Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1349–1353, March 1986
Cell Biology

Erythrocyte–neutrophil interactions: Formation of leukotriene B_4 by transcellular biosynthesis

(cell–cell communication/leukotriene A_4/epoxide hydrolase "suicide inactivation"/inflammation)

J. E. McGee and F. A. Fitzpatrick†

Lipids Research, The Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49007

Communicated by Sune Bergström, October 21, 1985

ABSTRACT **Studies on the mechanism of leukotriene B_4 biosynthesis in suspensions composed of neutrophils plus erythrocytes indicate that human erythrocytes convert neutrophil-derived leukotriene A_4 into leukotriene B_4. Leukotriene B_4 formation by neutrophils in the presence of erythrocytes exceeded that from corresponding suspensions of neutrophils alone. The increase was proportional to the erythrocyte content of the suspension. The erythrocyte-dependent increase in leukotriene B_4 biosynthesis did not equal the arithmetic sum of calcium ionophore-dependent biosynthesis by neutrophils plus calcium ionophore-dependent biosynthesis by erythrocytes, since erythrocytes produced no leukotriene B_4 upon incubation with ionophore A23187. Erythrocytes did not stimulate 5-lipoxygenase activity within neutrophils, since the erythrocyte effect was confined to enzymatic hydration: leukotriene B_4 increased coincident with decreased formation of 5,12-dihydroxyicosatetraenoic acids derived from nonenzymatic hydration. Biosynthesis of leukotriene B_4 within the erythrocyte, from neutrophil-derived leukotriene A_4, was established by comparing the effect of normal erythrocytes with erythrocytes containing a leukotriene A_4 hydrolase that was inactivated by the substrate. In the latter case, leukotriene B_4 formation increased by only 30–40%; in the former case, it increased by 100–200%. Transcellular biosynthesis of leukotriene B_4 from erythrocyte–neutrophil interactions (*i*) explains the paradoxical presence of leukotriene A_4 hydrolase within erythrocytes, a cell incapable of synthesizing leukotriene A_4; (*ii*) affords a mechanism to overcome rate limitations or "suicide inactivation" of leukotriene A_4 hydrolase in neutrophils; (*iii*) exploits a cryptic capacity within erythrocytes, provisionally dormant cells in terms of icosanoid biosynthesis; (*iv*) indicates that the biosynthetic capacity of cell combinations is not necessarily equivalent to the sum of their separate capacities.**

Enzymatic cooperation between separate cell types may be a significant, but neglected, mechanism for the biosynthesis of certain prostaglandins or leukotrienes. This concept originated from studies on platelet–endothelial cell interactions, which showed that prostaglandin I_2 (PGI_2), an antithrombotic icosanoid, was produced by endothelial cells from platelet-derived prostaglandin endoperoxide H_2 (1–5). Analogous transcellular biosynthetic processes are conceivable for other cell combinations and for other arachidonic acid metabolites; however, few biologically relevant examples exist. Requirements for this process include "acceptor" cells with a constitutive enzymatic capacity for the formation of a biologically active icosanoid product but a deficient, or disabled, capacity for substrate generation, plus auxiliary "donor" cells that can fulfill that corresponding substrate requirement. In this context, neutrophil–erythrocyte interactions attracted our attention as potential sources for transcellular biosynthesis of leukotrienes (6).

