Eicosanoid Transcellular Biosynthesis: From Cell-Cell Interactions to in Vivo Tissue Responses

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Abstract——The biosynthesis of the biologically active metabolites of arachidonic acid involves a number of enzymes that are differentially expressed in cells. Prostaglandins and thromboxanes are derived from the chemically unstable prostaglandin (PG) H_2 intermediate synthesized by PGH synthases (cyclooxygenase-1/2) and leukotrienes from chemically unstable leukotriene A_4 by 5-lipoxygenase. Additional enzymes transform these reactive intermediates to a variety of chemical structures known collectively as the lipid mediators. Although some cells have the complete cassette of enzymes required for the production of biolog-

ically active prostaglandins and leukotrienes, the actual biosynthetic events often are a result of cell-cell interaction and a transfer of these chemically reactive intermediates, PGH$_2$ and leukotriene A$_4$, between cells. This process has come to be known as transcellular biosynthesis of eicosanoids and requires a donor cell to synthesize and release one component of the biosynthetic cascade and a second, accessory cell to take up that intermediate and process each into the final biologically active product. This review focuses on the evidence for transcellular biosynthetic events for prostaglandins, leukotrienes, and lipoxins occurring during cell-cell interactions. Evidence for arachidonic acid serving as a transcellular biosynthetic intermediate is presented. Experiments for transcellular events taking place in vivo that reveal the true complexity of eicosanoid biosynthesis within tissues are also reviewed.

I. Introduction

Eicosanoids are potent arachidonate-derived mediators in both physiologic and pathophysiologic situations because of specific receptors that initiate signal transduction events that lead to coordinated cellular responses to specific stimuli. Transcellular biosynthesis of eicosanoids is one term that has been used to describe the dynamic situation taking place that results in the production of these active molecules through cooperation of multiple cells. Since the discovery of specific pathways for prostaglandins, thromboxanes, and leukotrienes, understanding of biosynthesis focused on identification of specific enzymes present within a single cell that could account for that cell's capacity to generate the biologically active eicosanoid starting from arachidonic acid stored in phospholipids to the release of the oxygenated metabolite product. However, shortly after the structure elucidation of each of the active eicosanoids as well as of each biochemical pathway involved in their synthesis, it was clear that production of eicosanoids within a tissue could take place in a more complex manner in which covalent transformations of arachidonate occurred in one cell type (donor cell) and then an intermediate was passed onto a second cell (acceptor cell) to complete the covalent formation into the biologically active mediator. Such complexity was unexpected, especially considering that in many cases the "eicosanoid intermediate" that was passing between cells was a chemically reactive intermediate, unlikely to exist outside of a protected membrane environment in which it was first synthesized. In addition, this was unexpected because in all cases the intermediates are highly lipophilic and would require some mechanism to facilitate their movement across phospholipid membrane barriers. Nonetheless, it became clear that this transfer of intermediates was a common event and could often significantly alter the biosynthetic spectrum of active products made by a tissue in response to a specific stimulus.

Several terms have emerged to describe this process of cell-cell interaction leading to eicosanoid production, including "endoperoxide shunting" and "endoperoxide steal," as well as the term used here, "transcellular biosynthesis." We will define this term based upon several criteria that have emerged from various experiments designed to study this process:

1. Two or more cells must occur in a tissue in vivo, in an appropriate ratio to each other, so that together they have the complete enzymatic cascade to generate a particular eicosanoid.
2. The donor cell must have the enzymatic activity to convert a cellular stimulus into free arachidonate or an arachidonate intermediate (precursor).
3. The acceptor cell must have the enzymatic capacity to convert the arachidonate intermediate into the biologically active eicosanoid but need not have the capacity to generate the initial precursor (intermediate).
4. The donor cell must be activated to begin this process, but the acceptor cell need not be.
5. Both donor and acceptor cells need specific mechanisms to export and import the highly lipophilic precursor and the acceptor cell must be able to release the active eicosanoid.

II. Prostaglandin Biosynthesis and Prostaglandin H$_2$

A. Biosynthesis Pathway

Prostaglandins are synthesized from arachidonic acid (Fig. 1), which is released from cellular phospholipids by cPLA$_{2\alpha}$,[1] then oxygenated by the enzyme cyclooxygenase. There are two cyclooxygenases expressed in various cells, cyclooxygenase-1 and cyclooxygenase-2, which are also called PGH synthase 1 and 2. These enzymes generate a reactive intermediate PGH$_2$ which has a reasonably long half-life (90–100 s) but is highly lipophilic. PGH$_2$ is converted into the biologically active prostaglandins by prostaglandin isomerases, yielding PGE$_2$, PGD$_2$, and PGF$_2$, or by thromboxane synthase to make

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[1] Abbreviations: cPLA$_{2\alpha}$, cytosolic phospholipase A$_{2\alpha}$; PG, prostaglandin; TX, thromboxane; HUVEC, human umbilical cord endothelial cell; CGS 13080, imidazo(1,5-2)pyridine-5-hexanoic acid; LT, leukotriene; HPLC, high-performance liquid chromatography; HpETE, hydroperoxyeicosatetraenoic acid; fMLP, N-formyl-L-methionyl-L-leucyl-L-phenylalanine; GM-CSF, granulocyte-macrophage colony-stimulating factor; E-FABP, epithelial fatty acid binding protein; LX, lipoxin; COX, cyclooxygenase; ATL, aspirin-triggered lipoxin; AEC, alveolar endothelial cell; AM, alveolar macrophage; HETE, hydroxyeicosatetraenoic acid; s, soluble; MK886, 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-tert-butylthioindol-2-yl]-2, 2-dimethylpropanoic acid; PNM, polymorphonuclear neutrophil; BAY X1005, (R)-2-[4-(quinolin-2-yl-methoxy)phenyl]-2-cyclopentyl acetic acid; cys-LT, cysteinyl leukotriene; Bay u9773, 6(R)-(4-carboxyphenylthio)-5(S)-hydroxy-7(E),9(E),11(Z),14(Z)-eicosatetraenoic acid; 5-LO, 5-lipoxygenase.
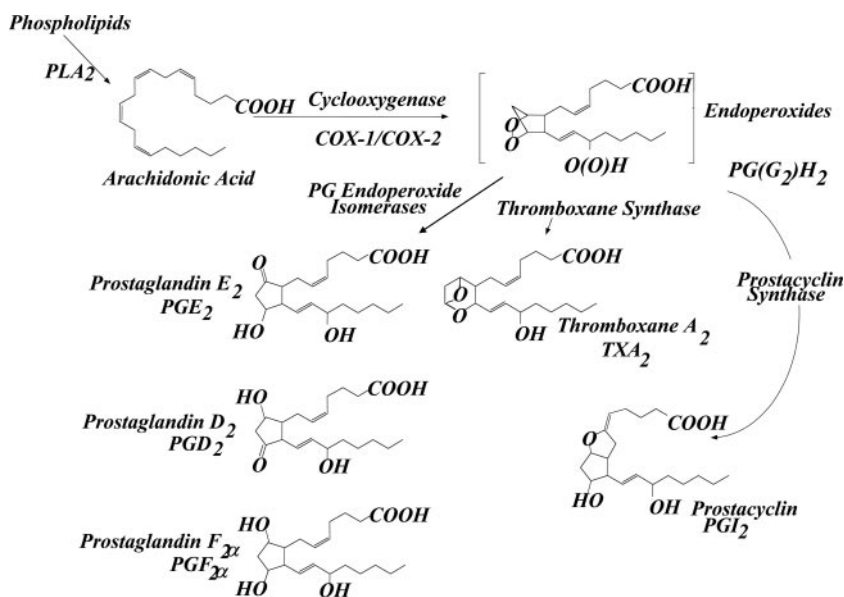
FIG. 1. Biosynthetic pathway of prostanoids from arachidonate-containing phospholipids and formation of biologically active prostacyclin, thromboxane, and prostaglandins.

TxA_2 or by prostacyclin synthase to make PGI_2. Most nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin inhibit both PGH synthase 1 and 2.

