE. The total quantities of these metabolites surpassed those of the 4-series products in response to AA-alone application (LTB₄ 6.3 ± 4.0 pmol/ml; LTC₄ 1.7 ± 1.3 pmol/ml; LTE₅ 6.7 ± 5.3 pmol/ml; 5-HEPE 67.1 ± 27.7 pmol/ml). In the absence of EPA, PAF stimulation
provoked no 5-series LT or 5-HEPE formation whether administered in lungs with or without PMN infusion. Combined administration of PAF and EPA elicited exclusive generation of 5-series LTs and 5-HEPE similarly amplified in relation to EPA-alone challenge as noted for PAF plus AA provocation. LTB5, LTC5, LTE5, and 5-HEPE were increased 3.4-fold, 3.5-fold, 9.4-fold, and 1.9-fold, respectively, and some LTD5 became detectable. Again, an even more impressive potentiation of LT synthesis was noted when PAF plus EPA was administered in lungs with PMN preapplication. The total sum of EPA-derived 5-lipoxygenase products elicited under these conditions surpassed that in response to EPA-alone challenge by >6.5-fold, and the summed 5-series products exceeded the summed AA metabolites in the corresponding experiments with dual challenge 1.8-fold. Within the array of EPA-derived products, 5-series cysteinyl-LTs again represented the predominant compounds.

Combined administration of PAF, AA, and EPA resulted in a release of both 4-series and 5-series LTs and 5-HET(P)E into the recirculating perfusate. LTB5 and 5-HEPE surpassed their corresponding AA derivatives ~2.1-fold and ~6.0-fold. LTC5 and LTE5 were ~3.0 times higher compared with LTC4 and LTE4, respectively, whereas only LTD5 was detectable (Figs. 1–3). Levels of each 4- and 5-series product were lower under the conditions of combined application of both fatty acids and PAF compared with the respective metabolite on application of PAF plus AA or PAF plus EPA.

8-, 9-, 11-, 12-, and 15-HET(P)Es, 5S,12S-diHET(P)E, 5,15-diHET(P)E, and nonenzymatic hydrolysis products of LTA4/5 (diastereomeric pair of 6-trans-LTB4/5 and

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**Fig. 2.** Time course of 4-series (A) and 5-series (B) cysteinyl-LT release in response to PAF and precursor fatty acid application. LTC/D/E4, sum of LTC4, LTD4, and LTE4; LTC/D/E5, sum of LTC5, LTD5, and LTE5. Lungs with and without infusion of neutrophils were stimulated with AA, EPA, PAF, or a combination of stimuli. Note different scale for LTC/D/E4 and LTC/D/E5. Values are means ± SE of 4 experiments; error bars not shown are within symbol. AA and PAF + AA provoked exclusive synthesis of LTC/D/E4. EPA and PAF + EPA provoked exclusive generation of LTC/D/E5. Application of PAF + AA + EPA yielded generation of both LTC/D/E4 and LTC/D/E5. LTC/D/E4/5 levels provoked by PAF + PMN + AA or EPA differed significantly from those in response to PAF + AA or EPA and PAF + AA + EPA. LTC/D/E4/5 levels after PAF + AA or EPA challenge surpassed those in the PAF-alone, AA + EPA, and PAF + AA + EPA studies (P < 0.05).

**Fig. 3.** Time course of 5-hydroxyeicosatetraenoic acid (HETE; A) and 5-hydroxyeicosapentaenoic acid (HEPE; B) release in response to PAF and precursor fatty acid application. Lungs with and without infusion of PMNs were stimulated with AA, EPA, PAF, or a combination of stimuli. Note different scale for 5-HETE and 5-HEPE. Values are means ± SE of 4 experiments; error bars not shown are within symbol. AA and PAF + AA provoked exclusive synthesis of 4-series product. EPA and PAF + EPA provoked exclusive generation of 5-HEPE. Application of PAF + AA + EPA yielded generation of both 5-HETE and 5-HEPE.
Baseline pulmonary arterial pressures ranged between 8 and 10 mmHg in all experiments. Due to the presence of acetylsalicylic acid, AA and EPA provoked only very moderate pressor responses, ranging <5 mmHg in all experiments. PAF administration caused a pulmonary arterial pressure increase to 20.3 ± 6.1 mmHg within 5 min. This feature corresponds to the previous notion that the PAF-elicited pulmonary vasoconstriction is not substantially inhibited by cyclooxygenase inhibition in rabbit lungs (33). Combined application of PAF and AA evoked a pulmonary arterial pressure of 17.1 ± 1.4 mmHg compared with 14.7 ± 1.8 mmHg when PAF and EPA were infused. After acute sequestration of human neutrophils, the injection of PAF and AA resulted in a pulmonary arterial pressure of 20.3 ± 1.6 mmHg; with the infusion of PAF plus EPA, the pulmonary arterial pressure rose to 18.7 ± 0.5 mmHg. In both cases, the influence of the fatty acids on the difference of pulmonary arterial pressure did not reach the level of significance, but the assessment of pulmonary arterial pressure was limited due to the presence of acetylsalicylate. When the reduction in pulmonary arterial pressure in the PAF plus EPA group was compared with the pressure response in the PAF group, a trend was observed that did not reach significance. PAF- and fatty acid-provoked edema formation never surpassed 4 g within the 30-min observation period in all experiments. The lung weight gain did not differ significantly between AA and EPA application. Because the study was planned for biochemical analyses, no interfering oncotic agent (e.g., albumin or hydroxyethyl starch) was added to the perfusate, thus rendering measurement of the capillary filtration coefficient unavailable. Ventilation pressures remained unchanged throughout the studies.