Ordinarily, biosynthesis of leukotriene B_4 (LTB_4) occurs within an individual cell type via sequential enzymatic reactions including phospholipase-dependent liberation of arachidonic acid, regioselective 5-lipoxygenation, rearrangement into leukotriene A_4 (LTA_4), and enzymatic hydration of LTA_4 by an epoxide hydrolase to yield LTB_4, a biologically active diol[(5*S*,12*R*)-5,12-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid] (7). Spontaneous nonenzymatic hydration yielding two inactive 5,12-dihydroxyicosatetraenoic acid (5,12-diHETE) isomers competes with the enzymatic hydration step. Conceptually, biosynthesis of LTB_4 could occur by a similar enzymatic pathway even if different parts of the enzymatic sequence were segmented within two separate cell types. Neutrophils and erythrocytes in combination possess the enzymatic attributes appropriate for such transcellular biosynthesis of LTB_4. For instance, neutrophils are a prominent source of the substrate, LTA_4, and they secrete it, intact, because of rate limitations in their capacity for LTB_4 formation (8, 9). Erythrocytes, by contrast, lack a demonstrable capacity for biosynthesis of LTA_4, yet they have a paradoxical capacity to convert it, when available, into LTB_4 (6). We aimed to determine whether human erythrocytes could produce the chemotactic myotropic agent LTB_4 from neutrophil-derived LTA_4.

MATERIALS AND METHODS

Materials. Synthetic LTA_4 methyl ester (Upjohn) was purified prior to use (10); LTA_4 lithium salt was prepared as described (11). Synthetic leukotrienes and prostaglandin B_1 (PGB_1; Upjohn) and microcrystalline cellulose, α-cellulose, and calcium ionophore A23187 (Sigma) were used as received. Water, ethyl acetate, and methanol, all distilled in glass (Burdick and Jackson, Muskegon, MI), and C_{18} reversed-phase HPLC (RP-HPLC) columns (250 × 6 × 4 mm) (Rainen Instruments, Woburn, MA) were used for metabolite isolation and quantification.

Preparation of Purified Human Erythrocyte Suspensions. Human venous blood (50 ml) collected in sterile 3.8% (wt/vol) sodium citrate (5.5 ml) was centrifuged at 200 × *g* for 30 min, and the platelet-rich plasma was removed. Sedimented erythrocytes, leukocytes, and platelets were resuspended in sterile 0.9% (wt/vol) NaCl (50 ml) and centrifuged at 200 × *g* for 20 min. The supernatant was discarded. After two washes the cell suspension was depleted of residual leukocytes and platelets by filtration through columns containing microcrystalline cellulose and α-cellulose (1:1, wt/wt) (12). Erythrocytes were resuspended at 2×10^9 cells per ml. The purified erythrocyte suspension contained $\leq 5 \times 10^4$ leuko-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LTB_4 and LTA_4, leukotriene B_4 and A_4; diHETE, dihydroxyicosatetraenoic acid; PGI_2 and PGB_1, prostaglandin I_2 and B_1; RP-HPLC, reversed-phase HPLC.

*To whom reprint requests should be addressed.

cytes per ml and $\leq 5 \times 10^6$ platelets per ml. Differential cell counts were determined with a Coulter Diffz system.

Isolation of Human Polymorphonuclear Neutrophils. Polymorphonuclear neutrophils were isolated from human whole blood by dextran sedimentation, centrifugation over Ficoll/Hypaque (Sigma) gradients, and hypotonic lysis (13). Neutrophils were resuspended (2×10^7 cells per ml) in Hanks' balanced salt solution (HBSS), 0.02 M Hepes buffer, pH 7.2, with 1.4 mM $CaCl_2$ and 0.8 mM $MgCl_2$.

Transcellular Metabolism of Neutrophil-Derived LTA_4. Neutrophils alone (1.5 ml, 2×10^7 cells per ml), erythrocytes alone (1.5 ml, 2×10^9 cells per ml), or neutrophils plus erythrocytes (1.5 ml, 2×10^7 neutrophils per ml, 1×10^9 erythrocytes per ml) were incubated at 37°C for 5 min, then calcium ionophore A23187 (5 μM) was added to stimulate leukotriene biosynthesis. After 2.5 min at 37°C biosynthesis was quenched by adding ethyl acetate (5.0 ml) containing PGB_1 (0.50 μg) as a quantitative internal standard for RP-HPLC. LTB_4 and related diols were extracted three times with 5 ml of ethyl acetate each time. Solvent was evaporated and the residue was reconstituted in methanol/water (70:30, vol/vol) (1.0 ml). Leukotrienes were determined by RP-HPLC (7). To measure erythrocyte concentration dependence, the neutrophil concentration was fixed at 16×10^6 cells per ml while the erythrocyte concentration was varied between 0 and 0.8×10^9 cells per ml. To establish neutrophil concentration dependence, the neutrophil content was varied from 0 to 20×10^6 cells per ml in the presence or absence of a fixed erythrocyte content (0.8×10^9 cells per ml). For kinetic studies samples were quenched at intervals between 0 and 30 min.

