Epidermal Cell–Polymorphonuclear Leukocyte Cooperation in the Formation of Leukotriene B₄ by Transcellular Biosynthesis

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The cellular origin of Leukotriene B₄, a potent pro-inflammatory agent that is present in psoriatic lesions, has not been completely ascertained. The present study was performed in order to assess the possible contribution of epidermal cells to leukotriene B₄ synthesis through 5-lipoxygenase or by means of transcellular metabolism of the epoxide intermediate leukotriene A₄ from activated polymorphonuclear leukocytes. The metabolism of exogenous arachidonic acid in fresh human epidermal cells, polymorphonuclear leukocyte or mixed suspensions was determined by means of high-performance liquid chromatography. Epidermal cells transformed arachidonic acid mainly into 12-hydroxy-eicosatetraenoic acid and prostaglandin E₂. Formation of prostaglandins F₂α and D₂, 12-hydroxy-epotadecatrienoic acid, and 15- and 11-hydroxy-eicosatetraenoic acids was also detected. We did not detect any eicosanoid derived from 5-lipoxygenase pathway. Mixed suspensions of polymorphonuclear leukocytes and epidermal cells (ratio 1:4) produced 1.72 times more leukotriene B₄ than leukocytes alone under the same experimental conditions. Epidermal cells incubated with 5 µM authentic leukotriene A₄ for 3 min yielded 2.954 ± 0.27 pmoles/10⁶ cells of leukotriene B₄, which was characterized by co-elution with authentic standard and its ultraviolet absorption spectrum. These data demonstrate the existence of a leukotriene A₄ epoxide hydrolase activity in human epidermal cells. Our results suggest that epidermal cells could cooperate in leukotriene B₄ biosynthesis by transcellular metabolism of leukotriene A₄ in lesions of psoriasis, and possibly other inflammatory dermatoses characterized by increased leukotriene B₄ levels and abundant polymorphonuclear leukocyte infiltrates. J Invest Dermatol 98:333–339, 1992

Since 1974 [1] there have been numerous reports in the literature showing the presence of large amounts of eicosanoids in psoriatic lesions. In this context, stable lesions are mainly characterized by the presence of several mono-hydroxylated unsaturated fatty acids and lesser amounts of leukotrienes and prostaglandins [1–9]. Leukotriene B₄ (LTB₄), has been shown to be one of the most potent chemoattractants for polymorphonuclear leukocytes (PMN) [10]. LTB₄ also stimulates other neutrophil responses such as chemokinesis, aggregation, adhesion to endothelium, degranulation, etc. [11,12], through binding to its specific receptors [13,14]. Human keratinocytes may also be a target for LTB₄ proliferative action, because specific binding sites have been identified on their membranes [15].

The metabolic pathways of arachidonic acid (AA) in freshly isolated human epidermal cells (EC) are still controversial [16–18]. Although it has been reported that human EC can synthesize LTB₄ from AA [18–20], the actual cellular origin of LTB₄ found in psoriatic lesions has not been definitively established yet.

Infiltration of PMN is one of the earliest events in the pathogenesis of psoriatic lesions and their accumulation can precede the appearance of the characteristic epidermal changes [21,22]. PMN are an important source of LTB₄ and other AA derivatives such as 5-hydroxy-eicosatetraenoic acid (5-HETE) via 5-lipoxygenase (5-LO) [23,24]. LTB₄ and peptido-leukotrienes are produced from an epoxide intermediate [leukotriene A₄ (LTA₄)] by the activity of an epoxide hydrolase and a glutathione S-transferase, respectively. LTA₄ is synthesized from AA by the action of 5-LO [23–26]. Epoxide hydrolase and glutathione S-transferase activities have been found in some cellular types that are unable to oxidize AA via 5-LO, such as human erythrocytes [27], some human T-cell lines [28], vascular smooth muscle cells [29], murine mast cells [30], or human endothelial cells [31].

The conversion of PMN-produced LTA₄ to LTB₄ by EC could contribute to the increased amounts of LTB₄ present in lesions of
psoriasis or other inflammatory dermatoses. In this study, we explore the capability of EC to synthesize LTBA from AA and/or transform PMN-derived LTAD into LTBA.