B. Platelet-Endothelial Cell Interactions

The first evidence for transcellular biosynthesis came from studies in which mesenteric artery rings from indomethacin-treated rabbits were incubated with human platelet-rich plasma followed by stimulation of the platelets with ADP (Bunting et al. 1976). There was no observed platelet aggregation, suggesting that PGH_2 from the platelet was being converted into PGI_2 by the indomethacin-treated arterial rings. This capacity, as measured by bioassay, suggested that the biochemical event to accept PGH_2 to make PGI_2 was found to be highest in the intimal layer of the arterial rings, consistent with a role for endothelial cells in converting platelet-derived PGH_2 into PGI_2 (Moncada et al., 1977). This experiment was repeated using purified human umbilical cord endothelial cells (HUVECs) treated with indomethacin that could also accept platelet-derived PGH_2 in activated platelet-rich plasma to generate PGI_2 (Weksler et al., 1978). Direct biochemical evidence for the ability of PGH_2 to be taken up by the endothelial cells to synthesize PGI_2 followed shortly, with the use of radiolabeled PGH_2 (Marcus et al., 1978). Platelet-derived PGH_2 was found as PGI_2 when platelets containing radioactive arachidonate were coincubated with aspirin-treated endothelial cells, followed by challenge with various agonists (Marcus et al., 1980). The aspirin-treated HUVECs did not make any PGI_2 measured as the 6-keto-$PGF_{1\alpha}$ metabolite, but when radiolabeled platelets were added to the aspirin-treated HUVECs, there was clear production of radiolabeled 6-keto-$PGF_{1\alpha}$ (Marcus et al., 1980).
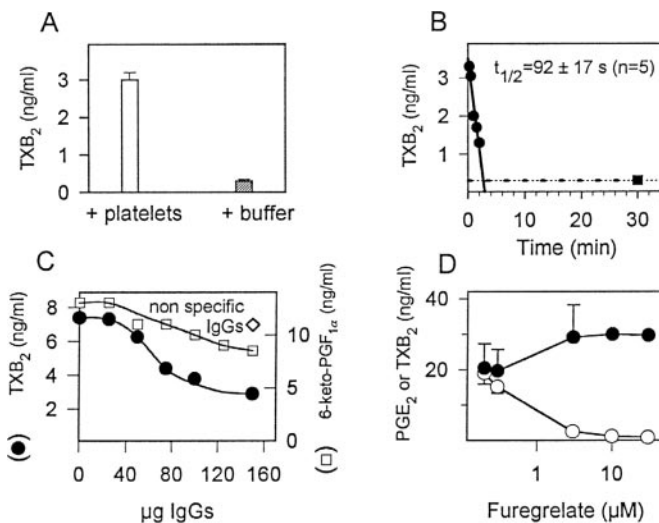
FIG. 2. Synthesis of thromboxane by aspirin-treated platelets from PGH_2 released from thrombin-stimulated endothelial cells. A, presence of a metabolic intermediate in the supernatant of thrombin stimulated endothelial cells. B, the supernatant was transferred to a polypropylene tube, and at the indicated times an aliquot was added to platelets and incubated for 2 min. The apparent half-life of the metabolic intermediate was calculated from the formation of thromboxane in each experiment ($n = 5$). C, IgGs directed against PGH_2 analog decreased the synthesis of thromboxane from a coincubation of platelets with endothelial cells. ●, coincubation and stimulation by thrombin in the presence of increasing amounts of IgGs directed against a stable PGH_2 mimic. Analysis of 6-keto-$PGF_{1\alpha}$ (□) was performed in the same samples. D, effect of a thromboxane synthase inhibitor on TxB_2 produced during coincubation of platelets with HUVECs. ○, TxB_2; ●, PGE_2. Reproduced with permission from the *Journal of Biological Chemistry* (Karin et al., 1996).

Complementary studies of platelet-endothelial cells were also carried out using aspirin-treated platelets coincubated with endothelial cells followed by thrombin treatment (Karim et al., 1996). These aspirin-treated platelets had no remaining activity of cyclooxygenase to make TxA_2 (measured as the stable metabolite TxB_2)

and, of course, no capacity to make PGH_2. However, after incubation with HUVECs and thrombin challenge, there was clear production of TxB_2 (Fig. 2). A transfer of the lipophilic PGH_2 between cells was suggested to occur so that platelet thromboxane synthase could convert endothelial PGH_2 into TxA_2. In time course studies, it was found that the capacity of thrombin-stimulated endothelial cells to induce production of TxB_2 in aspirin-treated platelets decreased with a half-life of 92 ± 17 s, consistent with the known half-life of PGH_2 (Karim et al., 1996). Similar studies extended the transcellular biosynthesis of prostaglandins in lymphocyte-HUVEC coincubations. In these studies, the transfer of lymphocyte-derived PGH_2 was demonstrated by the formation of PGI_2 (Merhi-Soussi et al., 2000).

C. In Vivo Transcellular Prostanoid Production

Direct evidence for PGH endoperoxide participation in transcellular biosynthesis in human subjects was obtained in studies of the production of TxA_2 and PGI_2 in a wound model in which a thromboxane synthase inhibitor was used to uniquely prevent the formation of TxA_2 by the platelet while blood was being taken. A small skin incision (normal blood collection) induced a platelet vascular interface known to stimulate platelets to synthesize PGH_2 and consequently, TxA_2 as well as endothelial cells to generate PGH_2 and subsequently PGI_2 (Nowak and FitzGerald, 1989). These active eicosanoids were measured in blood leaving the wound site as TxB_2 and 6-keto-$PGF_{1\alpha}$, respectively (Fig. 3). In untreated human subjects, the production of TxB_2 was quite high but after treatment with a thromboxane synthase inhibitor, the level of TxB_2 dropped precipitously. However, there was a significant increase in 6-keto-$PGF_{1\alpha}$ and PGE_2, most likely due to the diversion of platelet-derived PGH_2 into endothelial cells and transcellular biosynthesis into PGI_2 and PGE_2. Although, in this study the origin of the cells participating in this transcellular biosynthesis was unknown; a transcellular biosynthetic event was interpreted as operating in vivo.

III. Leukotrienes and Leukotriene A₄ Transfer

A. Biosynthesis Pathway

Leukotrienes are the products of the 5-lipoxygenase cascade. In this biosynthetic pathway, arachidonic acid is converted by a two-step mechanism into the conjugated triene epoxide leukotriene (LT) A_4 (Fig. 4). LTA_4 is then subsequently metabolized by LTA_4 hydrolase into LTB_4 and by leukotriene C_4 synthase into LTC_4. The half-life of LTA_4 in simple buffers has been estimated to be <3 s (Fitzpatrick et al., 1984).

B. Neutrophil-Red Blood Cell Interactions

The generality of transcellular biosynthetic mechanism for eicosanoid production became evident with the studies of leukotriene generation that involved intercel-
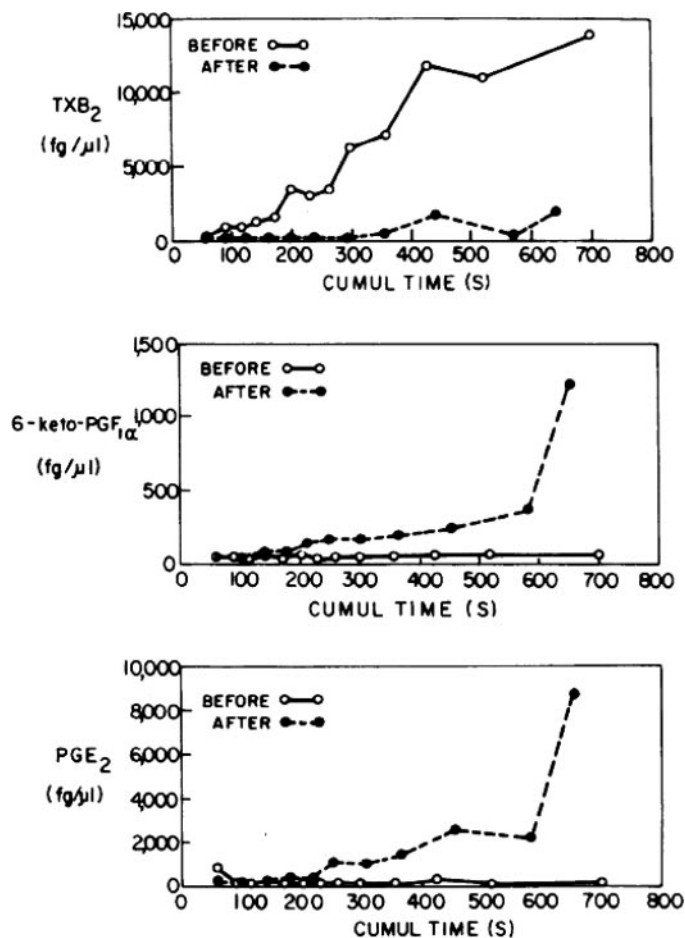