"Suicide Inactivation" of Erythrocyte LTA_4 Epoxide Hydrolase. Erythrocytes (1.0 ml, 2×10^9 cells per ml) were incubated with 30 μM LTA_4 for 2.0 min at 37°C. Under these conditions the epoxide hydrolase inactivates itself. Erythrocytes were then washed twice with 10 ml of 0.9% (wt/vol) NaCl containing human serum albumin at 25 mg/ml to sequester metabolites of synthetic LTA_4. The content of LTB_4 in the supernatant was determined as described (6), and the erythrocytes were resuspended in 0.9% (wt/vol) NaCl (2×10^9 cells per ml). The extent of suicide inactivation of LTA_4 epoxide hydrolase in erythrocytes was determined by comparing LTB_4 biosynthesis from control erythrocytes incubated with 10 μM LTA_4 to LTB_4 biosynthesis from suicide-inactivated erythrocytes incubated with 10 μM LTA_4. Transcellular metabolism of LTA_4 from neutrophils stimulated with 5 μM A23187 was characterized as described above. Control erythrocytes were compared to erythrocytes with suicide-inactivated LTA_4 epoxide hydrolase to establish the involvement of erythrocytes as acceptor cells for LTA_4 donated by neutrophils.

RESULTS

Human neutrophils stimulated with calcium ionophore A23187 produced three 5,12-diol metabolites of LTA_4, including the enzymatic hydration product LTB_4 and the two nonenzymatic products (5*S*,12*R*)-5,12-dihydroxy-6,8,10-*trans*-14-*cis*-icosatetraenoic acid (peak I) and (5*S*,12*S*)-5,12-dihydroxy-6,8,10-*trans*-14-*cis*-icosatetraenoic acid (peak II) (Fig. 1*A*), consistent with the established characteristics of these cells (7). Human erythrocytes incubated with A23187 did not produce detectable amounts of these or other leukotrienes, confirming that erythrocytes alone did not synthesize LTA_4 from endogenous arachidonic acid (Fig. 1*C*). When erythrocytes and neutrophils together were incubated with A23187, LTB_4 biosynthesis increased with a corresponding decrease in the formation of nonenzymatic isomers I and II, relative to suspensions of neutrophils alone (Fig. 1*B*). Ten separate experiments gave equivalent chromatographic profiles. Quantitative and qualitative results were confirmed by UV spectrophotometry, radioimmunoassay, and bioassay. Results were descriptively similar for stimuli other than calcium ionophore A23187. For instance,