**MATERIALS AND METHODS**

**Cell Preparations**

Preparation of Epidermal Cell Suspensions: EC were isolated from breast and abdomen of human skin obtained by plastic surgery, using the technique described by Liu and Karasek [32]. Briefly, narrow strips of skin were cut and rinsed twice in phosphate-buffered saline, pH 7.4, free of Ca²⁺ and Mg²⁺ (PBS). The strips were then placed in PBS containing 0.5% trypsin (Difco laboratories, Paisley, Scotland) w/v and kept at 37°C for 50–60 min. When dermo-epidermal detachment occurred, epidermal strips were transferred to a culture medium (Dulbecco’s modified Eagle’s medium (DMEM) (Flow Laboratories, Irvine, Scotland) + 2 mM glutamine + 1 mM sodium pyruvate) containing 10% fetal bovine serum (Flow laboratories, Irvine, Scotland). The fragments of epidermis were then gently stirred for 10–15 min in the aforementioned culture medium solution. The cellular suspension thus obtained was then filtered through a sterile gauze to discard fragments of whole epidermis and then kept at 37°C until incubation.

Cell counts were carried out by means of a Coulter Blood Cell counter and cell viability was assessed by Trypan blue dye exclusion. Only cell suspensions with a percentage of viability greater than 95% were used.

Polymorphonuclear Leukocyte Isolation: Peripheral venous blood, anti-coagulated with ACD-A solution (0.73% w/v citric acid, 2.2% w/v trisodium citrate, 2H₂O, 2.24% w/v glucose; Grifols Laboratories, Barcelona, Spain), was processed as described previously [33].

**Chromatographic Procedures**

Equipment: A reverse-phase C-18 column UltraspHERE ODS 250 × 4.6 mm (Beckman, San Ramon, CA) was used for all analyses. HPLC device was composed of a Spectra-Physics SP8700 solvent delivery system, a Spectra-Physics SP8700 pump module, a Beckman 165 UV-Vis detector, and a Beckman 171 radiosotope detector provided with a liquid scintillation cell. Scintillation cocktail (Ready Flow III, Beckman, Fullerton, CA) was pumped at a flow rate of 3 ml/min.

**Standards:** 15 (S)-hydroxy-5,6,8,9,12,14,15(15)-H-eicosatetraenoic acid, 285 Ci/mmol; 5(S)-hydroxy-5,6,8,11,12,14,15(15)-H-eicosatetraenoic acid, 183 Ci/mmol; 12(S)-hydroxy-5,6,8,11,12,14,15(15)-H-eicosatetraenoic acid, 119 Ci/mmol; 5,6,11, 12,14,15(15)-H prostaglandin E₂, 185 Ci/mmol; 5,6,8,9,12,14,15(15)-H prostaglandin D₂, 193 Ci/mmol; 1-14C prostaglandin F₂α, 60 mCi/mmol; and [5,6,8,9,11,12,14,15(15)-H]-Leukotriene B₄, 100 Ci/mmol were purchased from New England Nuclear (Boston, MA). Prostaglandin B₂, leukotriene B₄, leukotriene C₄, leukotriene D₄, 6-trans-leukotriene B₄, 12-hydroxy-eicosatetraenoic acid, and 5-hydroxy-eicosatetraenoic acid were purchased from Sigma (St. Louis, MO). 20-carboxy-leukotriene B₄, 11(S)-hydroxy-eicosatetraenoic acid, and 6-trans-12-epi-leukotriene B₄ were supplied by Paesel (Frankfurt, Germany).

**Chromatographic Procedure 1 (CP1):** This procedure was used to analyze mono- and di-hydroxy derivatives of AA. Chromatography was performed isocratically at a flow rate of 1 ml/min with methanol/water/trifluoroacetic acid/triethylamine 75: 25:0.1:0.05 (methanol, HPLC, Scharlau, Barcelona, Spain); trifluoroacetic acid, spectroscopy, Merck, Darmstadt, Germany; triethylamine, Analyticals, Carlo Erba, Milano, Italy).

