Transcellular Biosynthesis of Sulfidopeptide Leukotrienes During Receptor-Mediated Stimulation of Human Neutrophil/Platelet Mixtures

By Jacques Maclouf, Robert C. Murphy, and Peter M. Henson

The ability of different cell types to cooperate in the metabolism of reactive intermediates of arachidonic acid such as leukotriene A₄ (LTA₄) is currently receiving considerable attention. Of critical importance is the demonstration that transfer of LTA₄ could occur under conditions when relatively low amounts of LTA₄ are produced such as would be expected for in vitro receptor-mediated stimulation. Stimulation of human neutrophils with a combination of chemotactic factor (formyl-methionyl-leucyl-phenylalanine, FMLP) and phagocytosable particles (opsonized zymosan) resulted in little production of LTC₄ alone, but measurable quantities appeared when platelets were coincubated. When these agonists were added to platelets alone in the absence of neutrophils, no LTC₄ was produced. In the presence of stimulated neutrophils, the final synthesis of LTC₄ was shown to occur within the platelets (from neutrophil-derived LTA₄) by experiments using platelets that had been prelabeled with ³⁵S-cysteine to label intracellular platelet glutathione. Other ³⁵S-labeled sulfidopeptide leukotriene metabolites were also produced in this coinoculation of neutrophils and platelets. The observed synergy between FMLP and opsonized zymosan in the production of LTC₄ when neutrophils and platelets were coincubated may involve priming the neutrophil for LTA₄ production. Activation of platelets or endothelial cells with thrombin did not alter the capacity of either cell to convert exogenously added LTA₄ into LTC₄. This would support the suggestion that even when platelets are activated they retain their capacity to metabolize LTA₄ into LTC₄. Finally, previous exposure of the platelets to LTA₄ did not affect subsequent metabolism of arachidonic acid by the cyclooxygenase pathway to thromboxane A₂ (TXA₂) except at very high concentration of LTA₄. These results suggest that cell–cell interactions may be critical determinants of the profile of eicosanoids produced in physiologic and pathophysiologic circumstances. In particular, we believe that both endothelial cells and platelets can, together with neutrophils, contribute relatively large amounts of sulfidopeptide leukotrienes to inflammatory and thrombotic events. These cell–cell interactions are aspirin-insensitive; therefore, aspirin-treated platelets are capable of synthesizing the vasoconstrictor LTC₄ from neutrophil LTA₄ at a time when they can no longer produce thromboxane.

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MATERIALS AND METHODS

Materials. Synthetic LTA₄, methyl ester, a gift of Dr F. A. Fitzpatrick (Upjohn, Kalamazoo, MI), was hydrolyzed according to a published procedure to yield the sodium salt of the free acid.¹⁰ ³⁵H-LTC₄ (138 Ci/mmol) was obtained from New England Nuclear (Boston, MA). LTC₃, LTD₃, and LTE₄ were a gift of Dr J Rokach (Merck Frost, Montreal, Canada). The rabbit anti-LTC₄ was a gift from Dr E. Hayes (Merck, Sharp and Dohme, Rahway, NJ). The rabbit anti-LTE₄ was a gift from Dr Philippe Pradelles (CEA, Inserm U 150, Paris, France). Materials and National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

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### Transcellular Synthesis of Leukotrienes

Human thrombin was obtained from Roche (Basel, Switzerland). Thrombin (Sigma) was purchased from Amersham (Chicago, IL). Formyl-methionyl-leucyl-phenylalanine (FMLP) and bovine serum albumin (BSA) were obtained from Sigma (St Louis, MO). Zymosan (ICN, Cleveland, OH) was prepared as described and was boiled for 30 minutes before opsonization. Serum-opsonized zymosan was prepared from a pool of saline and kept in Hank's balanced salt solution (HBSS) containing 40 mmol/L EDTA at 0°C until use (within <2 weeks after preparation). Prostaglandin E, (PGE,) was purchased from Cayman Chemical (Ann Arbor, MI). High-performance liquid chromatography (HPLC)-grade solvents were obtained from Fisher Scientific (Springfield, NJ). Apex II octadecyl reversed-phase HPLC cartridges (5 μm, Jones Chromatography, Mid Glamorgan, England) were used for quantitation and characterization of leukotrienes after being washed with EDTA.