FIG. 3. The time-dependent formation of TxB_2 and 6-keto-$PGF_{1\alpha}$ in the same human subject (○) and 1 h after (●) administration of the thromboxane synthase inhibitor CGS 13080. Reproduced with permission from the *Journal of Clinical Investigation* (Nowak and FitzGerald, 1989).

lular transfer of LTA_4. The first indication that LTA_4 could be transferred into cells was obtained from the investigations of Fitzpatrick et al. (1982) with human erythrocytes. When LTA_4 that had been stabilized by albumin (Fitzpatrick et al., 1984), was incubated with red blood cells, there was robust production of LTB_4. These studies revealed for the first time that the red blood cells were not inert to eicosanoid biosynthesis but suggested that LTA_4-hydrolase was present as a cytosolic protein in the red blood cell and that it could participate in LTB_4 biosynthesis. The identity of the red blood cell epoxide hydrolase as LTA_4 hydrolase was later confirmed by Haeggstrom (2004) once it had been cloned and expressed. Shortly thereafter, bone marrow-derived mast cells were found to take up exogenous LTA_4 (also stabilized by albumin) and convert this reactive intermediate into the sulfidopeptide leukotriene LTC_4 (Dahinden et al., 1985). In the course of these studies, evidence was provided that substantial quantities of LTA_4 could be released from human neutrophils stimulated with the calcium ionophore (A23187) and subsequently stabilized in the incubation media when albumin (10
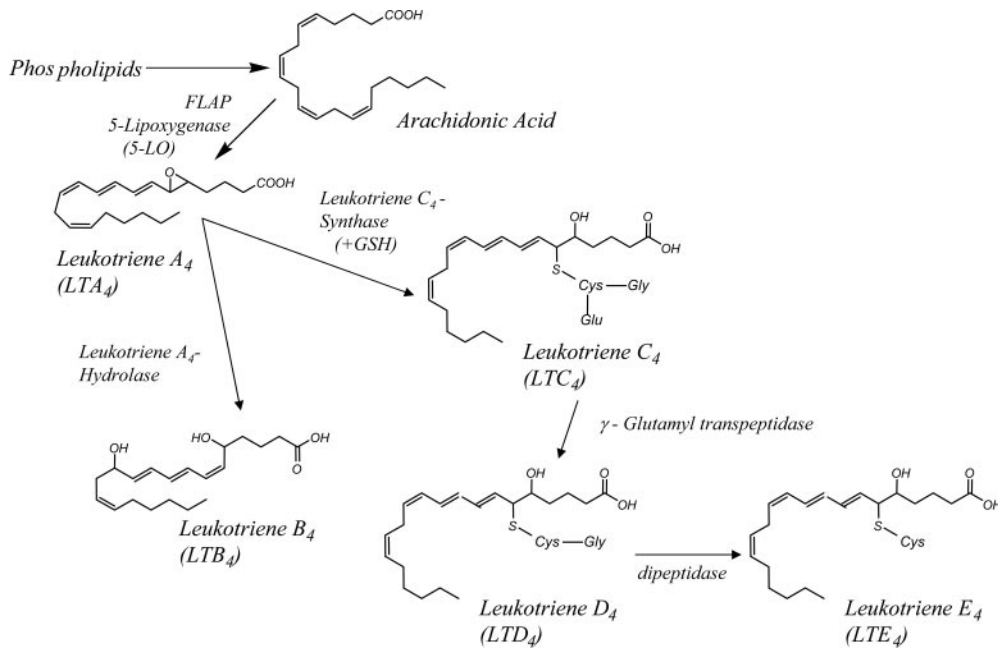
FIG. 4. Biosynthetic pathway of biologically active leukotrienes starting from cellular phospholipids containing arachidonic acid. FLAP, 5-lipoxygenase activating-protein.

mg/ml) was present. The level of intact LTA_4 released by the calcium ionophore was found to be surprisingly high (136 pmol/10^7 neutrophils) (Dahinden et al., 1985).

Coincubations of cells that expressed different components of the leukotriene cascade were first demonstrated in incubations of red blood cells with neutrophils (McGee and Fitzpatrick, 1986). Initiation of leukotriene biosynthesis occurred by stimulating the neutrophil with the calcium ionophore. Enhanced production of LTB_4 was observed compared with that

when the same number of neutrophils was stimulated by A23187 in the absence of red blood cells (Fig. 5). One interesting experiment described in these studies was that suicide inactivation of LTA_4 hydrolase present in the red blood cell, obtained by prior exposure of the red blood cells to LTA_4, led to a significant reduction in the total amount of LTB_4 generated. These results led to the suggestion that the red blood cell LTA_4 hydrolase provided a mechanism to continue LTB_4 biosynthesis in the face of LTA_4 hydrolase inac-
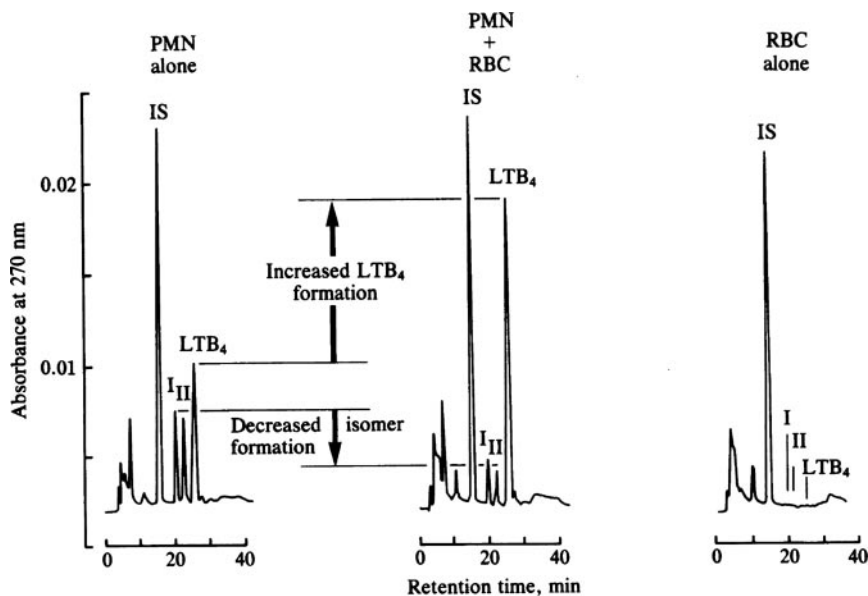
FIG. 5. Reversed phase HPLC analysis of LTB_4 and 5,12-diHETE (6-*trans*-LTB_4 isomers) biosynthesized during neutrophil/erythrocyte coincubations. Neutrophils (PMN) and erythrocyte (RBC) were incubated at 37°C for 5 min then the calcium ionophore A23187 (5 μM) was added to stimulate leukotriene biosynthesis for 2.5 min at 37°C. LTB_4 production increased and nonenzymatic hydration decreased relative to neutrophils alone, when erythrocytes were present. Reproduced with permission from the *Proceedings of the National Academy of Sciences of the United States* (McGee and Fitzpatrick, 1986).

tivation present in the neutrophil (McGee and Fitz-patrick, 1986).

C. Neutrophil-Endothelial Cell Interactions

The generation of LTC_4 by transcellular biosynthesis was first reported in mixtures of endothelial cells and human neutrophils, studied initially by Feinmark and Cannon (1986). An active leukotriene biosynthetic cascade had not been previously observed in the HUVECs, but when these cells were incubated with LTA_4, LTC_4 could be generated based upon reversed phase HPLC and ultraviolet spectroscopy as well as bioassay, viz., contraction of lung parenchymal strip. Additional reports followed in which HUVECs were coincubated with neutrophils followed by challenging of the cell mixture with A23187. In each case the production of significant amounts of LTC_4 was observed (Feinmark and Cannon, 1986; Claesson and Haeggstrom, 1988; Maclouf et al., 1989). Clearly, the HUVECs expressed LTC_4 synthase and Feinmark and Cannon (1986) very convincingly showed that when intracellular glutathione present in HUVECs was labeled with $[^{35}S]$cysteine, the production of $[^{35}S]LTC_4$ could be observed from neutrophil derived LTA_4 (Feinmark and Cannon, 1986).