**Chromatographic Procedure 2 (CP2):** This procedure was used to analyze prostaglandins and w-oxidation products. Eluents: A) acetonitrile: water: acetic acid 33:67:0.01 (acetonitrile, Scharlau, Barce-

lona, Spain) and B) acetonitrile: water: acetic acid 90:10:0.01. The composition of the mobile phase was 100% A for the first 16 min after injection. Thereafter, the percentage of B was increased linearly to reach 65% 26 min after injection, and then maintained until minute 45. Mobile phase flow rate was 1 ml/min.

**Data Processing:** Chromatographic peaks were integrated by means of a System Gold Chromatography Software (Beckman) and 171 Radioisotope Detector Chromatography Software (Beckman) in an IBM-PC XT 286 coupled to the scintillation detector.

**Preparation of Leukotriene A₄** Saponification of unlabeled LTAA methyl ester (98% purity, 50 μg/ml in 2% triethylamine in hexane, Paez, Frankfurt, Germany) was performed as described by Maycock et al [34]. Saponification was monitored a) measuring the non-enzymatic transformation of LTAA into 6-trans-LTB₄ epimer (2 μl aliquots of reaction mixture were added to 0.5 ml of Hank's buffer, pH 2.5, and kept at room temperature for 5 min and finally injected into the chromatographic column, using CP1 with UV detection at 280 nm); and b) by determination of the UV absorption spectrum of the reaction mixture after 60 min (Beckman DU-70 spectrophotometer). The concentration of LTAA (ε₄₅ 40,000 at 279 nm) was determined from its UV spectrum. Aliquots of the reaction mixture after 60 min were added directly to the cellular suspensions.

**Incubation Procedures**

**Metabolic Profile of Arachidonic Acid in Human Epidermal Cells:** In order to establish the suitable incubation time, two preliminary time-course experiments were performed. 10⁶ EC in 5 ml of RPMI 1640 (Flow laboratories, Irvine, Scotland), pH 7.4, plus 2 mM CaCl₂ were pre-incubated for 5 min at 37°C with gentle agitation. EC suspensions were then incubated with 2.86 μCi of 1⁴C-arachidonic acid (1⁴C-AAA, final concentration 10 μM) (56.6 mCi/mmol, Amersham, Buckinghamshire, England) plus 5 μM calcium ionophore A23187 (Sigma, St. Louis, MO) for several intervals of time. After 0.167, 0.5, 1, 5, 10, 20, 40, or 60 min, 0.5 μl aliquots of the suspension were transferred to tubes containing 0.5 ml methanol at 0°C. After centrifugation at 15,000 × g for 2 min, 500 μl of supernatant were directly injected into the chromatographic column.

The metabolic profile of AA in EC suspensions was determined according to the following two procedures.

I. EC suspensions (cell density 10⁶ cells/ml) in RPMI 1640 were warmed at 37°C for 5 min. Then 1 ml aliquots of cell suspensions were transferred into tubes containing 0.572 or 1.43 μCi 1⁴C-AAA in 10 μl ethanol, to yield final concentrations of 10 or 25 μM, respectively, in the presence or absence of 5 μM calcium ionophore A23187. Cells were then incubated at 37°C for 10 min. The reaction was stopped by adding 1 volume of methanol at 0°C. The samples were treated as described above until analysis.

II. A second group of incubations were performed as described by Rosenbach et al [18] with small variations. 2 × 1⁰⁷ EC in 500 μl of RPMI containing 1 mM CaCl₂ were incubated with 100 μM 1⁴C-AAA plus 1 μM A23187 at 37°C. After 10 min the samples were centrifuged at 200 x g for 10 min and supernatants were collected. The pellets were resuspended in 500 μl of RPMI plus 1 mM CaCl₂, and then incubated at 37°C for another 30 min in the presence of 100 μM of 1⁴C-AAA plus 1 μM A23187. The samples were centrifuged at 4500 X g for 3 min, the supernatants were collected, and HCl 1 N (to yield pH 3.4) plus 500 μl of methanol were added. Pellets were washed twice with 250 μl of cold methanol and 500 μl of acidified RPMI were added to the methanolic extracts before analysis. For analysis, 500 μl of supernatants or pellet extracts were injected onto the chromatographic column.