**Preparation of human platelets.** Human venous blood (6 vol) was obtained from adult human volunteers who had not received medication for at least 1 week. Blood was anticoagulated with acid-citrate-dextrose anticoagulant (ACD, National Institutes of Health formula A) (1 vol) and centrifuged for 15 minutes at 120 g at room temperature. The platelet-rich plasma (PRP) was transferred to a polypropylene tube with a plastic pipette and was then further acidified to pH 6.5 by addition of 1/10 vol ACD-A and centrifuged for 15 minutes at 400 g. The platelets were washed using the method of Patscheke in 36 mmol/L citric acid, 5 mmol/L glucose, 5 mmol/L KCl, 2 mmol/L CaCl₂, 1 mmol/L MgCl₂, 103 mmol/L NaCl, and 4 g/L BSA, 100 mmol/L PGE₂, pH 6.5. The platelet pellet was finally resuspended in HBSS buffer containing 4 g/L BSA (HBSS/BSA), pH 7.4. The concentrations of platelets used for each experiment are indicated in the legends to Figs 1 through 3. Lack of contamination by leukocytes or RBCs was checked by light microscopy. In some experiments, platelets (1 to 2 × 10⁶ cells/mL) were preincubated with ³⁵S-cysteine, 20 μCi/mL in the washing buffer. After 90-minute incubation at 37°C, the cells were washed and incubated as described below with addition of 50 μmol/L unlabeled cysteine to the incubation medium.

**Preparation of human polymorphonuclear leukocytes.** Suspensions containing neutrophils (>98% viable) were prepared using a plasma Percoll gradient by techniques that minimize exposure to bacterial endotoxin as previously described. The contamination of these cells by nonadhering platelets or RBCs was less than 3% and 5%, respectively, as estimated by phase-contrast microscopy. The cells were resuspended in HBSS/BSA to cell concentrations indicated in the legends to Figs 1 through 3. Experiments were performed to examine the production of thromboxane B₂ (TXB₂) by neutrophil and neutrophil/platelet mixtures stimulated maximally with A23187. Neutrophils alone produced 2.1 ± 0.8 ng TXB₂/10⁶ cells when stimulated with 1 μmol/L ionophore (n = 4). In the presence of 2 × 10⁶ platelets, 1.509 ± 240 ng TXB₂/10⁶ neutrophils was produced. From these figures, we can estimate that it would require a contamination of only one platelet per 20 neutrophils to achieve the levels of TXB₂ observed with neutrophil preparations. This degree of platelet contamination is typical.

**Isolation and culture of endothelial cells.** HUVEC were harvested from umbilical veins cultured in MCDB 131, supplemented with 5% fetal bovine serum (FBS), 10 ng/mL epidermal growth factor (Collaborative Research, Bedford, MA) 1 μg/mL hydrocortisone (Sigma) and 100 U/mL penicillin with 100 U/mL streptomycin (GIBCO, Grand Island, NY). Primary cultures were grown to confluence and subcultured at a split ratio of 1:2 into assay wells. Both cobblestone morphology and the presence of factor VIII-related antigen, measured by indirect immunofluorescence using goat anti-human factor VIII immunoglobulin (Atlantic Laboratories, Scarborough, MA) were used to identify the cells. They were plated in 35-mm wells (Costar, Cambridge, MA) and maintained in a 37°C, 5% CO₂ humidified atmosphere.

**Transcellular metabolism of neutrophil-derived LTA₄.** Combinations of platelets (0.35 × 10⁶) and neutrophils (7 × 10⁶) and 1 mL HBSS, pH 7.4, containing 1 g/L BSA (final volume) were allowed to equilibrate for 5 minutes at 37°C. Incubations were initiated by addition of opsonized zymosan (30 to 35 particles per neutrophil) and/or FMLP at concentrations from 10⁻⁴ to 10⁻⁶ mol/L. All incubations were performed at 37°C for 30 minutes. The reaction was stopped by addition of 1 vol ice-cold methanol to each tube, which was then allowed to stand for 1 hour at -20°C. The cells and any precipitated material were removed by centrifugation at 12,000g for 2 minutes. Incubations were performed in duplicate with three different donors. The supernatants were dried under vacuum after previous addition of 20,000 dpm of [³¹H]-LTC₄. The dried extract was suspended in 1 mL 0.1 mol/L phosphate buffer (pH 7.4) containing 1 g/L BSA. To these tubes was added 0.1 U each of γ-glutamyl transpeptidase and leucine aminopeptidase, and
Exogenous LTA₄ was then added at different concentrations to the platelet suspensions (0.5 mL) for 10 minutes at 37°C. Thrombin (0.1 U/mL) was then added, and platelets were incubated for 3 minutes more. The cells were transferred to tubes containing ice-cold 0.1 mol/L EDTA and centrifuged for 1 minute at 12,000 g. The supernatant was then analyzed for TXB₂ content using a previously described enzyme immunoassay (EIA).²⁶ Two separate experiments were performed on platelets obtained from two different donors. In other experiments, platelets or endothelial cells in 35-mm wells were allowed to equilibrate for 5 minutes at 37°C in 1 mL HBSS/BSA and then incubated with various amounts of thrombin (0.01 to 10 U/mL). After this stimulation, the cells were incubated at 37°C for 30 minutes with LTA₄ at the concentrations described in legends to Figs 1 through 3. At the end of this incubation, 1 mL ice-cold methanol was added to the cells and the tubes were kept frozen for analysis by HPLC or by EIA for LTC₄ (described below). In Figs 1 through 3, the values represent the average of three to five different experiments.