**DISCUSSION**

The principal finding of the present study is a dramatic amplification of LT synthesis in the lung vasculature after sequestration of ligand-responsive PMNs on simultaneous application of precursor fatty acid and the inflammatory ligand PAF. Cumulative evidence suggests cooperative (transcellular) eicosanoid synthesis as the underlying metabolic pathway, resulting in the predominance of cysteinyl-LT formation. Physiologically, the critical dependency on the presence of both the inflammatory ligand and free AA may help to restrict the burst of lipid mediator formation to sites of inflammatory events. Notably, substitution of EPA for AA resulted in a complete shift of product formation to 5-series LTs and 5-HETE. Moreover, EPA turned out to be the preferred substrate compared with AA.

In vitro studies in human PMNs demonstrated that inflammatory ligands such as PAF and fMLP activate 5-lipoxygenase without major stimulation of neutrophil phospholipases (11). Exogenous AA may then compensate for the restricted availability of endogenous precursor to be readily metabolized to the hydroperoxy intermediate 5-hydroperoxyeicosatetraenoic acid (HPETE) and, subsequently, the unstable 5,6-epoxide LTA₄, the latter being converted to LTB₄ as the major enzymatic secretion product. As alternate pathways, 5-HPETE is metabolized to 5-HETE, and excess LTA₄ is directly secreted into the extracellular compartment where it undergoes rapid nonenzymatic hydrolysis to the diastereomeric pair of 6-trans-LTB₄ and 5,6-diHETEs, with a half-time of only a few seconds (27). 4-Series cysteinyl-LT synthesis is not detectable on neutrophil stimulation with inflammatory ligands and AA. Similar to the preceding in vitro studies, LT or 5-HETE synthesis in the rabbit lung vasculature after sequestration of freshly isolated PMNs was dramatically amplified on simultaneous application of PAF and free AA. The total sum of 5-lipoxygenase products elicited on dual-ligand or AA challenge even surpassed that in response to optimal calcium ionophore A-23187 stimulation, the most potent stimulus of lung vascular LT generation known to date (42). There are, however, notable differences between PAF- and/or AA-evoked PMN stimulation in vitro and in the current model of pulmonary leukostasis. First, nonenzymatic degradation products of LTA₄ were not detectable in the ex vivo perfused lungs. Thus LTA₄, known to be secreted from PAF- and/or AA-challenged neutrophils, evidently completely escapes extracellular decay due to avid uptake and enzymatic conversion by adjacent lung vascular cells. This interpretation is supported by a preceding study (10) with a bolus injection of synthetic LTA₄ in perfused rabbit lungs, showing virtually complete metabolic conversion of this unstable LT intermediate in the lung capillary bed. Second, although amplification of LTB₄ and 5-HETE formation was known to be from PAF and/or AA stimulation of PMNs in vitro, the even more striking finding was the predominance of a burst of cysteinyl-LT synthesis. This change in LT profile suggests a shift of LTA₄ from ligand- and/or AA-activated PMNs to adjacent acceptor cells equipped with glutathione S-transferase for conversion of the unstable LT intermediate to cysteinyl-LTs. Likely candidates as acceptor cells are endothelial cells, shown to convert exogenous LTA₄ to LTC₄ in vitro (5). It may be speculated that such intercellular cooperation also contributes to the appearance of impressive quantities of LTB₄; in contrast to PMN-endothelial cell cocultures, the metabolic conversion to LTB₄ predominates in rabbit alveolar macrophages (16) and type II pneumocytes (17), receiving LTA₄ from adjacent “feeder” neutrophils. In addition, these cell types as well as mast cells might also be directly affected by the PAF stimulus, thereby provoked to contribute to the currently observed lipid mediator synthesis. Predominance of 4-series cysteinyl-LT appearance over LTB₄ generation was similarly noted in response to a bolus injection of synthetic LTA₄ in the perfused lung model (10). The rapid conversion of LTC₄ via LTD₄ to accumulating LTE₄ has been previously described for the rat and rabbit lung vasculature (20, 34). Due to the recirculating system, not only precursors but also intermediate metabolites of LT synthesis may have a relevant impact on emerging product
formation. However, in vitro data suggest that the majority of product formation stems from direct cell-to-cell contact and microenvironmental exchange.