**Transcellular Metabolism of LTAA:** Suspensions of PMN alone (2 × 1⁰⁷ PMN/ml) or PMN with EC (2 × 1⁰⁷ PMN/ml plus 8 × 1⁰³ EC) in Hanks’ buffer, pH 7.4, containing 2 mM CaCl₂, 1.5 mM MgCl₂, and 0.1% bovine serum albumin (BSA) were warmed at 37°C for 5 min. Then 0.5 ml aliquots of cellular suspensions were transferred to tubes containing ethanolic solutions of unlabeled AA (Sigma, St. Louis, MO), A23187, and prostaglandin B₂ (PGB₂).
Figure 1. Time course of eicosanoid production by EC suspensions incubated with 10 μM 14C-AA plus A23187. Results are expressed as pmoles of 14C-AA transformed by 10⁶ cells into (A) prostaglandins (sum of PGE₂₃, PGE₂, and PGD₂) or (B) 12-HETE. n = 2; mean, solid lines; individual experiments, dotted lines.

Figure 2. RP-HPLC profile of human EC incubated for 10 min at 37°C in the presence of 25 μM exogenous 14C-AA plus 5 μM A23187. Top chromatograms correspond to samples, bottom chromatograms correspond to standards. CP1 was used to separate mono and di-hydroxy acids (A). CP2 was used to analyze prostanooids and w-oxidized LTB₄ (B). 1, LTB₁; 2, HHT; 3, 15-HETE; 4, 12-HETE; 5, 5-HETE; 6, PGE₂₃, PGE₂, PGE₂; 8, PGD₂; 9, PGB₂; U, unknown compound derived from PGE₂.

The microwave-assisted synthesis procedure shown in Fig 1. Complex production of the sum of all prostanooids was observed to occur following incubation times between 2 and 10 min. 12-hydroxyeicosatetraenoic acid (12-HETE) formation reached a maximum at 10 min of incubation. A progressive decrease in the amount of prostanooids produced, especially of 12-HETE, was observed at incubation times longer than 10 min of incubation.

Figure 2 shows typical radiochromatograms obtained with the above-mentioned techniques after incubation of EC suspensions with 25 μM AA plus 5 μM A23187 for 10 min (incubation procedure I, see Materials and Methods). Three peaks co-eluting with the authentic standards of 12-hydroxy-epi-12TET (HHT), 15-HETE, and 12-HETE were found in chromatograms of samples derived from EC suspensions, obtained using CP1 (Fig 2A). No peak was found with a retention time corresponding to any AA derivative from 5-LO activity. Measurable peaks corresponding to HHT or 15-HETE were only occasionally present in the samples incubated with 10 μM of 14C-AA. Figure 2B shows the profile obtained using CP2, which allows for complete separation of prostanooids. Three peaks were observed that were found to co-elute with prostaglandins (PG) F₂₀, E₂, and D₂, respectively, a less polar unidentified peak (U), and a small peak with the retention time of PGB₂. Peak U was shown to be a non-enzymatic transformation product of PGE₂ by incubating labeled PGE₂, in the presence or in the absence of EC, for 15 min. Peak U appeared in both cases whereas the peak of PGE₂ decreased concomitantly. The decrease in PGE₂ production was found to be independent of the presence or absence of EC.

The profiles observed are shown in AA alone and with simultaneous addition of 5 μM A23187 were identical. In every case no detectable peaks corresponding to 5-LO products appeared. Peaks identified in Fig 2 as 2, 6, 7, 8, 9, and U totally disappeared when EC were pre-treated with 50 μM of indomethacin for 5 min before the addition of 14C-AA plus A23187. 12-HETE and PGE₂ were the eicosanoids produced by human EC in major amounts, as shown in Table I.