Instrumental analysis. RP-HPLC was performed using a Beckman gradient HPLC system and an ultraviolet (UV) detector (Spectromonitor III, LDC, Riviera Beach, FL). A linear gradient was used starting with 100% solvent A (methanol/water/phosphoric acid, 30:70:0.02, buffered to pH 5.7 with ammonium hydroxide) and proceeding to 100% methanol in 30 minutes with a flow rate of 1 mL/min. The identity of the peaks was assessed by coelution with authentic standards, UV spectroscopy, immunoactivity and, when appropriate, radioactivity content was followed by continuous radioactive monitoring using an on-line HPLC scintillation detector (Flo-one, Radiomatic, Tampa, FL). In some elutions, the UV-absorbing radioactive peaks were analyzed by EIA.

Quantitative determination of sulfidopeptide leukotrienes. Quantitation of LTC₄ by HPLC was made using PGB₂ as an internal standard as described previously.¹⁹ A sensitive EIA technique with acetyl cholinesterase coupled to LTC₄ as the enzymatic tracer was also used (gift from Dr P. Pradelles, CEA, Saclay, France). The EIA protocol used a double-antibody technique with 96-well microtiter plates coated with an anti-rabbit IgG monoclonal antibody (MoAb) (a gift from Dr Pradelles) and was performed as described previously.¹⁹ The quantitation of LTE₄ was made with a newly developed EIA for LTE₄, which essentially follows that previously described for other eicosanoids.¹⁸ Statistical analyses were made with Student’s unpaired t test.

**Fig 2.** Leukotriene biosynthesis after stimulation of neutrophils with FMLP and/or opsonized zymosan with or without added platelets. Neutrophils (1.0 mL, 8 to 10 × 10⁶ cells/mL) were incubated alone or in the presence of 0.7 to 0.8 10⁶ platelets/mL. After 30-minute stimulation with FMLP (at the doses indicated) and/or opsonized zymosan (25 to 40 particles per neutrophil), the cells were centrifuged for 1 minute at 12,500 g. LTE₄ was analyzed directly by EIA after an overnight incubation with γ-glutamyl transpeptidase and leucine aminopeptidase. Values are the mean ± SEM of six different experiments. Unstimulated cells, ie, control neutrophils, in the presence of platelets, generated less LTE₄ than 0.02 ± .002 pmol LTE₄/10⁶ neutrophils). *Significantly different (P < .05) from opsonized zymosan-treated cell incubations without FMLP (control values). **Significantly different (P < .01) from opsonized zymosan-treated cell incubations without FMLP (control values).

**Cell incubation with LTA₄.** Platelets were prepared according to the procedures described above. After washing, they were suspended at 0.2 × 10⁹ cells/mL in HBSS, pH 7.4, containing no BSA.