D. Neutrophil-Platelet Interactions

Platelets were also found to participate in LTC_4 biosynthesis when incubated with exogenous LTA_4 (Edenius et al., 1988a; Maclouf and Murphy, 1988). Furthermore, when platelets and neutrophils were coincubated at various ratios of cells, stimulation of the neutrophils to initiate leukotriene biosynthesis resulted in the production of LTC_4 in quantities dependent upon the number of platelets present in the coincubation (Maclouf and Murphy, 1988). Before this time, the platelet was thought to only generate the eicosanoids TxA_2 and 12-HpETE by cyclooxygenase and 12-lipoxygenase, respectively. Clearly, platelets also contained LTC_4 synthase as well as the mechanisms to permit uptake and stabilization of LTA_4. These latter capacities permitted LTC_4

generation by the transcellular biosynthetic process. Subsequent studies revealed that activation of neutrophils with fMLP and opsonized zymosan in platelet coincubations could result in LTC_4 production in levels related to the number of platelets present (Maclouf et al., 1990). However, stimulation of the platelets with thrombin had no effect on the transcellular biosynthetic event, in terms of quantity as well as types of products observed (Fig. 6). For example, activation of the thromboxane synthase cascade within the platelet had no effect on transcellular biosynthesis of LTC_4 and metabolic conversion of exogenous LTA_4. Detailed kinetic studies of LTA_4 metabolism by the human platelet revealed a K_m of 1.3 μM and V_{max} of 174 pmol/mg protein/min for the formation of LTC_4, whereas the HUVECs had a K_m of 5.8 μM and a V_{max} of 15 pmol/mg protein/min (Habib and Maclouf, 1992). Interestingly, platelets had a very high efficiency in performing conversion of LTA_4 into LTC_4, much higher than that observed for the HUVECs. The basis for this was probably related to the extent of protein expression in these cells, but one cannot discount differences in LTA_4 transport and stabilization.

E. Additional Cell Interactions

Transcellular biosynthesis of leukotrienes in various other cell-cell coincubations has also been reported. Kidney-derived endothelial cells (tumor necrosis factor-activated) were found to generate LTC_4 when coincubated with GM-CSF-primed neutrophils followed by challenge with fMLP (Brady and Serhan, 1992). Interestingly, when two monoclonal antibodies, one specific for L-selectin and the other specific for CD-18, were added to the endothelial cell-neutrophil coincubation, a significant reduction in the amount of LTC_4 generated was observed. This suggested that there was an important role for cell adhesion for transcellular biosynthesis to be efficient. Keratinocytes (Iversen et al., 1994) and chondrocytes (Amat et al., 1998) were also found to metabolize LTA_4 into LTB_4 and LTC_4 when coincubated with neutrophils, suggesting that these cells also expressed LTA_4
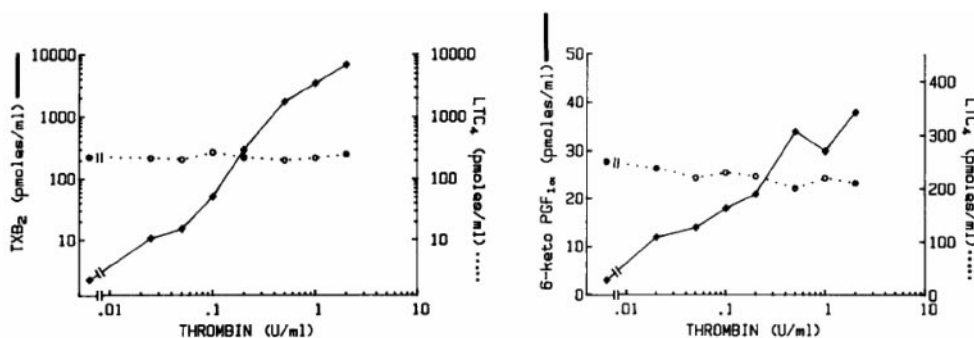
FIG. 6. Effect of thrombin stimulation on transformation of LTA_4 into LTC_4 by platelets (left) or endothelial cells (right). Platelets or endothelial cells were stimulated by increasing concentrations of thrombin. After 10 min, LTA_4 was added to platelets or endothelial cells for 20 min. The supernatants were analyzed by enzyme immunoassay for their content of TxB_2 (platelets) or 6-keto-$PGF_{1\alpha}$ (endothelial cells) as well as for LTC_4. This figure was originally published in *Blood*. Maclouf J, Murphy RC, and Henson PM (1990) Transcellular biosynthesis of sulfidopeptide leukotrienes during receptor-mediated stimulation of human neutrophil/platelet mixtures. *Blood* **76:**1838–1844. © The American Society of Hematology.

hydrolase and LTC_4 synthase. Alveolar macrophages were found to avidly take up exogenous LTA_4 and generate predominantly LTB_4 (Grimminger et al., 1991). When a coincubation of neutrophils with alveolar macrophages was stimulated with the calcium ionophore, there was a significant increase in LTB_4 production, similar to that observed in experiments in which red blood cells were incubated with human neutrophils. The blood peripheral monocyte was also found to be capable of releasing LTA_4 and providing this reactive intermediate to platelets for transcellular biosynthesis of LTC_4 (Bigby and Meslier, 1989).

The cells known to express 5-lipoxygenase are quite limited but include circulating neutrophils, monocytes, eosinophils, tissue macrophages, and mast cells. All of these cells have now been found through various studies, to release LTA_4 and participate in transcellular biosynthesis of either LTC_4 or LTB_4.

F. Intermediate Stabilization and Transport

A key feature for eicosanoid transcellular biosynthesis is the export of PGH_2 or LTA_4 from the donor cell as well as the uptake of these reactive intermediates by the acceptor cell. Very little is known about either process despite the demonstrated importance of both events. Lam et al. (1989) did report that LTA_4, stabilized by albumin, could be taken up by eosinophils even at 4°C. At this temperature there was enzymatic conversion of LTC_4 over a 1-h period, but the LTC_4 was not exported. Both the release and uptake process of LTA_4 are areas that need to be understood in some detail if the process of transcellular biosynthesis is to be fully understood.

Recent studies were performed to identify those proteins responsible for stabilization of LTA_4 expressed within cells known to generate leukotrienes led to the identification of epithelial fatty acid binding protein (E-FABP) as a protein that had such properties. Purification of soluble proteins from rat basophilic leukemia cells, using an assay for LTA_4 half-life to drive protein separation, led to the purification of E-FABP and its characterization by mass spectrometry (Dickinson-Zimmer et al., 2004b). Fatty acid binding proteins are a family of closely related proteins, abundantly expressed in most cells, although the type of FABP is specific for the cell type. Five different FABPs, including adipocyte, epidermal, heart, liver, and intestinal FABPs, were examined for their ability to increase the half-life of LTA_4 and at 4°C, each of these proteins was found to increase the half-life to approximately 20 to 30 min. Studies of the competition of this LTA_4-stability/binding revealed that whereas arachidonic acid could readily compete for bound LTA_4 at the binding site in E-FABP, other eicosanoids, including LTB_4, 5-hydroxyeicosatetraenoic acid, or 5,6-dihydroxyeicosatetraenoic acid had no effect on the stability of LTA_4 (Dickinson-Zimmer et al., 2004a). Because many cells express these fatty acid binding proteins, it is likely that FABPs could account

for much if not all of the ability of cells to stabilize LTA_4 once it enters the cell and once stabilized LTA_4 could find the enzymes responsible for conversion into the active eicosanoids.

IV. Lipoxin: Unique Transcellular Eicosanoids

A. Biosynthesis Pathway

The discovery of lipoxins came from studies of the metabolism of 15(S)-HpETE, the primary metabolite of 15-lipoxygenase, by human neutrophils in the presence of calcium ionophore (Serhan et al, 1984). The ionophore triggered 5-lipoxygenase activation and formation of new metabolites that were structurally characterized and called "lipoxin A_4" and "lipoxin B_4" (LXA_4 and LXB_4) (Fig. 7). Thus, these lipoxins are derivative of the combined action of 15-lipoxygenase as well as 5-lipoxygenase (Serhan, 1997). Early studies also revealed that the calcium ionophore-stimulated neutrophil-platelet coincubations led to the formation of lipoxin A_4 that had been derived from neutrophil-generated LTA_4 that was transferred to the platelet for subsequent conversion into lipoxin A_4 by the platelet 12-lipoxygenase (Edenius et al., 1988b). In fact, incubation of LTA_4 with platelets led to the formation of LXA_4. Thus, lipoxin biosynthesis became a unique example of cell-cell interaction and with transcellular biosynthesis playing a central role in the production of novel lipid mediators. The biologically active lipoxins required an active 5-lipoxygenase and either a 15-lipoxygenase or 12-lipoxgyenase and these activities could be either in the donor cell or acceptor cell.