Figure 3 reproduces a series of chromatograms corresponding to an experiment using incubation procedure II (see Materials and Methods) in which internal labeled standards were introduced before sample injection onto the column (Fig 3E,F). No peak whatsoever corresponding to a metabolite derived from 5-LO activity was observed.

| Table I. Production of Eicosanoids by Human EC in Suspension After Incubation with 10 or 25 μM 14C-AA Plus 5 μM A23187 for 10 min |
|-------------|----------------|----------------|
| Compound    | [AA]            | [AA]            |
|             | (n = 10)        | (n = 4)         |
| PGE₂₀       | 3.60 ± 1.4      | 3.02 ± 2.2      |
| PGE₂        | 5.40 ± 4.3      | 9.10 ± 5.3      |
| PGE₂        | 2.10 ± 0.7      | 2.14 ± 1.6      |
| U           | 2.60 ± 1.8      | 6.10 ± 3.3      |
| HHT         | ND              | 2.04 ± 8.2      |
| 12-HETE     | 48.63 ± 13.2    | 69.20 ± 18.3    |
| 15-HETE     | ND              | 8.06 ± 6.2      |

* Results are expressed as pmoles of 14C-AA transformed into each compound by 10⁶ cells in 10 min, mean ± SD.
* Not detected in every case.

Statistics Statistical significance was assessed using the two-tailed Student t test. A p value below 0.05 was considered significant.

RESULTS

The results from time-course experiments are shown in Fig 1. Maximal production of the sum of all prostanooids was observed to occur following incubation times between 2 and 10 min. 12-hydroxyeicosatetraenoic acid (12-HETE) formation reached a maximum at 10 min of incubation. A progressive decrease in the amount of prostanooids produced, especially of 12-HETE, was observed at incubation times longer than 10 min of incubation.
found under these experimental conditions. In one out of three experiments, a peak appeared in the supernatant obtained following 10 min of incubation, with a retention time identical to that of 11-HETE. This peak always appeared following 30 min of incubation of the resuspended 200 X g pellet with 100 μM 14C-CAA (see Fig 5). Using CP2, the qualitative analysis of prostanoids did not differ from the results obtained using incubation procedure I (see Materials and Methods). Quantitative results are shown in Table II.

Table II. Production of Eicosanoids by Human EC in Suspension After Incubation with 100 μM 14C-CAA Plus 1 μM A23187 Following Incubation Procedure II Described in Materials and Methods

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sup 10 min</th>
<th>Sup 30 min</th>
<th>Pel 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>6.49 ± 3.89</td>
<td>6.51 ± 2.98</td>
<td>1.79 ± 0.4</td>
</tr>
<tr>
<td>PGE₃</td>
<td>14.89 ± 10.59</td>
<td>11.76 ± 6.73</td>
<td>1.72 ± 0.48</td>
</tr>
<tr>
<td>PGD₂</td>
<td>3.26 ± 1.8</td>
<td>1.87 ± 1.03</td>
<td>0.56 ± 0.32</td>
</tr>
<tr>
<td>U</td>
<td>4.48 ± 1.59</td>
<td>3.62 ± 1.2</td>
<td>0.57 ± 0.5</td>
</tr>
<tr>
<td>HHT</td>
<td>4.06 ± 2.97</td>
<td>2.94 ± 1.45</td>
<td>1.70 ± 1.47</td>
</tr>
<tr>
<td>12-HETE</td>
<td>9.85 ± 1.67</td>
<td>12.96 ± 3.42</td>
<td>19.09 ± 4.61</td>
</tr>
<tr>
<td>15-HETE</td>
<td>2.87 ± 1.1</td>
<td>4.38 ± 1.68</td>
<td>4.76 ± 1.42</td>
</tr>
<tr>
<td>11-HETE</td>
<td>ND¹</td>
<td>5.53 ± 1.11</td>
<td>7.14 ± 0.4</td>
</tr>
</tbody>
</table>

*After 10 min supernatants were analyzed (Sup. 10 min). Pellets were resuspended and incubated again with 100 μM 14C-CAA plus 1 μM A23187 for 30 min, supernatant (Sup 30 min) and pellet washes (Pel 30 min) were analyzed separately. Results are expressed as pmol of 14C-CAA transformed into each compound by 10⁶ cells, mean ± SD, n = 3. ¹ Detected only in one case.

