Formation of Lipoxins and Leukotrienes During Receptor-mediated Interactions of Human Platelets and Recombinant Human Granulocyte/Macrophage Colony-stimulating Factor–primed Neutrophils

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Summary

The generation of lipoxygenase products of arachidonic acid is considered an important event in inflammation. This study demonstrates the levels of both lipoxins and leukotrienes (LTC_4, LTD_4, LTB_4, and ω-oxidized LTB_4) generated from endogenous sources of arachidonate by PMN primed with recombinant human granulocyte/macrophage colony-stimulating factor and in coincubations with platelets (1:1 to 1:100 ratio). Upon exposure to receptor-mediated stimuli (FMLP and thrombin), the levels of lipoxins generated were within the range of both LTB_4 and LTC_4. Co-incubation of [1-^{14}C]arachidonate–labeled platelets with primed polymorphonuclear leukocytes (PMN) followed by addition of thrombin and FMLP led to the formation of both 5- and 15-LO products that carried ^{14}C label. Thus, in addition to the transcellular conversion of LTA_4 to platelet-derived lipoxins and LTC_4, PMN can use platelet-derived arachidonate to generate lipoxygenase products. These results are the first to document the relationship between the levels of lipoxins and leukotrienes generated by receptor-mediated activation of cytokine-primed PMN interacting with platelets. Moreover, they indicate that PMN-platelet interactions utilize bidirectional transcellular routes to contribute to lipoxin formation.

The generation of eicosanoids by lipoxygenase-catalyzed reactions is associated with the activation of a wide range of human cell types that are involved in both physiologic and pathophysiologic events (1). It is now recognized that cell–cell interactions can initiate transcellular pathways for the biosynthesis of lipoxygenase products (2). These transcellular pathways may not only serve to amplify the levels of eicosanoids within a local milieu but may also lead to the generation of biologically active metabolites with functions different from those in its cell of origin. Studies on the interactions between human platelets and leukocytes have revealed several mechanistic models for transcellular eicosanoid biosynthesis, which may also be operative within other tissues (2–8). Platelets, for example, can utilize leukocyte-derived leukotriene A_4 to generate both peptido-leukotrienes (5, 6) and lipoxins (7, 8).

The ionophore of divalent cations A23187 is a useful tool in examining the cellular biosynthesis of eicosanoids, and in particular, transcellular pathways of formation because it (*a*) is not cell type specific in its actions, which enables activation of combined cell types and (*b*) it provokes the formation of individual eicosanoids in amounts that facilitate their isolation and structural elucidation by physical methods (1–7). By virtue of its ability to translocate divalent cations across cell membranes, it initiates a variety of responses that require an elevation in the intracellular levels of Ca^{2+}. In this regard, when added to human neutrophils and platelets, A23187 activates both the release of arachidonic acid from endogenous stores and lipoxygenation, which are two limiting events in the biosynthesis of lipoxygenase products by these cells (1–8). Since A23187 activates cells by circumventing receptor-ligand interactions in both cell types, potential regulatory events between individual lipoxygenases which may be operative following receptor activation remain to be fully appreciated. Previous studies have shown that platelet-directed stimuli (i.e., thrombin) can initiate metabolic interactions between platelets and neutrophils (4). Thus, receptor-mediated activation of combined cell types may lead to both quantitative and qualitative differences in the profiles and individual eicosanoids generated by transcellular biosynthesis. Recent results have shown that PMN primed by the cytokine granulocyte/macrophage colony-stimulating factor (GM-CSF)[1] can generate LTB_4 and

[1] *Abbreviations used in this paper:* DNCB, 2,4-dinitro-1-chlorobenzene; ED, electrochemical detector; GC, gas chromatography; GM-CSF, granulocyte/macrophage colony-stimulating factor; LO, lipoxygenase; LT, leukotriene; MS, mass spectrometry; NICI, negative ion chemical ionization.

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its ω-oxidation products following receptor-mediated stimulation by either the complement component (C5a) or the chemoattractant (FMLP), in quantities that are detectable by UV-RP-HPLC (9, 10). The enhanced levels of LTB_4 generated by stimulated PMN primed with GM-CSF observed in these studies are believed to be the result of increased availability of unesterified arachidonic acid generated during priming of the PMN (9, 10). Co-incubations of platelets and PMN stimulated with thrombin and FMLP lead to lipoxin generation without addition of substrates (8). To gain insight into the relationship between leukotrienes and lipoxins generated by receptor-mediated signals, we have determined the levels formed during co-incubations of platelets with GM-CSF–primed PMN and have examined their routes of formation.

Materials and Methods

FMLP, glutathione, and nitroprusside were from Sigma Chemical Co. (St. Louis, MO). DNCB and methyl formate were from Eastman Kodak Co. (Rochester, NY). Thrombin and LSM were from Enzyme Research Laboratories, Inc. (South Bend, IN) and Organon Teknika (Durham, NC), respectively. Human recombinant GM-CSF was purchased from Genzyme Co. (Boston, MA). Synthetic eicosanoids used as reference materials were purchased from Biomol Research Labs, Inc. (Philadelphia, PA) and [1-^{14}C]arachidonic acid was from New England Nuclear (Boston, MA).