**Fig 3.** Effect of thrombin stimulation on transformation of LTA₄ into LTC₄ by platelets (left) or endothelial cells (right). Platelets (0.6 × 10⁹/mL) or endothelial cells were stimulated by increasing concentrations of thrombin. After 10 minutes, LTA₄ (0.6 and 5 μmol/L, respectively) was added to platelets or endothelial cells for 20 minutes. The supernatants were analyzed by EIA for their content of TXB₂ (platelets) or 6-keto-PGF₁α (endothelial cells) as well as for LTC₄. Results are the mean of triplicate incubations.
TRANSCELLULAR BIOSYNTHESIS OF LEUKOTRIENES

RESULTS

Transcellular metabolism of neutrophil-derived LTA₄ by platelets. Previous work has suggested that LTA₄ from neutrophils can be metabolized within the platelet to yield sulfidopeptide leukotrienes. To test the hypothesis that production of LTC₄ resulted from platelet conversion of neutrophil-derived LTA₄ after physiologic stimulation, ³⁵S-cysteine-labeled platelets were used in neutrophil coincubations. Exposure of platelets to radiolabeled cysteine resulted in production of intracellular ³⁵S-glutathione,²⁰ which was then available as a substrate for LTC₄ synthetase within the platelet. Formation of ³⁵S-labeled LTC₄ as a final product (Fig 1), would therefore indicate that the LTA₄ had been converted within the platelet. Because the amount of LTC₄ generated in these types of experiments was at the UV detection limit for leukotrienes by HPLC analysis, separated HPLC fractions were analyzed by EIA and radioactivity was analyzed using an on-line HPLC scintillation detector. To minimize any transfer of ³⁵S-cysteine or ³⁵S-glutathione from the labeled platelets during coincubation with the neutrophils, 50 μmol/L cysteine (unlabeled) was added to the incubation medium in these experiments.²¹ In separate experiments, we verified that addition of cysteine (50 μmol/L) did not affect the quantitative production of LTC₄.

When the neutrophils alone were stimulated with opsonized zymosan and FMLP (10⁻⁶ mol/L), almost no immunoreactive material could be detected (described below). When platelets were added, however, the ³⁵S radioactivity profile, as well as analysis of the HPLC fractions by EIA, indicated radioactive and immunoreactive peaks corresponding to the elution of LTC₄ had appeared (Fig 1). These results were consistent with our previous data of transcellular metabolism of LTA₄ by neutrophils and platelets when the calcium ionophore A23187 was used as the stimulus.⁶ In some other experiments, we also found immunoreactive LTD₄ and E₄, probably caused by further metabolism of LTC₄ into those compounds.

Owing to the potential presence of multiple metabolites of the sulfidopeptide leukotrienes, subsequent experiments involving quantitative measurements of sulfidopeptide leukotrienes were assessed after conversion of LTC₄ and LTD₄ into LTE₄ by addition of γ-glutamyl transpeptidase and leucine aminopeptidase to the postincubation supernatants. This is known to metabolize LTC₄ to LTE₄ efficiently.¹⁷ In representative experiments, the extent of metabolism was monitored by HPLC and scintillation counting to assess conversion of ³⁵H-LTC₄ added as an internal standard. More than 80% conversion was always observed.

As shown in Fig 2, stimulation of human neutrophils with FMLP (10⁻⁶ mol/L) or opsonized zymosan (25 to 40 particles per cell) alone did not result in formation of significant amounts of sulfidopeptide leukotrienes (as measured by LTE₄ equivalents) although the levels obtained from opsonized zymosan stimulation were higher than those obtained from unstimulated cells (0.114 ± 0.035 pmol/10⁶ neutrophils as compared with 0.054 ± 0.002 for the control, mean ± SEM, n = 6). Such data are in good agreement with previous reports demonstrating that, in contrast to eosinophils, neutrophils alone have only a small capacity to synthesize LTC₄.²¹ Addition of platelets to the suspension resulted in little alteration in sulfidopeptide leukotriene biosynthesis induced by FMLP alone (0.042 ± 0.005 pmol/10⁶ neutrophils LTE₄ equivalents), but a slight increase in these products was observed when neutrophil/platelet suspensions were stimulated with opsonized zymosan (0.182 ± 0.037 pmol/10⁶ neutrophils LTE₄). The combination of FMLP (10⁻⁴ mol/L) and opsonized zymosan added to a mixed suspension of neutrophils and platelets resulted in a substantial increase in sulfidopeptide leukotriene biosynthesis, however (0.70 ± 0.20 pmol/10⁶ neutrophils), as compared with neutrophils stimulated in the absence of platelets (0.18 ± 0.03 pmol/10⁶ neutrophils). Furthermore, a dose-dependent increase occurred in production of sulfidopeptide leukotrienes from 10⁻⁸ to 10⁻⁴ mol/L FMLP when opsonized zymosan and platelets were present in the incubation system. Indeed, even at 10⁻⁸ mol/L FMLP (with opsonized zymosan and platelets) there appeared to be greater than threefold enhancement in production of sulfidopeptide leukotrienes as compared with that obtained without FMLP present.