Figure 4 depicts chromatograms obtained from a sample of mixed EC and PMN (A), or from the same PMN suspension alone (B), following incubation with 10 μM AA plus 5 μM A23187 for 3 min. Quantitative results are given in Table III. A significant (p < 0.05) increase in LTB₄ production was found when ionophore-activated PMN were co-incubated with EC, in comparison to the synthesis of LTB₄ by PMN alone under the same stimulating conditions. This...
Table III. Production of 6-Trans-LTB4 Isomers, LTB4, 5-HETE, and 12-HETE by Incubations of 10^7 PMN Alone or 10^7 PMN Plus 4 x 10^7 EC in 0.5 ml of Hanks’ Buffer, pH 7.4, in the Presence of 10 µM Exogenous AA + 5 µM A23187 + 0.1% BSA + 0.075 µM PGB2 (internal standard)\(^*\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>PMN</th>
<th>PMN + EC</th>
<th>EC</th>
<th>PMN + EC/PMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-trans-LTB4</td>
<td>53.7 ± 19.2</td>
<td>32.9 ± 1.4</td>
<td>0.61</td>
<td>—</td>
</tr>
<tr>
<td>LTB4</td>
<td>152.7 ± 31.8</td>
<td>263.3 ± 34.6</td>
<td>1.72*</td>
<td>—</td>
</tr>
<tr>
<td>5-HETE</td>
<td>509.8 ± 61.2</td>
<td>441.1 ± 53.6</td>
<td>0.86</td>
<td>—</td>
</tr>
<tr>
<td>12-HETE</td>
<td>407 ± 61.3</td>
<td>439 ± 44.1</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(* No products from 5-LO activity were found in the samples of EC alone (4 x 10^7 cells). CP (see Materials and Methods) was used in these experiments detecting peaks by absorption at 280 or 234 nm as indicated in Fig 4, as a consequence prostanoids were not analyzed in these experiments. The only peak detected in these conditions from incubations of EC alone was 12-HETE. Results are expressed as total pmole of AA transformed into each compound in 3 min, mean ± SD (n = 5). \(p < 0.05\).

The data presented herein show that the main products of AA metabolism in human EC suspensions were 12-HETE and PGF\(_{2\alpha}\), with PGG\(_{2\alpha}\) and PGD\(_2\) also produced in minor amounts. The only monohydroxy derivatives produced, as identified by their chromatographic behavior, were HHT, 12-HETE, 15-HETE, and 11-HETE. The amount of AA metabolized into 12-HETE was greater than that metabolized through the cyclooxygenase pathway. In no case could any 5-LO product be detected following incubation of EC suspensions with exogenous \(^{14}\)C-AA plus A23187, even after 1 h of incubation. Co-incubation with indomethacin diverted AA metabolism towards an increase in 12-HETE production; even in this case no 5-LO products were detectable. W-oxidation derivatives of LTB\(_4\) could not be found either. The detection limit of our analytical system was about 300 cpm/peak, which in the experimental conditions described (10^7 EC per incubation) corresponds approximately to a production of 1 pmole/10^7 EC.

There are no major qualitative differences between our results and those from Holtzman et al [17]. Nevertheless, these authors found greater production of PGE\(_2\) by EC. This may be due to the higher ratio [AA]/cell number in their incubation mixture, which can favor the production of the minor compounds because of greater substrate excess. Rosenbach et al [18] described PGD\(_2\) as the major prostanoid produced from exogenous AA. This finding may be due to the different chromatographic procedures they used. It is possible that in the work of Rosenbach et al the broad spot corresponding to PGD\(_2\) became over-evaluated by thin-layer chromatography because it was not well separated from other prostaglandins such as PGA\(_2\) and/or PGB\(_2\) derived from PGE\(_2\).