Notably, the amplification of LT synthesis with predominance of cysteinyl-LT formation was also obvious on combined PAF and AA challenge in lungs without an infusion of human PMNs, although the total sum of 5-lipoxygenase metabolites elicited under these conditions ranged at only approximately one-fifth of that after preapplication of freshly prepared human neutrophils. These data should be interpreted in view of the recent finding that ex vivo perfused rabbit lungs harbor a large capillary pool of neutrophils even after extensive extracorporeal rinsing with buffer fluid; morphometric analysis estimated $1.4 \times 10^6$ PMNs entrapped in this compartment (7). For comparison, $2 \times 10^8$ human PMNs were presently injected. Although rabbit neutrophils were presently injected. Although rabbit neutrophils synthesize less LTA₄ than human PMNs (20% in response to AA-23187 (12) and PAF and/or AA challenge; Grimminger, unpublished data), this difference may not fully account for the limited 5-lipoxygenase product formation in the lungs without preapplication of freshly prepared human PMNs. As an additional explanation, prolonged PMN entrapment in the lung microvasculature may cause some loss of responsiveness to receptor-operated challenge; E-type prostaglandins and nitric oxide, e.g., were repeatedly demonstrated to suppress neutrophil activation (9, 28), and a baseline level of prostaglandin E₂ as well as of nitric oxide is assumed to be continuously released from capillary endothelial cells (1, 32, 36). In contrast to PAF, *Escherichia coli* hemolysin (HlyA), a bacterial exotoxin known to bypass cellular (receptor-operated) signal transduction pathways, was able to stimulate the resident rabbit neutrophils in our model. Used in analogy to the present study, HlyA and AA provoked a comparable quantity of lipoxygenase product in the absence of freshly isolated human neutrophils (13). Thus the sensitivity of neutrophils to PAF might be downregulated by the aforementioned mechanisms, whereas the direct activation of neutrophils by HlyA was not affected.

Analogous to AA, exogenous EPA was rapidly and extensively converted to 5-series LTs and 5-HEPE when applied simultaneously with PAF, and the total sum of 5-lipoxygenase products liberated from neutrophil-infused lungs greatly surpassed that without PMN preapplication. Within the array of EPA-derived metabolites, predominance of cysteinyl-LT formation was again noted, and the low quantities of tetraenoic LTs provoked by PAF-alone challenge were completely suppressed on dual stimulation with ligand plus EPA. This complete shift of the metabolite profile from 4-series to 5-series products on disposal of free exogenous EPA strongly supports the notion that the extracellularly provided precursor fatty acids served as direct substrates for the 5-lipoxygenase pathways rather than being subjected to preceding acylation-deacylation cycles in membrane lipid pools. Notably, EPA was revealed to be the preferred substrate compared with AA. 1) The total sum of n-3 fatty acid-derived 5-lipoxygenase products in response to PAF plus EPA surpassed that of AA metabolites in response to PAF plus AA nearly twofold. 2) When both AA and EPA (plus PAF) were simultaneously applied, the levels of all 5-series products surpassed the amounts of their corresponding 4-series product with ratios of 2.1 (LTB₅ to LTB₄), 3.0 (sum of LTC₅, LTD₅, and LTE₅ to sum of LTC₄, LTD₄, and LTE₄), and 6.0 (5-HEPE to 5-HETE). These findings in the PMN-harboring pulmonary microvasculature fully agree with in vitro studies in ligand-challenged human neutrophils in which EPA was preferentially metabolized via 5-lipoxygenase and effected suppression of the metabolic conversion of endogenous and exogenous AA (11). Higher affinity of the purified neutrophil 5-lipoxygenase for EPA over AA has been previously described (29). The resulting LTA₄ is, however, metabolized less effectively via the neutrophil LTA hydrolase than LTA₄, resulting in reduced LTB₅ formation in relation to the 5-HEPE levels (11).

In conclusion, the present experiments in a model of lung microvascular leukostasis support the concept of a critical dependency of inflammatory ligand-evoked LT generation on the simultaneous disposal of free precursor fatty acid (11). Profiles of the rising LTs strongly suggest a major contribution of PMN-related transcellular metabolic pathways to the extent and quality of LT synthesis in this intact organ vasculature. Notably, intravascular disposal of free EPA effected a complete shift of the metabolite profile to pentaenoic LTs, with concomitant suppression of 4-series product formation. Considering the strong inflammatory potencies of the AA-derived lipoxygenase metabolites and the markedly reduced or even antagonistic biological properties of the corresponding n-3 derivatives (21, 25), these findings may be of interest for the development of anti-inflammatory therapies. The use of n-3 fatty acid-enriched (fish oil-derived) lipid emulsions rather than the conventional n-6 fatty acid-rich preparations may be a plausible strategy to combine parenteral nutrition and suppression of PMN-related inflammatory events.

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