Preparation of Cell Suspensions. For each experiment, fresh peripheral blood was obtained from healthy donors who had not taken aspirin or other medications for at least 7 d. When either platelets alone, or both platelets and PMN were isolated, acid citrate dextrose was used as anticoagulant and the isolation begun by first obtaining platelet-rich plasma from the whole blood (2). Washed platelets were prepared from PRP as described (2) and enumerated using a Coulter Counter model ZM (Coulter Electronics, Inc., Hialeah, FL). Platelets were adjusted to 3×10^9 cells/ml before co-incubation with PMN. Platelets were labeled (2) with 0.2 μCi/ml of [1-^{14}C]arachidonate. PMN were isolated from the remaining blood by Ficoll-Hypaque gradient centrifugation and dextran sedimentation. These suspensions contained 98 ± 1% PMN as scored by light microscopy before incubations with GM-CSF.

Analysis of Lipoxins and Leukotrienes. All incubations were terminated by addition of cold methanol (2 vol) containing PGB_2 as internal standard and extracted (8). After elution from C18-Sep Pack (Waters Associates, Milford, MA), materials within the methyl formate fractions (i.e., LTB_4, LXA_4) were concentrated and chromatographed separately by RP-HPLC from materials and eluting within the methanol fractions (i.e., LTC_4 and LTD_4). The ω-oxidation products of LTB_4, LTB_4, and the lipoxins were resolved utilizing ED-UV detection with RP-HPLC (11–13). This system was equipped with both a Lambda Max UV detector model 481 and an on-line Electro Chemical detector (ED) model M 460 operated with a $Ag/AgCl_2$ reference electrode (Waters Associates). The column, a Beckman Ultrasphere-ODS (4.5 mm × 25 cm) was eluted with $MeOH/H_2O$ (65:35, vol/vol), trifluoroacetic acid (1 mM) with a flow rate of 1 ml/min. This system permits detection of lipoxins and leukotrienes in the picogram range (11, 12). The electrode potential was set at 1.345 V and the UV detector was set at 270 nm (to monitor leukotrienes) and 300 nm (for lipoxins). Peptidoleukotrienes (LTC_4, LTD_4, and LTE_4) were resolved using an RP-HPLC system equipped with an LC-75 UV detector (Perkin-Elmer, Norwalk, CT) and a pump model 110A (Beckman Instruments, Berkeley, CA). The column was an Altex Ultrasphere-ODS eluted with $MeOH/H_2O$/acetic acid (65:35:0.01, vol/vol/vol) and adjusted to pH 5.7 as mobile phase. Mono-HETEs (5-, 12-, 15-HETE) were resolved using another separate RP-HPLC system that used an isocratic mobile phase of $MeOH/H_2O$/acetic acid (75:25:0.01, (vol/vol/vol) with the UV detector set at 235 nm. Lipoxygenase products were routinely identified by comparison of their individual retention times with those obtained for synthetic standards in each HPLC system. Their quantities were determined by comparing peak areas obtained for calibrated standards in each HPLC system and the corresponding products from individual incubations following correction for the recovery of internal standard.

Pentafluorobenzyl (PFB) ester trimethysilyl (TMS)–ether derivatives of the materials which coeluted with LXA_4 and LXB_4 on HPLC were collected and prepared as described (14, 15). The PFB ester derivatives were converted to their respective (TMS) derivative with bis (TMS) trifluoroacetamide (BSTFA)/pyridine (1:1) (15) and analyzed by gas chromatography–mass spectrometry. The gas chromatography–mass spectrometry (GC-MS) (Hewlett-Packard model 5988A equipped with an HP 59970 workstation) was operated in the negative ion mode. Negative ion chemical ionization (NICI) spectra were recorded with an electron energy of 377 eM volts and a relative resulting voltage of 2,677.

Results and Discussion

GM-CSF-primed PMN. GM-CSFrh-primed PMN exposed to FMLP (10^{-7} M) generated both leukotrienes and lipoxins without the addition of exogenous substrates (Table 1). PMN were exposed to GM-CSFrh for 90 min at 37°C before addition of FMLP in the present experiments since previous results have shown that maximal priming occurs between 1 and 2 h (9, 10). The present results with LTB_4 and its ω-oxidation products, namely their quantities amounting to major products generated by GM-CSFrh–primed PMN exposed to FMLP in vitro, are consistent with those recently reported (9, 10). Peptidoleukotrienes (LTC_4, LTD_4, and LTE_4) were not detected in these incubations, while the levels of LXA_4, LXB_4, and their all-trans-isomers were obtained in the range of amounts similar to those observed for native LTB_4. The average sum of the lipoxins was within the same order of magnitude as the sum obtained for LTB_4 and its ω-oxidation products generated by GM-CSF–primed PMN. The levels of lipoxins obtained here and their relationship to LTB_4 production with GM-CSF–primed PMN stimulated via a receptor-mediated agonist (i.e., FMLP) are drastically different from their relationship in ionophore-stimulated (A_{23187}) cells. In the case of the ionophore A_{23187}, which bypass receptor activation, PMN generate LTB_4 as a dominant product and LXA_4 is generated in amounts 50–100 times less than LTB_4 (13). Therefore the present observation indicates that receptor-mediated formation of lipoxygenase products by human PMN leads to a different balance between leukotrienes and lipoxins than that observed with ionophore-stimulated cells.