As has been mentioned above, no products of 5-LO activity in EC suspensions could be detected, in contrast with the findings of other authors [18–20], who have reported the detection of LTB\(_4\) and 5-HETE by means of HPLC techniques [18,19] or the presence of minimal amounts of LTB\(_4\)-like activity [20]. We also performed experiments using an incubation procedure similar to that used by Rosenbach et al [18] (incubation procedure II, see Materials and Methods) in an attempt to find an explanation for the discrepancies between our results and those from these authors. They found chromatographic peaks corresponding to LTB\(_4\) and 5-HETE in double incubations of human keratinocytes or Langerhans cells with 0.8 µM \(^{14}\)C- AA plus a very high concentration of unlabeled AA (100 µM) plus A23187. Under these experimental conditions but using 100 µM \(^{14}\)C- AA exclusively, we observed the appearance of three peaks that were not present at lower AA concentrations. Two of these peaks were of pmole order of magnitude and their retention times were close to and greater than that of LT (see Fig 3). The third new peak eluted at the same time as 11-HETE. 11-HETE can come from cyclooxygenase activity in the presence of a great excess of substrate [35]. We do not have a coherent explanation for the discrepancies between our results and those of Rosenbach et al. In our experimental conditions, loss of eicosanoids during manipulation was avoided by direct chromatography with no prior extraction or purification procedures (recovery for PGB\(_2\) was always greater than 99%). Moreover, due to the low water solubility of the monohydroxy acids, which could cause their partial retention in the cell pellet [36], we washed the cell pellets twice with methanol after the second incubation.

The release of LTA\(_4\) from PMN has been demonstrated in vitro using trapping experiments with methanol or ethanol added to the incubation supernatants, and analyzing the formation of 12-methoxy- or 12-ethoxy-derivatives of LTA\(_4\) [37]. LTA\(_4\) released by PMN has been shown to be transformed to LTB\(_4\) or peptido-leukotrienes by different cellular types when albumin is present in the incubation medium. Albumin increases dramatically the half life of LTA\(_4\) in aqueous medium, protecting LTA\(_4\) from non-enzymatic degradation because of its hydrophobic interaction [38].

We have shown that transcellular metabolism of LTA\(_4\) by cooperation between PMN and EC is produced in vitro, because EC efficiently transform LTA\(_4\) produced by PMN stimulated with 5 µM A23187. When EC were co-incubated with PMN in the presence of A23187, the peak-area corresponding to LTB\(_4\) was 1.72 times that obtained when PMN were incubated alone, whereas peaks corresponding to 6-trans-LTB\(_4\) isomers decreased concomitantly. These results, together with the fact that 5-HETE production was the same in the presence or in the absence of EC, strongly suggest that enzymatic transformation of LTA\(_4\) is enhanced in the mixed incubations, whereas only PMN are able to synthesize LTA\(_4\).

In addition, we describe in this paper the production of LTB\(_4\) by EC suspensions incubated with commercial LTA\(_4\) in the absence of any stimulating agent. However, no peptido-leukotriene appeared in the chromatograms, which suggest that an epoxide hydrolase activity exists in human EC. Epoxide hydrolase is present in the cytosolic fraction of most of the cells that possess the enzyme, but more investigation is needed in order to characterize this enzymatic activity and to determine its cellular location in human EC.

Our findings suggest that transcellular metabolism of eicosanoids might provide an additional source of eicosanoids, and especially LTB\(_4\), in inflammatory dermatoses and other diseases with an important component of PMN in the inflammatory infiltrate such as psoriasis. The cooperation between EC and PMN in the synthesis of
LTB₄ in skin inflammatory lesions could presumably potentiate the effect of epidermal infiltrating PMN by increasing the local concentrations of LTB₄ and enhancing its chemotactic and proliferative effects. Further research is required to assess the effect of different PMN/EC ratios, correlate them with the in vivo situations and evaluate the actual biologic and pathophysiological relevance of PMN/EC biosynthetic cooperation. Transcellular metabolism of eicosanoids might prove to be an interesting area of research employing mixed cell suspensions as in vitro models more akin to the in vivo situation.

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REFERENCES