Influence of recipient cell activation on metabolism of LTA₄ into LTC₄. The experiments so far described resulted in data that could not rule out the additional possibility that metabolism of LTA₄ generated from neutrophils also required activation of endothelial cells or platelets, even though the glutathione-S-transferases are not believed to require activation.²² Accordingly, the capacity of platelets and endothelial cells (as recipient cells) to metabolize exogenous LTA₄ into LTC₄ was examined in the presence of increasing concentrations of thrombin, which is known to activate both platelets and endothelial cells, but not neutrophils.²³ Addition of thrombin to platelets induced their activation, as reflected by a dose-related production of TXB₂. Addition of a constant amount of LTA₄ 10 minutes after thrombin stimulation, however, was accompanied by LTC₄ production independent of the presence of thrombin (Fig 3A). Similar results were obtained with endothelial cells, in which 6-keto-PGF₁α production was dependent on the concentration of thrombin, whereas LTC₄ production was not affected (Fig 3B).

In the control experiments, production of LTC₄ from endothelial cells after addition of LTA₄ was evaluated by immunoreactivity and HPLC chromatography. A major peak of immunoreactivity eluting from the HPLC at the time expected for LTC₄ was evaluated by UV spectroscopy. In experiments using 5 μmol/L LTA₄ incubated with endothelial cells, quantitation of LTC₄ production measured by EIA was 210 pmol/mL and LTC₄ production measured by HPLC techniques was 230 pmol/mL. The EIA technique could detect 0.2 pmol/mL LTC₄, but the HPLC techniques were sensitive only for quantities greater than 20 pmol/mL. By the HPLC techniques, however, there was no evidence for LTD₄ or LTE₄. Although the production of LTC₄ by endothelial cells was in good agreement with results of a previous report,¹ we could find no evidence for the occurrence of TXB₂, LTD₄, or LTE₄ as reported by other investigators.⁸ Additional experiments were performed with endothelial cells.
cultured in 20% FBS, a concentration used by other investigators; however, identical results were obtained. Addition of 3H-LTC₄ to these high-serum-cultured cells did not show enhanced degradation to 3H-LTD₄ or 3H-LTE₄ during the incubation period.

The influence of LTA₄ concentration on formation of TXA₂ after thrombin activation was also evaluated. In these experiments, platelets were preincubated with LTA₄ at different concentrations for 10 minutes at 37°C. Thrombin was then added to platelets for another 3-min incubation period. Analysis of the supernatant (Table 1) indicated that LTC₄ production was linearly related to the concentration of LTA₄ added to the platelet incubations, as expected from previous experiments. Furthermore, thrombin-induced formation of TXA₂ (as measured by TXB₂) was not affected by previous exposure of the platelet to LTA₄. Only at the highest concentrations of LTA₄ (10 μmol/L) did there appear to be a decrease in thromboxane production.

### DISCUSSION

The finding that both endothelial cells and platelets have the capacity to metabolize LTA₄ into LTC₄ is of potential interest in relationship to the interaction of the vessel wall with blood cells under pathologic circumstances as well as in the pharmacologic effects of the peptidoleukotrienes on vessel tone and the alteration of vascular permeability. In our previous report, we showed that platelets efficiently metabolized LTA₄ generated from neutrophils by action of the calcium ionophore A23187. This nonphysiologic stimulus had previously been used to demonstrate transcellular cooperation in production of LTB₄ or LTC₄ by neutrophil-erythrocyte and neutrophil-endothelial cell interactions. The concentrations of leukotrienes achieved using the calcium ionophore were difficult to reconcile with the low production of arachidonate products obtained using receptor-specific inducer of neutrophil activation, however.

Direct evidence for transcellular metabolism of LTA₄ from neutrophils to platelets following a more relevant stimulus was illustrated by studies with platelets prelabeled with 35S-cysteine (Fig 1). Platelets possess a substantial concentration of endogenous glutathione that can be labeled by 35S-cysteine, and the observation of 35S-labeled LTC₄ resulting from these experiments could therefore be attributed to conversion of neutrophil-derived LTA₄ into LTC₄ using the glutathione in the platelets. Identification of transcellular-derived LTC₄ was made by HPLC coelution of 35S-radiolabeled with a 280-nm absorbing component at the LTC₄ retention time as well as immunoreactivity for LTC₄ by an EIA at the same retention time. Reutilization of 35S-cysteine was prevented by addition of excess unlabeled cysteine in the reaction mixture. Metabolism of LTA₄ in the medium by glutathione transferase (and glutathione) released from lysed platelets was not believed to be a factor because the glutathione concentrations would have been too low (even if all the platelets had lysed). Finally, uptake of labeled glutathione into the neutrophils was rendered unlikely because of the concentration issue described above and because this molecule does not readily penetrate cell membranes.