B. Aspirin-Triggered Lipoxins

More recently, it was observed that when COX-2 was covalently inhibited by aspirin by acetylation, arachidonate was metabolized to 15(R)-HpETE (Lecomte et al., 1994; O'Neill et al., 1994). Within the framework of lipoxin biosynthesis, 5-lipoxygenase and aspirin-treated COX-2 would coordinately act on arachidonic acid to form lipoxins with a somewhat different structure, namely having the unnatural stereochemistry at carbon-15, viz.. 15-epi-lipoxin A_4 and 15-epi-lipoxin B_4. These have now been termed "aspirin-triggered lipoxins" (ATLs). Nevertheless, the biosynthesis of ATLs has a prerequisite for transcellular biosynthesis involving donor cells or acceptor cells expressing 5-lipoxygenase and the alternative cell type expressing either 12-lipoxygenase, 15-lipoxygenase, or aspirin-inhibited cyclooxygenase-2.

C. Neutrophil-Endothelial Cell Interactions

The production of ATLs was reported in endothelial cells (HUVECs) that had been induced by interleukin-2 to express COX-2 and then treated with aspirin and exogenous arachidonic acid (Claria and Serhan, 1995). Coincubation of these treated HUVECs with human
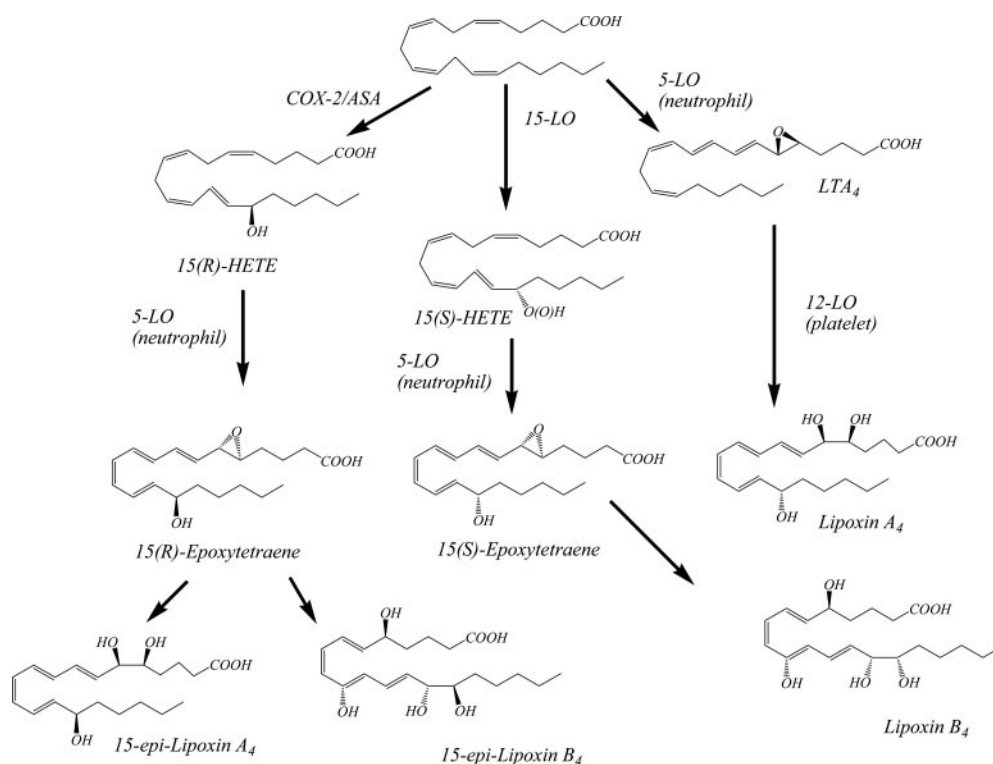
FIG. 7. Biosynthetic pathway of biologically active lipoxins starting from arachidonic acid. Three separate pathways are indicated based upon the initial oxidation mediated by 5-lipoxygenase, 15-lipoxygenase, or aspirin-inactivated PGH synthase (COX-2/ASA). The latter pathway results in the 15-epi-lipoxin metabolites.
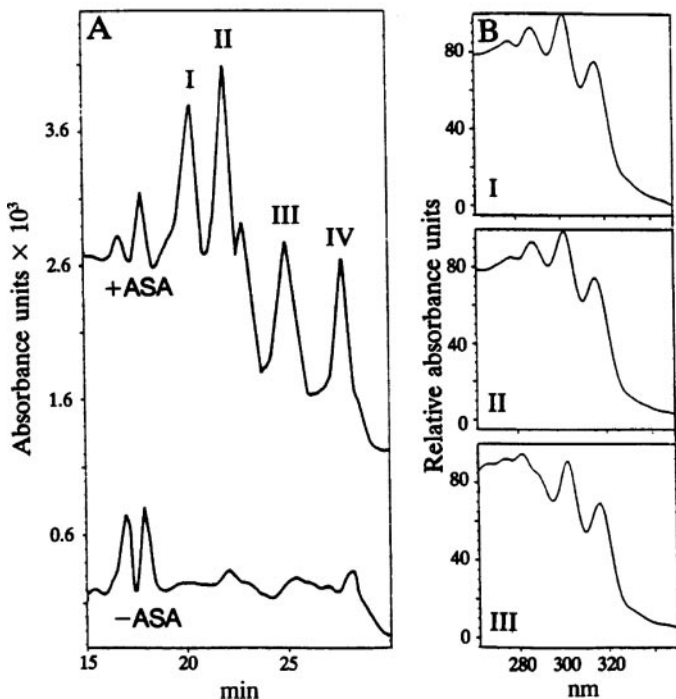

FIG. 8. A, reversed phase HPLC separation and profile of products from HUVEC and human PMN coincubations. Confluent HUVECs were exposed to interleukin-1β for 24 h and treated with 500 μM aspirin (ASA) or vehicle for 20 min at 37°C and 20 μM arachidonic acid for 60 s. Each was incubated with fresh isolated PMNs (HUVEC/PMN cell ratio of 1:50) followed by costimulation with 5 μM ionophore A23187 for 30 min at 37°C. B, UV spectra recorded during the elution of indicated peaks I to IV. Reproduced with permission from the National Academy of Sciences (Claria and Serhan, 1995).

neutrophils and stimulation of 5-lipoxgyenase by the calcium ionophore led to the production of at least four ATL products detected as conjugated tetraene eicosanoids (Fig. 8). These new transcellular eicosanoid products were found to markedly inhibit neutrophil adhesion to endothelial cells (Claria and Serhan, 1995), and extensive pharmacological studies have revealed numerous potent anti-inflammatory properties unique to the ATLs (Serhan, 1997).

V. Arachidonate and Transcellular Processes

It is clear that the movement of free arachidonic acid between cells is a significant process leading to eicosanoid production. Within our definition framework, transcellular biosynthesis would arise when donor cells were stimulated to release free arachidonic acid from membrane phospholipids and rather than immediate conversion into LTA_4 or PGH_2 or reacylation back into phospholipids, the free arachidonic acid is taken up by the acceptor cell followed by enzymatic oxygenation and transformation into various eicosanoid mediator pathways. At first glance, this type of transcellular biosynthesis may seem trivial or at least straightforward. However, it is far from that, in part due to the multiple classes and molecular species of phospholipids that contain esterified arachidonate that can serve as substrate for phospholipases, the number of phospholipases capable of releasing arachidonic acid, the location of both

substrate and enzyme within the cell, and the transport and uptake mechanisms for this highly lipophilic free fatty acid, as well as the time dependence of each individual event. One of the early examples of supporting these events was the formation of radiolabeled LTB_4 when platelets, prelabeled with [^{3}H]arachidonate were incubated with human neutrophils and then stimulated with A23187 (Peters-Golden and Feyssa, 1993). In addition, in this experiment [^{3}H]12-hydroxyeicosatetraenoic acid was metabolized by neutrophil 5-lipoxygenase to 5,12-dihydroxyeicosatetraenoate.