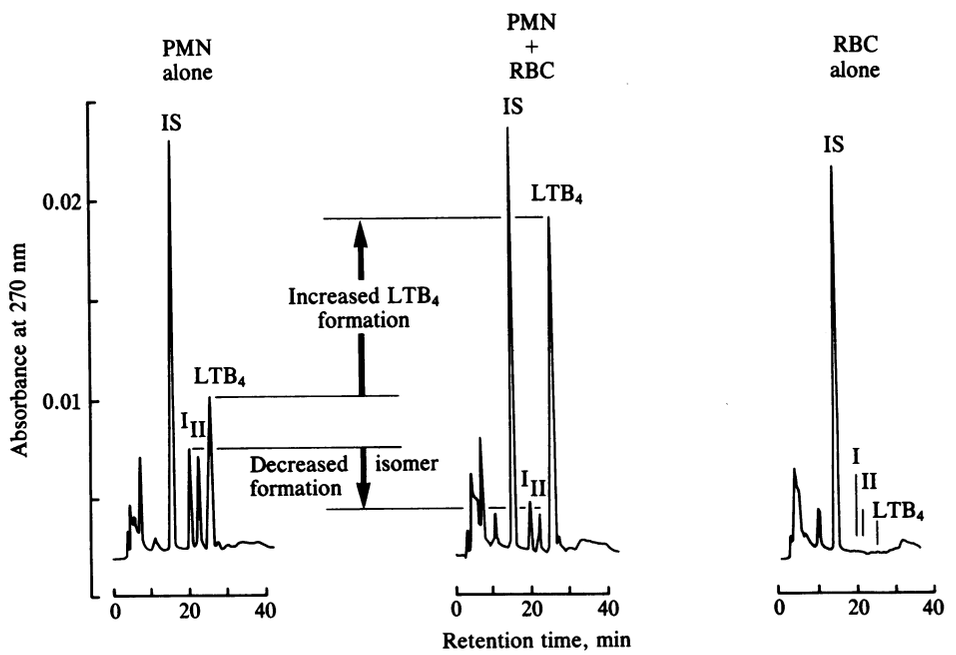

FIG. 1. RP-HPLC analysis of LTB_4 and 5,12-diHETE (isomers I and II) biosynthesis by neutrophils and erythrocytes. Neutrophils (PMN) were 2×10^7 per ml; erythrocytes (RBC) were 1×10^9 per ml. Cells were incubated at 37°C for 5 min, then calcium ionophore A23187 (5 μM) was added to stimulate leukotriene biosynthesis for 2.5 min at 37°C. Biosynthesis was quenched with ethyl acetate (5.0 ml) containing PGB_1 (0.5 μg) as a quantitative internal standard (IS) for RP-HPLC. Peaks designated I and II are 5,12-diHETE isomers formed by nonenzymatic hydration of LTA_4. LTB_4 is formed by enzymatic hydration. LTB_4 production increased and nonenzymatic hydration decreased, relative to neutrophils alone, when erythrocytes were mixed with neutrophils. Equivalent profiles were obtained from 10 separate donors. Qualitative and quantitative results were confirmed by UV spectrophotometry, bioassay, and radioimmunoassay for LTB_4.

human neutrophils (2×10^7 cells per ml) incubated for 5 min at 37°C with 0.2 μM formylmethionylleucylphenylalanine (fMet-Leu-Phe) produced 4 pmol of LTB_4 in the absence of erythrocytes and 54 pmol of LTB_4 in the presence of 0.8×10^9 erythrocytes per ml. Erythrocytes alone, incubated with 2 μM fMet-Leu-Phe, produced no detectable hydration products of LTA_4.

LTB_4 biosynthesis was directly proportional to the neutrophil content of cell suspensions in the presence or absence of erythrocytes (Fig. 2). However, LTB_4 biosynthesis in suspensions containing neutrophils and erythrocytes together consistently exceeded that from a corresponding suspension with neutrophils alone. For 2, 5, 10, 15, and 20×10^6 neutrophils per ml, LTB_4 biosynthesis increased 76 ± 10%, 87 ± 10%, 109 ± 15%, 156 ± 17%, and 202 ± 30% (mean ± SEM, $n = 7$), respectively, in the presence of 0.8×10^9 erythrocytes per ml.

Fig. 3 depicts the relative hydration of neutrophil-derived LTA_4 as a function of erythrocyte concentration. For a constant neutrophil concentration, enzymatic conversion of LTA_4 into LTB_4 increased in proportion to the erythrocyte concentration in the suspension. Neutrophils alone produced 0.49 ± 0.044 nmol of LTB_4 (mean ± SEM, $n = 15$) compared to 0.67 ± 0.05, 0.77 ± 0.06, 0.94 ± 0.09, 1.03 ± 0.10, and 1.10 ± 0.10 nmol of LTB_4 in the presence of 16, 32, 48, 64, and 80×10^7 erythrocytes per ml, respectively. The erythrocyte-dependent increases in LTB_4 biosynthesis were statistically significant ($P \leq 0.01$). Erythrocytes alone produced no detectable LTB_4 or related metabolites, confirming, again, that the increases were not attributable to ionophore-dependent production by separate cell types. Nonenzymatic hydration of LTA_4, reflected by the formation of 5,12-diHETE isomers I and II, was inversely proportional to the erythrocyte concentration (Fig. 3).