As is the case with other eicosanoids, identification of the lipoxins by GC-MS operated in the electron impact mode requires isolation of materials in the microgram range (1). In contrast, analysis based upon GC-NICI MS has proven

to be a highly specific and sensitive methodology to determine trace amounts (picogram) of eicosanoids present in biological samples (14, 15). Since both ED and UV detection with HPLC indicated that the lipoxins were formed in nanogram amounts (Table 1), it was necessary to use NICI-MS to confirm the identity of the materials generated by GM-CSF–primed PMN stimulated with FMLP that coeluted with synthetic lipoxins on ED-UV-RP-HPLC. To this end, the materials beneath the UV peaks (on RP-HPLC before ED) were collected, converted to PFB ester derivatives, treated with BSTFA (14, 15), and analyzed by NICI-MS. The mass spectrum of the PFB ester, TMS ether derivative of the material which coeluted with LXA_4 in RP-HPLC, showed an intense anion at m/e 567, which corresponded to the fragment $[M\text{-}PFB]^-$. Also present in its spectrum were ions at m/e 477 and m/e 387, corresponding to the loss of TMSOH and 2(TMSOH) from the parent anion $[M\text{-}PFB]^-$. There two ions were present in <5% of the spectrum (m/e 50–600), and the ion at m/e 567 was the base peak. Similarly, the PFB ester, TMS ether derivatives obtained from isolated materials that coeluted with LXB_4, and the all-trans-isomers on RP-HPLC also coeluted on GC with their respective PFB ester, TMS ether derivatives prepared from synthetic lipoxins. In each case, m/z 567 corresponding to $[M\text{-}PFB]^-$ was the base peak in the spectrum (Brezinski, D., and C.N. Serhan, manuscript submitted for publication). Taken together, the physical characteristics of these products on ED-UV-RP-HPLC as well as ions present in their respective mass spectra recorded on GC-NICI-MS suggest that primed PMN stimulated with FMLP generate lipoxins without addition of exogenous substrates.

Coincubation of GM-CSFrh–Primed PMN and Platelets. Simultaneous activation of PMN and platelets in suspension (1:10 cell ratio) with thrombin and FMLP leads to the formation of both LXA_4 and LXB_4 (8). In addition, evidence has been presented indicating that platelet 12-LO and its ω-6-oxygenase activity can play a role in the formation of lipoxins from PMN-derived leukotriene A_4 (7, 8). Since platelets can also utilize LTA_4 to generate LTC_4 (5, 6), we next evaluated the relationship between leukotrienes and lipoxins generated by GM-CSFrh–primed PMN coincubated with platelets (1:100 cell ratio). After addition of FMLP (10^{-7} M), the peptido-LTs (LTC_4, LTD_4, and LTE_4) were generated (Table 1). A 60% increase in ω-oxidized LTB_4 was also noted with PMN stimulated in the presence of platelets. Human platelets do not possess either the 5-lipoxygenase or the ω-oxidation system for LTB_4 (2–6); therefore, it is likely that the increased formation of LTB_4 and the peptido-LTs observed here with GM-CSF–primed PMN and platelets reflects, at least in part, the increased formation of LTA_4 by GM-CSF–primed PMN and the transfer of LTA_4 to platelets for LTC_4 production. The levels of LXB_4 and its trans-isomers were also increased in the presence of platelets, suggesting that multiple biosynthetic routes may contribute to the formation of lipoxins during the interactions of platelets with GM-CSF–primed PMN.

Addition of thrombin (0.1 U/ml) to co-incubations consisting of GM-CSF–primed PMN and platelets led to an increase in LXA_4 and a decrease in the amounts of peptido-LTs, while the level of LTBs was similar in both settings. For purposes of comparison, the relationship between LXs and

Table 1. *GM-CSFrh–primed Human PMN Co-incubations with Platelets: Formation of Lipoxins and Leukotrienes*