The subsequent metabolism and degradation of LTC₄ by activated neutrophils, which might have reduced the estimate of sulfidopeptide leukotrienes produced (Fig 1), has already been reported by other investigators. Although platelets were used throughout this study as a model for cellular interactions with neutrophils, endothelial cells appeared to share with platelets some common features of transcellular metabolism of LTA₄ into LTC₄.

In the present study, opsonized zymosan and FMLP alone initiated production of only low levels of leukotrienes in the presence of recipient cells such as platelets. The dose of FMLP used was sufficient to elicit an oxygen burst in the neutrophil, and in parallel experiments consistently does so. The results are in keeping with the low levels of eicosanoids that are generally produced by neutrophils stimulated in this fashion. Accordingly, a combination of the two stimuli was used. In part, the rationale for this approach derived from our previous work on the synergism of different stimuli in their interaction with neutrophils—the “priming” phenomenon. Seeger et al reported the combined effect of C5a and complement component C8 on LTB₄ generation by human neutrophils. In addition, we wished to examine the effects of these two commonly used activating agents because of the likely occurrence of mixed stimuli in vivo, especially mixes of chemotactic agents and phagocytosable particles. With these two stimuli, an increase of LTC₄ biosynthesis was achieved when platelets were included in the incubation mixture with the neutrophils. This observation further supports a possible occurrence of such transcellular events in vivo. Although the amount of LTC₄ obtained with opsonized zymosan and FMLP (~0.5 to 1 pmol/10⁶ neutrophils) was much lower than that achieved with the calcium ionophore (nearly 100 pmol/10⁶ neutrophils from A23187), these nanomolar concentrations are nonetheless compatible with significant biological activity. For example, the sulfidopeptide leukotrienes LTC₄ at 10⁻⁹ mol/L has been reported to reduce coronary blood flow significantly in isolated guinea pig heart. Our results indicate (Fig 2) that four to six times this concentration is produced in these in vitro incubations.

That conversion of LTA₄ into LTC₄ was unaffected by the degree of activation of the recipient platelets or endothelial cells is in good agreement with what is known of other

### Table 1. Influence of LTA₄ Exposure to Platelets on TXB₂ and LTC₄ Formation Before Thrombin Activation

<table>
<thead>
<tr>
<th>LTA₄ (μmol/L)</th>
<th>LTC₄ (pmol/mL)</th>
<th>TXB₂ (pmol/mL)</th>
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LTA₄ was added to platelets (0.5 mL at 0.2 × 10⁶ cells/mL) for 10 minutes. Thrombin (0.1 U/mL) was then added, and the system incubated for 3 minutes. Results are the mean of two different experiments with two different donors.
glutathione transferases.22 The description of a platelet function perhaps described as parallel to activation is also worth emphasis because most other capacities of these cells are closely related to their reactivity to stimuli. It also indicates that aggregated platelets (including those concentrated locally in a thrombus) are likely to retain their capacity to metabolize LTA₄, which could be released by a nearby neutrophil. Furthermore, we showed that transcellular production of LTC₄ can occur with aspirin-treated platelets. Transcellular biosynthesis of vasoconstictor LTC₄ from neutrophil LTA₄ can thus occur within platelets that can no longer synthesize thromboxane owing to cyclooxygenase inhibition, but such platelets still retain capacity to produce a vasoconstictor.

The interactions of neutrophils with endothelial cells and/or platelets may represent an important source of LTC₄ production in the systemic circulation. Thus, LTC₄ production by the latter cells may be as quantitatively important as that which is elicited from eosinophils, mast cells, or mononuclear phagocytes.31 Neutrophils, platelets, and endothelial cells clearly interact with each other in vivo. The reality of the proposed transcellular biosynthetic events under such pathophysiologic circumstances has not been clearly demonstrated, however. Likely possibilities may include aspirin-induced asthma, in which platelets are believed to be involved, myocardial infarction,33 or events associated with inflammatory reactions, in which platelets have been suggested to contribute as helper cells to neutrophil-dependent reactions.34

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