Results in Figs. 1–3 suggested that the erythrocyte-dependent increase in LTB_4 biosynthesis occurred by transcellular metabolism of LTA_4 derived from neutrophils. A distinctive feature of the erythrocyte epoxide hydrolase that catalyzes LTB_4 formation allowed a rigorous test of this proposal. Specifically, LTA_4 epoxide hydrolase in erythrocytes is susceptible to suicide inactivation (14). Our model for transcellular metabolism predicts that suicide inactivation of epoxide hydrolase by exposure of erythrocytes to synthetic LTA_4 would diminish their capacity to produce LTB_4 upon subsequent exposure to neutrophil-derived LTA_4. Our results confirm this prediction. Neutrophils alone incubated with 5 μM A23187 produced 0.606 ± 0.040 nmol of LTB_4 (mean ± SEM, $n = 8$); neutrophils plus autologous control erythrocytes produced 1.29 ± 0.060 nmol of LTB_4; and neutrophils plus autologous erythrocytes with an epoxide hydrolase that had been inactivated by prior exposure to 10 μM LTA_4 produced 0.83 ± 0.050 nmol of LTB_4. Thus, the relative increase in LTB_4 biosynthesis was 0.68 nmol of LTB_4 (Fig. 4), or 112%, with normal erythrocytes, which may be compared to 0.22 nmol of LTB_4, or 37%, with erythrocytes containing suicide-inactivated LTA_4 hydrolase. Control experiments with two successive additions of synthetic LTA_4 confirmed that erythrocytes were suicide inactivated by the first addition of synthetic LTA_4 (Fig. 4). It was noteworthy that nonenzymatic hydration products were relatively constant, or slightly increased, in suspensions of suicide-inactivated erythrocytes, indicating that the 5-lipoxygenase enzyme in neutrophils was not inactivated.

LTB_4 levels in suspensions containing erythrocytes remained high for at least 20 min. For example, at 5, 10, and 20 min, suspensions of neutrophils alone (1.5×10^7 cells per ml) contained 0.56, 0.46, and 0.21 nmol of LTB_4, which may be compared to 1.25, 1.25, and 0.87 nmol of LTB_4 in corresponding suspensions containing 6.4×10^8 erythrocytes per ml.

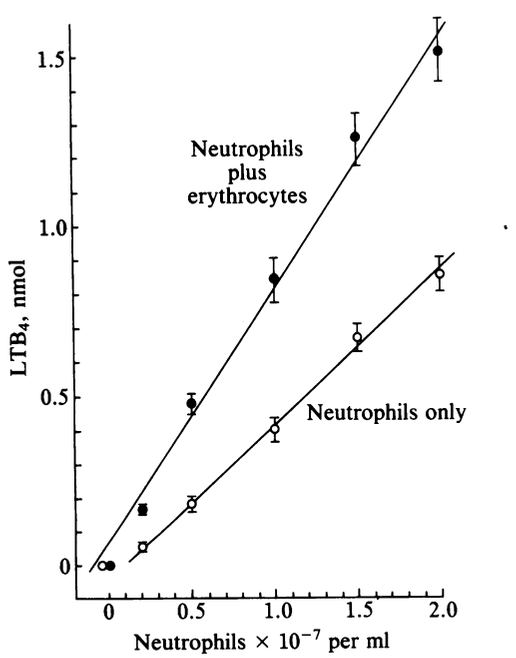

FIG. 2. Neutrophil concentration dependence of LTB_4 biosynthesis in the presence or absence of erythrocytes. Neutrophils (1.5 ml, 0–2 × 10^7 cells) plus or minus erythrocytes (0.8×10^9 cells) were incubated for 5 min at 37°C, then A23187 (5 μM) was added to stimulate leukotriene biosynthesis. After 2.5 min at 37°C samples were quenched and extracted as above, and LTB_4 biosynthesis (mean ± SEM, $n = 7$) in the presence (●) or absence (○) of erythrocytes was determined by RP-HPLC.