Incubation	t-LXB_4	LXB_4	t-LXA_4	LXA_4	LTC_4	LTD_4	LTE_4	ω_5-LTB_4	LTB_4
GM-CSF–primed PMN* + (FMLP 10^{-7} M)	11.4 ± 2.9	7.9 ± 2.8	14.2 ± 3.3	14.1 ± 5.9	—§	—§	—§	51.7 ± 4.5	13.8 ± 3.5
PMN‡ and platelets (FMLP + thrombin)	21.0 ± 1.5	24.1 ± 1.7	17.5 ± 3.2	18.6 ± 4.5	52.3 ± 8.1	28.7 ± 8.7	15.7 ± 7.9	60.3 ± 7.3	6.2 ± 2.1
GM-CSF–primed PMN* and platelets (FMLP)	103.7 ± 58.4	54.0 ± 8.4	26.7 ± 3.1	22.9 ± 6.7	47.5 ± 7.2	54.7 ± 34.2	13.0 ± 5.3	77.7 ± 11.1	10.5 ± 4.4
GM-CSF–primed PMN* and platelets (FMLP + thrombin)	76.3 ± 30.9	35.5 ± 4.6	23.5 ± 4.8	29.6 ± 7.8	37.6 ± 8.0	21.6 ± 5.1	13.5 ± 5.7	75.6 ± 12.7	12.2 ± 3.0
GM-CSF–primed PMN* and platelets (A_{23187}; 10^{-7} M)	134.1 ± 74.3	100.7 ± 26.3	45.2 ± 5.5	46.2 ± 12.4	88.3 ± 36.2	34.5 ± 9.6	21.5 ± 9.0	127.7 ± 35.6	21.3 ± 8.9

*,‡ Isolated PMN (30×10^6/ml) were incubated for 90 min at 37°C in the *presence or ‡absence of GM-CSFrh (200 pM). Next, aliquots (0.5 ml) of each cell suspension were combined with 0.5 ml aliquots of isolated platelet suspensions (3×10^9/ml). Cell ratios were approximately 1:100 (PMN/platelet) in 1 ml. The suspensions were exposed to either A_{23187} (0.1 μM), FMLP (10^{-7} M), or FMLP (10^{-7} M) in combination with thrombin (0.1 U/ml). ω_5-LTB_4 denotes [20-OH-LTB_4 + 20-COOH-LTB_4] and t-LX denotes the two coeluting all-trans-isomers of LXA_4 and LXB_4, respectively (see reference 13). The incubations (20 min at 37°C) were terminated and analyzed by RP-HPLC as described in Materials and Methods. Results are expressed in nanograms/incubation; mean ± SE of three separate experiments.
§ Products not detected.

LTs was determined for platelets co-incubated with PMN stimulated with FMLP (10^{-7} M) and thrombin (0.1 U) in the absence of the cytokine. In this case, both lipoxins and leukotrienes were generated with LTC_4 (which can be derived from platelets; references 5, 6) and ω-oxidation products of LTB_4) (formed by PMN; references 9, 10) registered as the dominant products. GM-CSF–primed PMN coincubated with platelets also generated lipoxins and LTs when exposed to ionophore (A_{23187}, 10^{-7} M) (Table 1; last row). Unlike the other stimuli examined, the ionophore does not interact with specific receptors and therefore may provide an index of the biosynthetic potential of these cells to generate individual lipoxygenase products during the interaction of GM-CSF–primed PMN with platelets. Together these results indicate that, during coincubation conditions (i.e., cell–cell interactions) with GM-CSF–primed PMN, lipoxins are generated in the range of amounts similar to that observed for individual leukotrienes when cells are exposed to physiologically relevant stimuli.

Next, we evaluated the role of cell ratio in receptor-mediated formation of lipoxygenase products during coinincubation of both PMN-platelets (Fig. 1, *left*) and GM-CSF–primed PMN-platelets (Fig. 1, *right*). Upon exposure to thrombin (0.1 U) and FMLP (10^{-7} M), the levels of LTBs (Σ LTB_4, ω-OH-LTB_4, and 20-COOH-LTB_4) and lipoxins (Σ LXA_4, LXB_4, and their all trans-isomers) were generated in excess of the amounts of peptido-LTs. The levels of peptido-LTs did not approach those observed for either the LXs or LTBs until the platelet count exceeded ~10 per PMN. At the cell ratio of 1:10, the increase in peptido-LTs appears to follow a reciprocal decrement in the levels of LTBs. Since LTA_4 is the common precursor to both LTB_4 and LTC_4, it appears that this shift to peptido-LTs is the result of LTA_4 transcellular metabolism. A similar reciprocal relationship between LTB_4 and LTC_4 has been established with suspensions of platelets and granulocytes stimulated with ionophore A_{23187} (6).

Coincubations of platelets with GM-CSF–primed PMN generated higher levels of lipoxins (Fig. 1, *right*) when exposed to FMLP (10^{-7}M) and thrombin (0.1 U) than coincubations with PMN that were not primed with GM-CSF. At cell ratios of 1:100 (PMN platelets), the levels of lipoxins exceeded those of either the LTBs or peptido-LTs. This finding together with previous observations (7, 8) suggests that, during the coincubation of GM-CSF–primed PMN and platelets, several transcellular biosynthetic routes may be operative to contribute to the production of lipoxins.