A. Alveolar Epithelial Cell-Alveolar Macrophage Interactions

One example of these events is the study of the calcium ionophore induced biosynthesis of PGH synthase products by alveolar epithelial cells (AECs) and 5-lipoxygyenase products by alveolar macrophages (AM), either in isolated culture or coincubation cultures (Antoine et al., 1992). When [^{14}C]arachidonic acid was incorporated into the AEC, challenge of these cells led predominantly to production of radiolabeled PGE_2 and 6-keto-$PGF_{1\alpha}$ with very little radiolabeled 5-lipoxygenase products, LTB_4 and 5-HETE. However, challenge of the coincubation of AEC with AM, led to the formation of a substantial quantity of radiolabeled LTB_4, 5-HETE, and even TXB_2 (Fig. 9). The radiolabeled arachidonate converted into LTB_4 had to be derived from AECs, whereas the enzymatic conversion by the 5-lipoxygenase pathway was required in the AMs, since these were the only cells in this system expressing 5-lipoxygyenase. Furthermore, when indomethacin was added to the cocultures, there was a substantial increase in 5-lipoxygenase products (Antoine et al., 1992), probably a result of "shunting" of the free arachidonic acid from the donor cell to the acceptor cell.

B. Neutrophil-Platelet Interactions

Platelet-neutrophil coincubations have been a useful model, revealing not only transcellular biosynthetic events centered around LTA_4 movement but also the complex transfer of free arachidonic acid between cells using radioactively labeled arachidonic acid to label phospholipids in platelets and quantitative mass spectrometry to measure absolute amounts of eicosanoids synthesized. The specific activities of 5-lipoxygenase products and neutrophil-platelet coincubations were found to change remarkably after initiation of LTA_4 biosynthesis by treatment of platelet-neutrophil coincubations with A23187 (Marcus et al., 1992). At early time points (1.5 min), a fairly low specific activity of LTC_4 was observed, which strikingly increased by 10 min, suggesting that platelet-derived arachidonic acid was used for LTA_4 production at the early time point; however, this situation changed later in the time course, with a higher proportion of arachidonate of neutrophil origin involved in the substrate mixture used for LTA_4 production. An
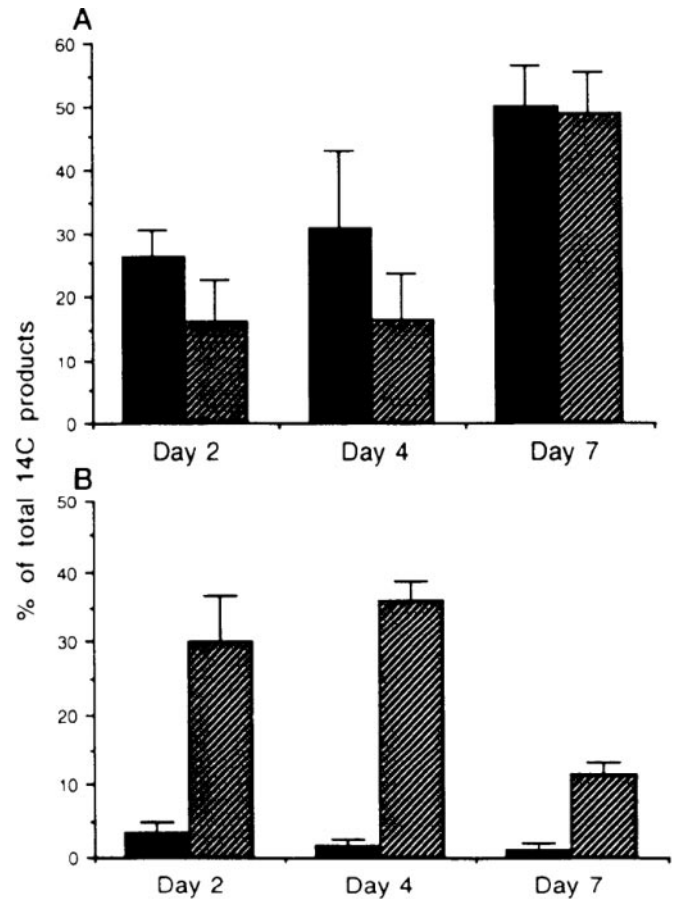

FIG. 9. Effect of coculture of AECs with AMs on metabolism of AEC-derived arachidonic acid. Cultures of [^{14}C]arachidonate-prelabeled AECs alone (■) or AEC/AM cocultures (▨) were established at AEC culture days 2, 4, and 7. A, data for major eicosanoids unique to AEC (PGE_2 + 6-keto-$PGF_{1\alpha}$). B, eicosanoids unique for AM (LTB_4 + 5-HETE). Reproduced with permission from the *American Journal of Physiology* (Peters-Golden and Feyssa, 1993).

even more complex picture of events taking place in such coincubations was revealed when fMLP was used as an agonist to specifically stimulate neutrophils present in a platelet-neutrophil coincubation mixture. In these studies, radiolabeled arachidonic acid was added to neutrophils with sufficient time to incorporate the free acid into phospholipids. This label was used to follow release of arachidonic acid specifically from neutrophil phospholipids. When fMLP was added to the coincubations, abundant production of [^{3}H]TXB_2 was observed (1619 ± 227 cpm) versus fMLP neutrophils treated alone (38 ± 15 cpm), revealing transfer of arachidonic acid from neutrophil to platelets and platelet metabolism by cyclooxygenase-1 and thromboxane synthase (Maugeri et al., 1994). In a similar coincubation experiment using only prelabeled platelet phospholipids, [^{3}H]LTC_4 was clearly produced, revealing the transfer of arachidonic acid in the opposite direction, namely from platelets to neutrophils (Krischer et al., 1998). Interestingly, inclusion of an antibody to P-selectin in the incubation system to prevent platelet-neutrophil adhesion resulted in a substantial decrease in the total level of LTC_4 biosynthesis

as well as TXB_2 production. Thus, close adhesion of these cells seems to be an important facet of transcellular biosynthesis, at least within the platelet-neutrophil system, which probably facilitated both free arachidonic acid exchange as well as LTA_4 transport.

C. Transport and Microparticles

The transport of free arachidonic acid across membranes has been an active area of investigation and not without considerable controversy (Barry et al., 1997). Nonetheless, evidence does suggest that the neutrophil uses a membrane protein to transport this highly lipophilic fatty acid across its plasma membrane. Support for a protein transporter has included demonstration of saturation of the process, sensitivity to proteases, as well as sensitivity to protein-modifying reagents (Barry et al., 1997). There have also been reports that microparticles from platelets may also be involved in the transport of arachidonic acid in platelet-acceptor cell systems (Reddy and Herschman, 1996). Interestingly, the presence of $sPLA_2$, the soluble low molecular weight phospholipase A_2 nonspecific for hydrolysis of phospholipids, was required to drive the production of 6-keto-$PGF_{1\alpha}$ in platelet-endothelial cell incubations (Reddy and Herschman, 1996). A role for $sPLA_2$ in eicosanoid production, probably by transcellular biosynthetic processes, has been the target of several studies, including studies of mast cell-endothelial coincubations (Fradin et al., 1989). More needs to be learned about how arachidonic acid freely moves between cells and actively participates in transcellular biosynthetic events and whether or not this process involves microparticles as a source of this mobile arachidonate. In this latter case, it will be important to understand how phospholipases such as $sPLA_2$ release arachidonic acid.

VI. Tissue and in Vivo Evidence for Transcellular Biosynthesis

A. Eicosanoid Biosynthesis in Whole Blood

Evidence for the occurrence of transcellular biosynthetic events in vivo has been surprisingly easy to demonstrate, supporting the concept that there is a highly effective mechanism for transferring arachidonic acid as well as chemically reactive intermediates between cells. Studies of eicosanoid biosynthesis in whole blood were reported using zymosan (yeast cell wall) phagocytosis to initiate eicosanoid biosynthesis. The major phagocytic cell in the blood is the neutrophil and, not surprisingly, a major eicosanoid product observed during whole blood phagocytosis was LTB_4 (Fradin et al., 1989). However, there was also significant production of LTE_4 by this tissue, and this was shown to be a result of platelet-neutrophil interaction and transcellular metabolism of LTA_4 by systematically removing each major cell component in whole blood before initiation of phagocytosis.