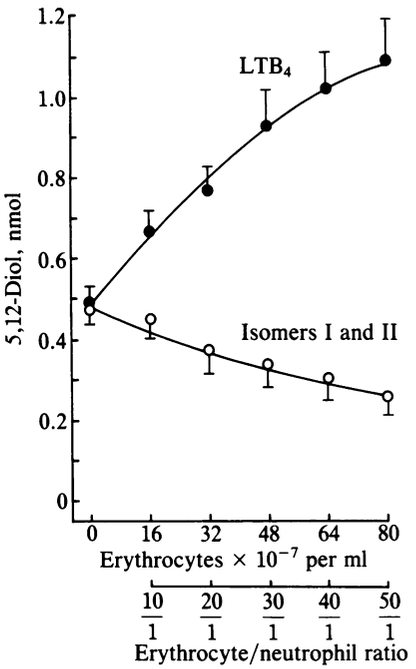

FIG. 3. Leukotriene biosynthesis as a function of erythrocyte content in neutrophil/erythrocyte suspensions. Neutrophils (1.0 ml, 2×10^7 cells per ml) were mixed with variable amounts of erythrocytes (0–0.8×10^9 cells per ml) and suspensions were stimulated with 5 μM A23187 to initiate leukotriene biosynthesis. Values represent the mean ± SEM ($n = 15$) of LTB_4, isomer I, and isomer II formation. Neutrophils alone produced 0.49 ± 0.05 nmol of LTB_4. Erythrocytes alone produced no detectable LTB_4 or diHETE isomers.

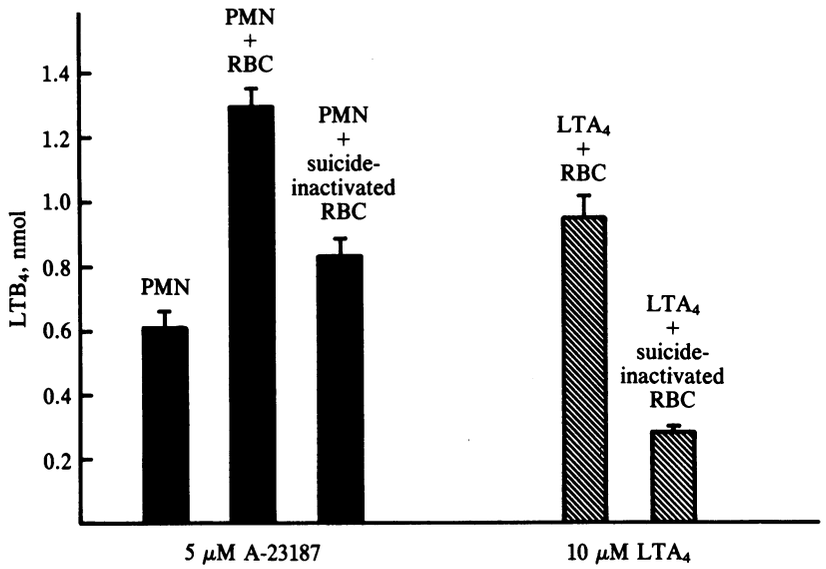

FIG. 4. Comparative transcellular metabolism of neutrophil-derived LTA_4 by normal erythrocytes and erythrocytes with suicide-inactivated LTA_4 epoxide hydrolase. Neutrophils (PMN) (1.0 ml, 2×10^7 cells per ml) were mixed with control erythrocytes (RBC) (0.5 ml, 2×10^9 cells per ml) or erythrocytes containing a suicide-inactivated LTA_4 epoxide hydrolase. Suspensions were stimulated with 5 μM A23187 to initiate leukotriene biosynthesis. The three solid bars indicate LTB_4 biosynthesis (mean ± SEM, $n = 6$) by neutrophils alone, neutrophils plus control erythrocytes, or neutrophils plus suicide-inactivated erythrocytes. The two shaded bars are controls that indicate the relative enzymatic hydration of LTA_4 by erythrocytes from two successive incubations with synthetic LTA_4 (10 μM).