Co-incubation of [1-^{14}C]arachidonate–labeled Platelets and Unlabeled Neutrophils. During coincubations stimulated with ionophore (A_{23187}), platelet-derived arachidonate can serve as a precursor for PMN-derived LTB_4 and 5-HETE (2), and platelets can convert exogenous LTA_4 to lipoxins (7, 8) as well as peptido-LTs (5, 6). To determine if these two biosynthetic routes are operative during receptor-mediated eicosanoid formation by cell–cell interactions, labeled platelets were co-incubated with both PMN and GM-CSF–primed PMN and the lipoxygenase products were analyzed for the content of [1-^{14}C].

Representative radiochromatograms of mono-HETEs obtained after stimulation of co-incubation with FMLP (10^{-7} M) and thrombin (0.1 U) are given in Fig. 2. Both 5-HETE and 15-HETE carried [1-^{14}C] platelet-derived radiolabel. These results illustrate that, after incubation with thrombin, platelets release [1-^{14}C]arachidonate, which was

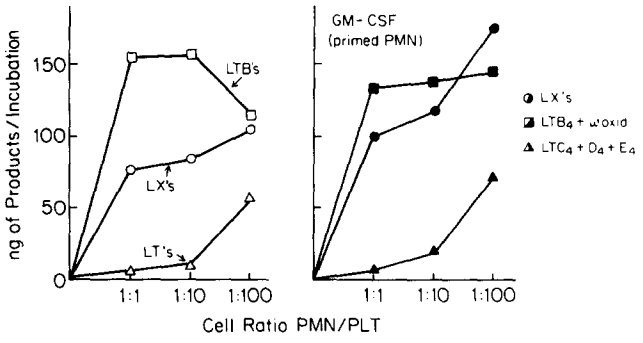

Figure 1. Formation of lipoxins and leukotrienes by PMN-platelet coincubations stimulated with FMLP and thrombin: effect of cell ratio and comparison with GM-CSF–primed PMN. (*Left*) PMN (15× 10^6 cells) were co-incubated with autologous platelets in 1 ml and stimulated with FMLP (10^{-7} M) and thrombin (0.1 U/ml) for 20 min at 37°C. (*Right*) shows similar co-incubations where PMN were exposed to GM-CSFrh (90 min, 37°C) before coincubations. Products were extracted and quantitated by combined ED-UV-HPLC in two separate RP-HPLC systems (see Materials and Methods). LTBs denotes the sum of LTB_4, 20-COOH-LTB_4 and 20-OH-LTB_4, LXs are the sum of LXA_4, LXB_4, and their all-trans-isomers and LTs represents the sum of LTC_4, LTD_4, and LTE_4. The results are representative of three separate experiments.

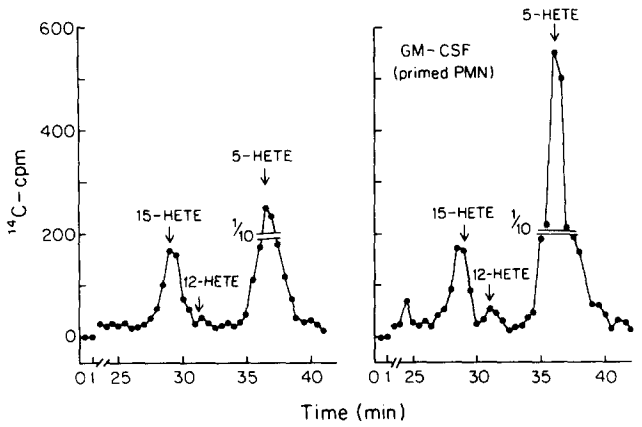

Figure 2. RP-HPLC profile of [1^{14}C]-labeled mono-HETEs from co-incubation of unlabeled PMN with [1-^{14}C]arachidonate-labeled platelets exposed to FMLP and thrombin. Isolated platelets were labeled (2) with [1-^{14}C]-arachidonate, washed two times and incubated with autologous PMN (1:100, PMN/platelets) in 1 ml PBS with 15 × 10^6 PMN. (*Left*) Co-incubations were exposed to FMLP (10^{-6} M) and thrombin (0.1 U/ml) for 20 min at 37°C. (*Right*): PMN were primed with GM-CSFrh (200 pM, 90 min, 37°C) before co-incubation with labeled platelets and addition of FMLP (10^{-6} M) and thrombin (0.1 U/ml). Products were extracted and chromatographed with MeOH/H_2O/acetic acid (75:25:0.01, vol/vol/vol) as the mobile phase. Arrows denote co-chromatography of synthetic standards. Results in each profile are representative of three separate experiments.