The level of LTB_4 was higher in whole blood than that produced by the same number of neutrophils (from the same human blood sample) stimulated by opsonized zymosan, suggesting that a portion of LTB_4 synthesis was the result of the tissue environment and not limited to a single cell. This suggested that even the LTB_4 was in part a transcellular product of the neutrophil-red blood cell interaction. Interestingly, the absolute quantity of LTE_4 (0.6 ± 0.07 ng/10^6 neutrophils) relative to LTB_4 (2.8 ± 0.3 ng/10^6 cells) differed only 3- to 4-fold despite a 20:1 ratio of red blood cells to platelets. These results suggested that the platelet very efficiently competed with the red blood cell in whole blood for LTA_4 made and released by the neutrophil.

B. Lung Transcellular Events

Evidence for the direct interaction of these same cell types was also obtained in isolated rat lung preparations (Voelkel et al., 1992) when animals were injected with *Salmonella enteritidis* endotoxin (2 mg/kg i.p.). Lungs were perfused with buffer in an antegrade, recirculating mode and then challenged with fMLP (100 nM). This agonist led to a pronounced increase in pulmonary artery perfusion pressure that could be prevented by a thromboxane receptor antagonist. When the concentration of fMLP was changed, dose-related production of TXB_2 and LTB_4 was measured as well as production of LTC_4 and 6-keto-$PGF_{1\alpha}$, but only at lower doses of fMLP (10 nM). These eicosanoids were not observed in the absence of prior endotoxin treatment of the animals. When endotoxin-treated, isolated lungs were perfused in a retrograde fashion, cell clumps that contained a core of neutrophils and platelets surrounded by a layer of red blood cells were collected in the perfusate, suggesting that adhesion of these cells took place after endotoxin treatment of the animal and these clumps became lodged in the pulmonary capillary network. These cell aggregates were felt to be responsible for the production of eicosanoids by transcellular biosynthetic mechanisms (Voelkel et al., 1992).

Isolated and perfused rat lungs have been previously studied for their capacity to metabolize LTA_4 that was injected as a bolus into the pulmonary artery (Grimminger et al., 1988). LTC_4 was the major LTA_4-derived product observed, revealing the capacity of the pulmonary vascular architecture, most likely the pulmonary endothelial cells, to take up and metabolize this 5-lipoxygenase intermediate. These studies were extended by adding 2×10^8 human neutrophils into the recirculating buffer that was perfusing a rabbit lung (Grimminger et al., 1990). After a short time, all neutrophils were trapped by the lung in this model of pulmonary leukostasis. Challenge of this organ preparation with 1 μM A23187 not only resulted in abundant production of LTB_4 expected for neutrophil activation of the 5-lipoxygenase cascade but also there was a significant quantity of LTE_4 generated (Grimminger et al., 1990). Control

experiments without prior addition of neutrophils to the isolated lung preparation revealed no LTA_4-derived products after challenge with calcium ionophore A23187. The appearance of the cysteinyl LTE_4 strongly supported operation of transcellular biosynthetic events, probably mediated by release of neutrophil-derived LTA_4 and uptake by pulmonary endothelial cells followed by conversion into LTC_4.

C. Cardiac Transcellular Events

The isolated rabbit heart (Langendorff preparation) was also studied to probe whether transcellular biosynthesis of leukotrienes in this organ could take place when human neutrophils were added to the recirculating buffer. These neutrophils were shown to adhere quite avidly to coronary endothelial cells in this model system (Sala et al., 1996). When A23187 (0.5 μM) was added to the preparation, there was immediate formation of LTC_4 but a delayed increase in coronary perfusion pressure (Fig. 10) (Sala et al., 1993). Both LTC_4 production and the increase in perfusion pressure induced by A23187 were prevented by pretreatment of the neutrophils used in the perfusion system with the 5-lipoxygenase activating-protein inhibitor MK886 (1 μM) before addition of the neutrophils to the rabbit heart preparation. Somewhat different results were obtained when fMLP (1 μM) was added to the isolated perfused rabbit heart. In these studies, the neutrophils were primed with GM-CSF (1 nM) before addition to the perfusion buffer; and there was a profound increase in cysteinyl leukotriene formation present in the perfusate as well as an increase in coronary perfusion pressure in response to this receptor-mediated stimulus specific to neutrophil activation. Because A23187 is known to stimulate nitric oxide production in this model and lead to multiple responses, including vasodilation and inhibition of PMN adhesion (Kubes et al., 1991), the fMLP/GM-CSF stimulus was more relevant since this stimulus does not lead to increased production of nitric oxide, which confounds interpretation of A23187 as a stimulus. Furthermore, addition of the monoclonal antibody directed against the CD-18 subunit of PMN-β_2 integrin to the neutrophils before their addition to the heart preparation resulted in a substantial reduction of cysteinyl leukotriene biosynthesis and virtually no increase in coronary perfusion pressure. These studies were consistent with a transcellular biosynthesis of cysteinyl leukotrienes that mediated substantial physiological changes in the coronary system when neutrophils are activated and release (or were activated and released) LTA_4 into the endothelial cells for production of LTC_4.

Evidence for transcellular biosynthesis of cysteinyl leukotrienes in the heart in vivo has come from studies of ligation of the left descending coronary artery in animal models. In these studies, the urinary excretion of LTE_4 was measured in sham-operated rabbits, 3 h after ligation and 3 h after ligation and infusion of anti CD-18 (1 mg/kg i.v.). A significant elevation of LTE_4 in the urine of these rabbits was observed when the coronary artery was ligated relative to the concentration of LTE_4 in urine of sham-operated animals (Sala et al., 2000). In addition, there was no elevation of urinary LTE_4 in the animals treated with anti-CD-18. In the same experimental model pretreated with the 5-lipoxygenase activating-protein inhibitor, BAY X1005, there was significant cardioprotection and reduced mortality, suggesting that inhibition of LTA_4 biosynthesis and, ultimately, of transcellular biosynthesis to the cysteinyl leukotrienes played a significant role in the cardiac events (Rossoni et al., 1996).

Elevation of urinary LTE_4 had been reported previously in human subjects with acute myocardial infarctions or unstable angina (Carry et al., 1992). Similar findings of elevation of urinary LTE_4 had been reported in human subjects with atherosclerotic coronary artery disease before bypass surgery (Allen et al., 1993). Although it is very difficult to carry out definitive studies proving or disproving the transcellular biosynthesis hypothesis of cysteinyl leukotrienes in human coronary disease, nonetheless, these results are consistent with the formation of LTE_4, most likely in a neutrophil-dependent manner consistent with LTA_4 uptake by acceptor cells in the heart.
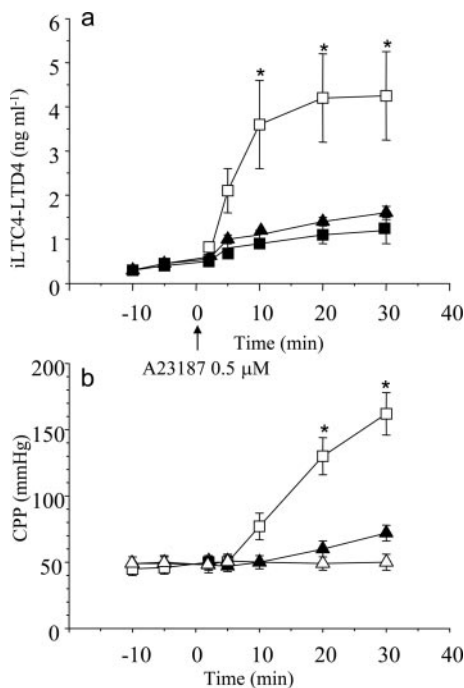
FIG. 10. a, time course for appearance of immunoreactive cysteinyl leukotrienes ($iLTC_4$-LTD_4) in the recirculating medium of buffer-perfused (■) or human PMNs-perfused rabbit isolated hearts (□), challenged with A23187. b, effect of PMN pretreatment with MK886 (1 μM) (▲). Coronary perfusion pressure (CPP) of rabbit isolated hearts perfused with unchallenged human PMNs (△) and after challenge with A23187 (□). Effect of PMNs pretreatment with MK886 (▲). Reproduced with permission from the *British Journal of Pharmacology* (Sala et al., 1993).