DISCUSSION

Collectively, these results demonstrate that transcellular biosynthesis of LTB_4 occurs along an erythrocyte–neutrophil axis. Neutrophils can produce and release both LTB_4 and LTA_4; erythrocytes can convert a portion of neutrophil-derived LTA_4 into additional LTB_4. Certain points support this conclusion. First, the erythrocyte-dependent increase in LTB_4 biosynthesis did not originate from the arithmetic sum of ionophore stimulation of neutrophil arachidonic acid metabolism plus ionophore stimulation of erythrocyte arachidonic acid metabolism. Human erythrocytes, lacking detectable phospholipase A_2 and 5-lipoxygenase activity, did not produce LTB_4 or related LTA_4 metabolites upon stimulation with ionophore A23187. Second, the increase in LTB_4 formation did not originate from erythrocyte-dependent stimulation of neutrophil metabolism. If erythrocytes had stimulated arachidonic acid release and 5-lipoxygenase metabolism within the neutrophil, increased nonenzymatic formation of 5,12-diHETE isomers would have accompanied the increase in LTB_4 biosynthesis. This was not the case. Instead, the erythrocyte effect was confined to enzymatic hydration of LTA_4; LTB_4 formation increased at the expense of nonenzymatic hydration. Third, a role for biosynthesis of LTB_4 by erythrocytes was established by comparing normal erythrocytes with erythrocytes whose LTA_4 epoxide hydrolase had been inactivated by the substrate. Erythrocytes with a disabled LTA_4 epoxide hydrolase did not augment LTB_4 biosynthesis as effectively as normal erythrocytes did. In view of these points, and the fact that neutrophils are the exclusive source of LTA_4 under our experimental conditions, one may conclude that erythrocytes convert neutrophil-derived LTA_4 into LTB_4.

Our results on erythrocyte–neutrophil interactions and transcellular biosynthesis provide one plausible explanation for the otherwise paradoxical presence of LTA_4 epoxide hydrolase within human erythrocytes. Furthermore, transcellular biosynthesis of LTB_4 affords a mechanism to supersede rate limitations or suicide inactivation within the neutrophil, and to recruit a provisionally inactive cell, the erythrocyte, for active contributions to leukotriene formation. The cellular and enzymatic traits that permit transcellular biosynthesis from erythrocyte–neutrophil interactions also facilitated a rigorous evaluation of the hypothesis. These traits include the compartmentalization of phospholipase and 5-lipoxygenase activities within neutrophils but not erythrocytes, the susceptibility of LTA_4 hydrolase to suicide inactivation, and the hydration of LTA_4 by both enzymatic and nonenzymatic processes. These or comparable traits may be useful for identifying other model systems of cell–cell interactions.

It is interesting to compare erythrocyte–neutrophil interactions with existing examples of transcellular metabolism of icosanoids. For instance, platelet–neutrophil interactions can also increase LTB_4 biosynthesis and form (5*S*,12*S*)-diHETE and 12,20-diHETE (15–18) products of uncertain biological significance. In contrast, neutrophil–erythrocyte interactions increase only LTB_4, a leukotriene with chemotactic and myotropic effects (19). Platelet–endothelial cell interactions yield PGI_2, an antithrombotic substance, but proofs that PGI_2 originates from platelet-derived prostaglandin H_2 have depended upon the use of pharmacological agents to modify platelet thromboxane synthetase or endothelial cell cyclooxygenase. In contrast, a transcellular origin for LTB_4 and a need for such an origin can be established exclusively in terms of cellular, enzymatic, and chemical attributes of erythrocyte and neutrophil LTA_4 hydrolase. The biological significance of transcellular metabolism remains to be established, but our results support Marcus' advocacy for cell–cell interactions as an important but novel form of icosanoid biosynthesis (3, 16). Since cell combinations encountered in disease may differ from those encountered in normal circumstances the study of transcellular metabolism assumes some significance (20).