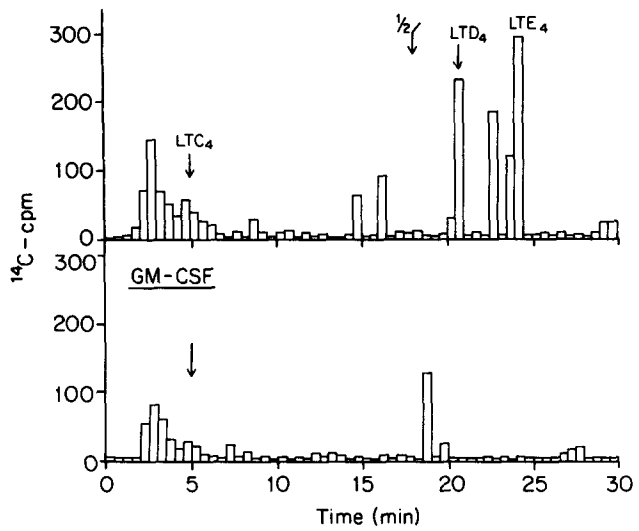

Figure 3. RP-HPLC profiles of [1-^{14}C]-labeled peptido-LTs from co-incubation of unlabeled PMN with [1-^{14}C]arachidonate-labeled platelets exposed to FMLP and thrombin. (*Top*) Co-incubations were exposed to FMLP (10^{-7} M) and thrombin (0.1 U/ml) for 20 min at 37°C. (*Bottom*) PMN were primed with GM-CSFrh (200 pM, 90 min at 37°C) before co-incubation with labeled platelets and addition of FMLP (10^{-7} M) and thrombin (0.1 U/ml). Here, MeOH fractions from incubations were chromatographed separately. Arrows denote retention times of synthetic standards. Results in each radiochromatogram are representative of three separate experiments.

transformed by both the 5- and 15-lipoxygenase of FMLP-activated PMN. With GM-CSF–primed PMN, a similar HPLC profile was observed after addition of stimuli and an enhanced level of [1-^{14}C]-5-HETE was generated during the co-incubations. These results are consistent with the demonstrated enhancement in 5-lipoxygenase–derived products with GM-CSF–primed PMN (9, 10) and provide further evidence indicating that platelet-derived arachidonate (2) is an important source of substrate for PMN lipoxygenase pathways. Further analysis revealed that peptido-LTs from these incubation conditions also carried [1-^{14}C] label (Fig. 3, *top*). Since these products are generated from PMN-derived LTA$_4$ (5, 6), it appears that, during receptor-mediated cell–cell interaction, platelet-derived [1-^{14}C]arachidonate is released and transformed by the PMN 5-LO to [1-^{14}C]-LTA$_4$, which is then transferred back to platelets for conversion to labeled peptido-LTs. In contrast, with GM-CSFrh–primed PMN and labeled platelets, labeled peptido-LTs were not generated in appreciable amounts (Fig. 3, *bottom*), although unlabeled products were formed. This observation suggests that labeled LTA$_4$ may have been diverted to other routes with the GM-CSF–primed PMN (see Table 1 and Fig. 1).

Along these lines, evidence for at least two transcellular routes has been presented for lipoxin production: one involves the transformation of exogenous 15-HETE by human PMN (13) and eosinophils (16), and the other involves the conversion of LTA$_4$ by human platelets (7, 8). Lipoxins from these incubation conditions also carried [1-^{14}C] label, demonstrating that platelet-derived arachidonate contributes to their formation during cell–cell interactions initiated by receptor-mediated mechanisms. However, since both [1-^{14}C]15-HETE and [1-^{14}C]LTC$_4$ (which can serve as an indicator of labeled LTA$_4$ formation by PMN) were detected, it was not possible to conclude from the present data which pathway predominates in the formation of lipoxins during PMN-platelet interactions. Nevertheless, the present findings provide evidence that both PMN and platelets generate lipoxins by transcellular routes. Agonist-induced generation of lipoxins by PMN appears to be enhanced by platelet-derived arachidonate, while the platelet generation of lipoxins is dependent upon leukocyte-derived LTA$_4$.

Conversion of LTA$_4$ by Thrombin-activated Platelets. We next determined the relationship between lipoxin and peptido-LT production and assessed factors that alter their formation by platelets (Table 2). Thrombin-activated platelets generated LTC$_4$ + LTD$_4$ in amounts greater than those of LXA$_4$ + LXB$_4$ from LTA$_4$. Conversion of LTA$_4$ to lipoxins can involve the platelet 12-LO (7, 8), while its conjugation to LTC$_4$ may involve a specific glutatione-*S*-transferase or platelet LTC$_4$ synthetase (5, 6). Depletion of GSH by treating platelets with DNCB does not alter platelet aggregation or

Table 2. *Conversion of LTA$_4$ by Thrombin-activated Platelets: Relationship between Lipoxins and Peptidoleukotrienes*

Incubations	LXB$_4$	LXA$_4$	LTC$_4$	LTD$_4$
Platelets +				
LTA$_4$ + thrombin 0.1 U/ml	15.9 ± 3.7	21.6 ± 7.9	111.8 ± 13.7	24.1 ± 9.1
DNCB (100 μM)	55.9 ± 6.4	23.7 ± 9.9	22.1 ± 6.2	21.0 ± 6.6
DNCB (500 μM)	149.3 ± 13.0	19.3 ± 7.6	21.0 ± 11.0	24.8 ± 8.4
Nitroprusside (10 μM)	31.7 ± 6.0	30.0 ± 1.8	14.5 ± 8.1	16.8 ± 5.3