D. Central Nervous System Transcellular Events

Recently, the potential for transcellular biosynthesis taking place in the brain was examined in the isolated perfused guinea pig brain with an intact and functional vascular endothelial barrier (Di Gennaro et al., 2004). When such brain preparations were infused with human neutrophils that had been primed with GM-CSF (1 nM) followed by addition of fMLP (100 nM) to the infusion buffer, a substantial amount of LTD_4 was found to be released into the perfusate as identified by specific mass spectrometric techniques. In addition, there was evidence for the production of LTB_4 and nonenzymatic products of LTA_4 hydrolysis (Di Gennaro et al., 2004). These results were consistent with the generation of LTA_4 by the neutrophils present in the perfusion media followed by brain endothelial cell uptake and metabolism into LTC_4. In this model there was an increase in brain wet weight, suggesting edema formation initiated by neutrophil activation. This edema was prevented by pretreatment of the perfused neutrophils with the inhibitor MK886 or by the dual cys-LT1/2 receptor antagonist Bay u9773 (Heise et al., 2000), but not the selective cys-LT1 receptor antagonist, iralukast (Capra et al., 1998).

E. Genetic Approach: Chimeric Mice

Perhaps the most striking support for eicosanoid transcellular biosynthesis in vivo has come from studies of knockout mice deficient in either 5-lipoxygenase or LTA_4 hydrolase (Fabre et al., 2002). In these studies, chimeric mice were made in which the bone marrow from either wild-type or the specific knockout mice was added to a recipient mouse (wild type or $5\text{-}Lo^{-/-}$) that had been exposed to a lethal dose of radiation (Fig. 11). Interestingly, when the bone marrow from animals that had no LTA_4 hydrolase was given to $5\text{-}Lo^{-/-}$ animals, there was clear production of LTB_4, suggesting that there was generation of LTA_4 from the bone marrow-derived cells, since cells from the recipient animals were unable to produce LTB_4 themselves (they were $5\text{-}Lo^{-/-}$). Whereas LTA_4 had to be synthesized within the $Lta_4\text{-}h^{-/-}$ bone-derived marrow cells (neutrophils), this leukotriene intermediate had to be transferred into cells of the $5\text{-}Lo^{-/-}$ mouse that did express LTA_4 hydrolase. Such cells would include the red blood cell. This is perhaps the first experiment to clearly demonstrate that transcellular biosynthesis does take place in vivo, and, furthermore, the extent of transcellular biosynthesis was at a sufficient level to contribute significantly to alter the inflammatory response (Fabre et al., 2002).

VII. Conclusions

Transcellular biosynthesis of eicosanoids represents a unique example of cell-cell communication that profoundly defines the profile of arachidonate products in tissues and organs. This is a system of remarkable ef-
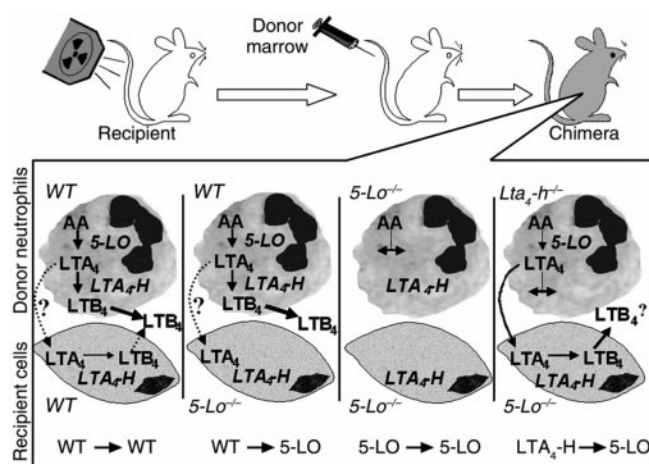

FIG. 11. Chimeric mice were generated by exposing mice to a lethal dose of ionizing radiation with subsequent injection of donor bone marrow. The following chimeras were generated: wild-type (WT) mice receiving wild-type marrow (WT → WT), $5\text{-}Lo^{-/-}$ mice receiving wild-type marrow (WT → 5-LO), $5\text{-}Lo^{-/-}$ mice receiving $5\text{-}Lo^{-/-}$ marrow (5-LO → 5-LO), and $5\text{-}Lo^{-/-}$ mice receiving $Lta_4\text{-}h^{-/-}$ marrow (LTA$_4$H → 5-LO). The illustration indicates the expected interaction between neutrophils of donor origin (top) and cells of recipient origin (bottom) that will lead to LTB_4 production. Reproduced with permission from the *Journal of Clinical Investigation* (Fabre et al., 2002).

fectiveness and flexibility; for example, a chemotactic stimulus can also turn into a vasoactive one or a stimulus for platelet aggregation and vasospasm can concomitantly evoke formation of vasodilating-antiaggregatory eicosanoids. Moreover, transcellular biosynthesis can occur among resident or circulating cells of the same type (homologous) or cells of different origin (heterologous). It is a very dynamic process that can integrate the biosynthetic capacity of a circulating donor cell (such as the leukocyte) to influence the lipid mediator profile released from cells that reside deeply in tissues. This unique process allows cells to integrate their expressed enzymes into the tissue response to insult even if a complete arachidonate cascade is not resident in the cell. All this can take place apparently with little energy expenditure, yet a sophisticated and coordinated cascade of biologically active lipids emerges within a given microenvironment. Transcellular biosynthesis of eicosanoids can operate in physiological conditions as well as in pathological ones. For example, platelets that are marginalized and have direct contact with the vessel wall (intact or partly damaged), become activated to synthesize endoperoxides (PGH_2) that can be converted to antiadhesive vasoprotective agents (PGI_2-based) by the endothelial cells in normal resolution. Transcellular biosynthesis mechanisms allow these antiadhesive vasoprotective mechanisms to become quickly and fully operative yet remain switched off when the PGH-donor platelet does not contact the acceptor endothelial cell.

In pathological conditions, such as during an inflammatory event, marginating leukocytes must undergo the complex process of diapedesis and tissue infiltration to fulfill their phagocytic duties. In cooperation with the

endothelial cells via transcellular biosynthesis mechanisms, leukocytes promote the formation of the permeability-altering cysteine leukotriene, resulting in edema. This represents a fundamental defense mechanism of the body that must become operative only when and where needed, but if not properly controlled can lead to tissue damage. Transcellular biosynthesis of eicosanoids may represent a common mechanism to switch on (via prostaglandins and leukotrienes) and switch off (via lipoxins) such complex events.

The profile of arachidonate products that are formed in tissues and organs cannot be predicted on the basis of the simple sum of the potential contributions of individual cells with intact arachidonate cascade pathways but rather is the result of the contribution of expressed enzymes within cells that participate together even though separated in the process of transcellular biosynthesis. This has obvious pathophysiological implications and may also affect drastically effective pharmacological intervention. For example, the presence of infiltrating neutrophils in an inflamed tissue might suggest that the use of LTB_4 receptor antagonists could optimally control phlogosis, whereas had the activated leukocytes contributed LTA_4 to acceptor cells (e.g., mast cells) for cysteinyl leukotriene synthesis, cys-LT1 or cys-LT2 receptor antagonists might be the correct therapeutic intervention.

Although it is clear that in vivo, the profile of "housekeeping" or "pathological" eicosanoid can only be defined by mechanisms that include transcellular biosynthesis, still much has to be understood to have a complete picture of events taking place. Intermediates such as LTA_4 are extremely labile yet can undergo export from a donor cell, transport to and uptake by the acceptor cell, and enzymatic transformation. An emerging story is that the transcellular biosynthesis of eicosanoids is facilitated by physical contact between cells where events may take place in a "polarized" way to optimize the final products. However, there is also evidence that intercellular transfer of arachidonic acid and/or stabilized intermediates may take place even under conditions of cell-cell proximity when transport through the extracellular medium must take place. In actual fact, little is known about either process, yet each could represent mechanisms for therapeutic attack. Currently, the only pharmacological intervention of proven clinical efficacy that can "disrupt" the process of transcellular biosynthesis of eicosanoids is inhibition of the oxygenases (cyclooxygenase or lipoxygenase) or cysteine leukotriene receptor antagonism. However, each single step that controls this process, including transcellular mechanisms, may have profound physiopathological implications and may represent a potential pharmacological target.

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