An important point from our results is that the biosynthetic capacity achieved by combining separate cell types is not simply the quantitative or qualitative sum of their individual capacities. In the extreme case represented, cells such as erythrocytes with "zero" capacity alone can contribute substantially when their constitutive biosynthetic capacity is allied with that of another cell type. This phenomenon is not necessarily restricted to erythrocytes and neutrophils. Furthermore, this type of cell–cell interaction is not necessarily restricted to icosanoids. The results for leukotrienes may

have heuristic value for studies of cell–cell interactions in other biosynthetic contexts.

1. Bunting, S., Gryglewski, R., Moncada, S. & Vane, J. R. (1976) *Prostaglandins* **12,** 897–913.
2. Marcus, A., Weksler, P., Jaffe, E. & Broekman, J. (1980) *J. Clin. Invest.* **66,** 979–986.
3. Marcus, A., Broekman, M. J., Weksler, B., Jaffe, E., Safier, L., Ullman, H. & Tack-Goldman, K. (1981) *Phil. Trans. R. Soc. London Ser. B* **294,** 343–353.
4. Needleman, P., Wyche, A. & Raz, A. (1979) *J. Clin. Invest.* **63,** 345–349.
5. Hornstra, G., Haddeman, E. & Don, J. (1979) *Nature (London)* **279,** 66–68.
6. Fitzpatrick, F., Liggett, W., McGee, J., Bunting, S., Morton, D. & Samuelsson, B. (1985) *J. Biol. Chem.* **259,** 11403–11407.
7. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3213–3217.
8. Radmark, O., Malmsten, C., Samuelsson, B., Goto, G., Marfat, A. & Corey, E. J. (1980) *J. Biol. Chem.* **255,** 11828–11831.
9. Sun, F. & McGuire, J. (1984) *Biochim. Biophys. Acta* **794,** 56–64.
10. McKay, S., Mallern, D., Shrubsall, P., Smith, J., Baker, S., Jamieson, W., Ross, W., Morgan, S. & Rackham, D. (1981) *J. Chromatogr.* **214,** 249–256.
11. Fitzpatrick, F., Morton, D. & Wynalda, M. (1982) *J. Biol. Chem.* **257,** 4680–4683.
12. Beutler, E., West, C. & Blume, K.-G. (1976) *J. Lab. Clin. Med.* **88,** 328–333.
13. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest. Suppl.* **97,** 77–89.
14. McGee, J. & Fitzpatrick, F. (1985) *J. Biol. Chem.* **260,** 12832–12837.
15. Marcus, A., Broekman, M., Safier, L., Ullman, H. & Islam, N. (1982) *Biochem. Biophys. Res. Commun.* **109,** 130–137.
16. Marcus, A., Safier, L., Ullman, H., Broekman, M., Islam, N., Oglesby, T. & Gorman, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 903–907.
17. Borgeat, P., Fruteau de Laclos, B., Picard, S., Drapeau, J., Vallerand, P. & Corey, E. J. (1982) *Prostaglandins* **23,** 713–724.
18. Wong, P., Westlund, P., Hamberg, M., Granstrom, E., Chao, P. & Samuelsson, B. (1984) *J. Biol. Chem.* **259,** 2683–2686.
19. Samuelsson, B. (1983) *Science* **220,** 568–575.
20. Needleman, P., Wyche, A., Bronson, S., Holmberg, S. & Morrison, A. (1982) *J. Biol. Chem.* **254,** 9772–9777.