Human platelets (1.5×10^9 cells/ml) were incubated (20 min, 37°C) with LTA$_4$ (20 μM), human albumin (0.1%), and thrombin (0.1 U/ml). Platelets were treated 30 min with DNCB or 3 min with nitroprusside at 37°C before addition of stimuli. Products were extracted and quantitated as described in Materials and Methods. Results are expressed in nanograms of product per incubation; mean ± SE of three separate experiments.

secretion (17), but enhances 12-lipoxygenase activity (18). Platelets treated with DNCB blocked the formation of LTC_4 and enhanced LXB_4 but not LXA_4 when compared with untreated cells (Table 2). The enzymatic generation of LTC_4 by platelets appears to be unlike that of macrophages, where DNCB does not inhibit LTC_4 formation by LTC_4 synthetase (19). Nitroprusside-treated platelets gave elevated levels of both LXA_4 and LXB_4 but generated less LTC_4. Since nitroprusside (10 μM) stimulates cGMP, inhibits Ca^{2+} mobilization, and blocks platelet aggregation (20), its ability to divert the fate of LTA_4, in favor of lipoxin production by platelets (Table 2), may be related to the vasodilatory actions of nitroprusside, since peptido-LTs are potent vasoconstrictors. Thus, the levels of both lipoxins and peptido-LTs generated by platelets may be altered by drugs that influence the glutathione status of platelets.

Counterregulatory actions of lipoxins have been documented in renal microcirculation, where LXA_4 stimulates vasodilation and antagonizes the vasoconstrictive activity of LTD_4 (21). LXA_4, at nanomolar levels, inhibits the chemotactic response of PMN to either LTB_4 or FMLP (22) and blocks the mobilization of Ca^{2+} and IP_3 in these cells (23). LXA_4 also inhibits LTB_4-induced inflammation (in vivo) in the hamster cheek pouch (24), and has recently been shown to cause relaxation of human pulmonary arteries precontracted with either prostaglandin $F_{2\alpha}$ or the vasoconstrictor peptide endothelin (25). The profile of bioactions observed for LXA_4 thus far and its identification in human bronchoalveolar fluids (26) suggest that the generation of lipoxins may play a functional role in vivo. Thus, the generation of physiologically relevant levels of lipoxins as documented here (in vitro) by receptor-mediated activation of cytokine-primed PMN interacting with platelets by at least two separate biosynthetic routes, namely one which involves the donation of platelet-arachidonate to the 15-lipoxygenase of PMN and the other PMN-derived LTA_4 transformation by platelets, may be important in both inflammation and in the interaction of these cells with the vessel wall.

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References

1. Samuelsson, B., and C.N. Serhan. 1987. On the formation and biological role of leukotrienes and lipoxins. *In* Prostaglandins in Cancer Research. E. Garaci, R. Paoletti, and M.G. Santoro, editors. Springer-Verlag, Berlin/Heidelberg. 3–11.
2. Marcus, A.J., M.J. Broekman, L.B. Safier, H.L. Ullman, N. Islam, C.N. Serhan, L.E. Rutherford, H.M. Korchak, and G. Weissmann. 1982. Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions in vitro. *Biochem. Biophys. Res. Commun.* 109:130.
3. Maclouf, J.A., B.F. de Laclos, and P. Borgeat. 1982. Stimulation of leukotrienes biosynthesis in human blood leukocyte by platelet-derived 12-hydroperoxy-icosatetraenoic acid. *Proc. Natl. Acad. Sci. USA.* 79:6042.
4. Marcus, A.J., L.B. Safier, H.L. Ullman, M.J. Broekman, N. Islam, T.D. Oglesby, and R.R. Gorman. 1984. 12S,20-Dihydroxyicosatetraenoic acid: A new icosanoid synthesized by neutrophils from 12S-hydroxyicosatetraenoic acid produced by thrombin- or collagen-stimulated platelets. *Proc. Natl. Acad. Sci. USA.* 81:903.
5. Maclouf, J.A., and R.C. Murphy. 1988. Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets: a potential cellular source of leukotriene C_4. *J. Biol. Chem.* 263:174.
6. Edenius, C., K. Heidvall, and J.A. Lindgren. 1988. Novel transcellular interaction: conversion of granulocyte-derived leukotriene A_4 to cysteinyl-containing leukotrienes by human platelets. *Eur. J. Biochem.* 178:81.
7. Edenius, C., J. Haeggström, and J.A. Lindgren. 1988. Transcellular conversion of endogenous arachidonic acid to lipoxins in mixed human platelet-granulocyte suspensions. *Biochem. Biophys. Res. Commun.* 157:801.
8. Serhan, C.N., and K.-A. Sheppard. 1990. Lipoxin formation during human neutrophil-platelet interactions: evidence for the transformation of leukotriene A_4 by platelet 12-lipoxygenase in vitro. *J. Clin. Invest.* 85:772.
9. Dahinden, C.A., J. Zingg, F.E. Maly, and A.L. de Weck. 1988. Leukotriene production in human neutrophils primed by recombinant human granulocyte/macrophage colony-stimulating factor and stimulated with the complement component C5a and FMLP as second signals. *J. Exp. Med.* 167:1281.
10. McColl, S.R., E. Krump, P.H. Naccache, and P. Borgeat. 1989. Enhancement of human neutrophil leukotriene synthesis by human granulocyte-macrophage colony-stimulating factor. *Agents Actions.* 27:465.
11. Herrmann, T., D. Steinhilber, J. Knospe, and H.J. Roth. 1988. Determinations of picogram amounts of lipoxin A_4 and lipoxin B_4 by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 428:237.

12. Hermann, T., D. Steinhilber, and H.J. Roth. 1987. Determination of leukotriene B_4 by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 416:170.
13. Serhan, C.N. 1989. On the relationship between leukotriene and lipoxin production by human neutrophils: evidence for differential metabolism of 15-HETE and 5-HETE. *Biochim. Biophys. Acta.* 1004:158.
14. Strife, R.J., and R.C. Murphy. 1984. Preparation of pentafluorobenzyl esters of arachidonic acid lipoxygenase metabolites. Analysis by gas chromatography and negative-ion chemical ionization mass spectrometry. *J. Chromatogr.* 305:3.
15. Waddell, K.A., S.E. Barrow, C. Robinson, M.A. Orchard, C.T. Dollery, and I.A. Blair. 1984. Quantitative analysis of prostanoids in biological fluids by combined capillary column gas chromatography negative ion chemical ionization mass spectrometry. *Biomed. Mass Spectrum.* 11:68.
16. Serhan, C.N., U. Hirsch, J. Palmblad, and B. Samuelsson. 1987. Formation of lipoxin A by granulocytes from eosinophilic donors. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 217:242.
17. Hill, T.D., J.G. White, and G.H.R. Rao. 1989. The influence of glutathione depleting agents on human platelet function. *Thromb. Res.* 53:457.
18. Hill, T.D., J.G. White, and G.H.R. Rao. 1989. Role of glutathione and glutathione peroxidase in human platelet arachidonic acid metabolism. *Prostaglandins.* 38:21.
19. Abe, M., and T.E. Hugli. 1988. Characterization of leukotriene C_4 synthetase in mouse peritoneal exudate cells. *Biochim. Biophys. Acta.* 959:386.
20. Morgan, R.O., and A.C. Newby. 1989. Nitroprusside differentially inhibits ADP-stimulated calcium influx and mobilization in human platelets. *Biochem. J.* 258:447.
21. Badr, K.F., D.K. DeBoer, M. Schwartzberg, and C.N. Serhan. 1989. Lipoxin A_4 antagonizes cellular and in vivo actions of leukotriene D_4 in rat glomerular mesangial cells: evidence for competition at a common receptor. *Proc. Natl. Acad. Sci. USA.* 86:3438.
22. Lee, T.H., C.E. Horton, U. Kyan-Aung, D. Haskard, A.E.G. Crea, and B.W. Spur. 1989. Lipoxin A_4 and lipoxin B_4 inhibit chemotactic responses of human neutrophils stimulated by leukotriene B_4 and N-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin. Sci.* 77:195.
23. Grandordy, B.M., H. Lacroix, E. Mavoungou, S. Krillis, A.E.G. Crea, B.W. Spur, and T.H. Lee. 1990. Lipoxin A_4 inhibits phosphoinositide hydrolysis in human neutrophils. *Biochem. Biophys. Res. Commun.* 167:1022.
24. Hedqvist, P., J. Raud, U. Palmertz, J. Haeggström, K.C. Nicolaou, and S.-E. Dahlén. 1989. Lipoxin A_4 inhibits leukotriene B_4-induced inflammation in the hamster cheek pouch. *Acta. Physiol. Scand.* 137:571.
25. Matsuda, H., J. Haeggström, K.C. Nicolaou, and S.-E. Dahlén. 1990. Lipoxins relax isolated arteries including the human pulmonary artery. *7th International Conference on Prostaglandins and Related Compounds,* Florence, Italy. (Abstr. No. 83)
26. Lee, T.H., A.E.G. Crea, V. Gant, B.W. Spur, B.E. Marron, K.C. Nicolaou, E. Reardon, M. Brezinski, and C.N. Serhan. 1990. Identification of lipoxin A_4 and its relationship to the sulfidopeptide leukotrienes C_4, D_4, and E_4 in the bronchoalveolar lavage fluids obtained from patients with selected pulmonary diseases. *Am. Rev. Respir. Dis.* 141:1453.