Decreased Leukotriene C4 Synthesis Accompanies Adherence-Dependent Nuclear Import of 5-Lipoxygenase in Human Blood Eosinophils

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*J Immunol* 1999; 162:1669-1676; ;
http://www.jimmunol.org/content/162/3/1669
Decreased Leukotriene C₄ Synthesis Accompanies Adherence-Dependent Nuclear Import of 5-Lipoxygenase in Human Blood Eosinophils¹

Thomas G. Brock,²*, James A. Anderson,‡ Francine P. Fries,‡ Marc Peters-Golden,* and Peter H. S. Sporn‡

The enzyme 5-lipoxygenase (5-LO) catalyzes the synthesis of leukotrienes (LTs) from arachidonic acid (AA). Adherence or recruitment of polymorphonuclear neutrophils (PMN) induces nuclear import of 5-LO from the cytosol, which is associated with enhanced LTB₄ synthesis upon subsequent cell stimulation. In this study, we asked whether adherence of human eosinophils (EOS) causes a similar redistribution of 5-LO and an increase in LTC₄ synthesis. Purified blood EOS examined either in suspension or after adherence to fibronectin for 5 min contained only cytosolic 5-LO. Cell stimulation resulted in activation of 5-LO, as evidenced by its translocation to membranes and LTC₄ synthesis. As with PMN, adherence of EOS to fibronectin for 120 min caused nuclear import of 5-LO. Unexpectedly, however, adherence also caused a time-dependent decrease in LTC₄ synthesis: EOS adhered for 120 min produced 90% less LTC₄ than did cells adhered for 5 min. Adherence did not diminish the release of [³H]AA from prelabeled EOS or reduce the synthesis of the prostanoids thromboxane and PGE₂. Also, inhibition of LTC₄ production caused by adherence could not be overcome by the addition of exogenous AA. Adherence increased, rather than decreased, LTC₄ synthase activity. However, the stimulation of adherent EOS failed to induce translocation of 5-LO from the nucleoplasm to the nuclear envelope. This resistance to activation of the nuclear pool of 5-LO with diminished LT production represents a novel mode of regulation of the enzyme, distinct from the paradigm of up-regulated LT synthesis associated with intranuclear localization of 5-LO observed in PMN and other cell types. The Journal of Immunology, 1999, 162: 1669–1676.

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Received for publication July 1, 1998. Accepted for publication October 23, 1998.

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¹ This work was supported by the American Lung Association (T.G.B.), the Department of Veterans Affairs (P.H.S.S.), and the Cornelius Crane Asthma Center of Northwestern University (P.H.S.S.). T.G.B. is a Parker B. Francis Fellow in Pulmonary Research, an Edward Livingston Trudeau Scholar of the American Lung Association, and Aberg’s Research Grant Awardee.

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³ Abbreviations used in this paper: LT, leukotriene; 5-LO, 5-lipoxygenase; AA, arachidonic acid; DPI, diphenylene iodonium; EIA, enzyme immunoassay; EOS, eosinophils; FLAP, 5-lipoxygenase-activating protein; PMN, polymorphonuclear neutrophils.

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source of cysteinyl LTs released into the airways in allergic asthma or rhinitis following allergen challenge (21, 22). However, the compartmentation of 5-LO has not been comprehensively examined in this cell type. Because a similar phenomenon in PMNs could be relevant to the overproduction of cysteinyl LTs by EOS, we examined the effect of adherence on the subcellular localization of 5-LO in human EOS in the current study. We then asked whether adherence up-regulates the synthesis of cysteinyl LTs in EOS. We report that although adherence of EOS to fibronectin causes nuclear import of 5-LO, it actually decreases, rather than increases, cysteinyl LT synthetic capacity. The decrease in LTC4 production cannot be ascribed to changes in AA availability, LTC4 synthase activity or oxidative metabolism of LTC4. Instead, the compartmentation of 5-LO within the nucleus appears to protect it from activation, because intranuclear 5-LO translocates poorly to the nuclear envelope upon cell stimulation. As translocation is thought to be essential for 5-LO action, the failure to translocate may explain the diminished metabolism of AA to LTs. These results, taken from in vitro experiments, support a role for nuclear import of 5-LO in regulating cysteinyl LT synthetic capacity in vivo both in normal immune responses and disease states such as asthma.

Materials and Methods
Preparation of human peripheral blood EOS
Blood for isolation of human EOS was obtained from volunteers with a clinical history of mild atopic disease (in most cases allergic rhinitis) and a peripheral eosinophilia of ≥3%. These subjects were all nonsmokers and were not taking oral or inhaled steroids, nonsteroidal anti-inflammatory drugs, LT synthesis inhibitors, or LT receptor antagonists. EOS were isolated by a modification of the method of Hansel et al. (23). Briefly, heparinized venous blood was centrifuged through Percoll (Pharmacia & Upjohn, Kalamazoo, MI), (density 1.084 g/ml), and erythrocytes were removed by hypotonic lysis. The remaining granulocytic fraction was washed, counted, and incubated with MACS CD16 MicroBeads (Miltenyi Biotec, Auburn, CA) on ice for 30 min. The granulocyte suspension was then applied to type CS magnetic columns (Miltenyi Biotec) in a 0.6 Tesla magnetic field, and the eluate containing EOS was collected. Eluates were washed and resuspended in HBSS containing 2% FCS. The resulting cell suspensions derived by this method were 97 ± 1% viable (mean ± SEM; n = 9).

Cell culture, adherence, and stimulation
For the culture of EOS, individual wells in tissue culture plates or Lab-Tek Permanox chamber slides (Fisher Scientific, Pittsburgh, PA) were coated with human plasma fibronectin (Collaborative Biomedical Products, Bedford, MA) by incubation overnight at 4°C with fibronectin at 100 μg/ml. Then the wells were washed twice with PBS. To block unoccupied binding sites, the wells were incubated for an additional 2 h at 4°C with 0.1% BSA, and washed twice more with PBS. Freshly purified EOS (10^6 cells in 200 μl) were either maintained in suspension in siliconized tubes or adhered to fibronectin-precoated 96-well plates in HBSS + 2% FCS and incubated at 37°C in 5% CO2/95% air for times ranging from 5 to 120 min. Cells were then washed, resuspended in HBSS + 2% FCS, and plated in fibronectin-precoated 24-well plates at 5 × 10^5 cells/ml/well.

Indirect immunofluorescent microscopy
As described previously (13), cells that were either maintained in suspension or adhered to fibronectin-coated slides were fixed in −20°C methanol, permeabilized in 1% acetone, and air dried. Cells were rehydrated and blocked with 1% BSA in PBS containing nonimmune goat serum. Rabbit polyclonal Ab raised against purified human leukocyte 5-LO (a generous gift from Dr. J. Evans, Merck Frosst Centre for Therapeutic Research, Pointe Claire-Dorval, Quebec, Canada; Ref. 24) was prepared in 1% BSA-PBS (titer, 1:200) and applied for 1 h, 37°C. Nonspecific sites were washed with 1% BSA-PBS and incubated with rhodamine-conjugated goat anti-rabbit Ab (1:200; Sigma) for 1 h, 37°C, then washed extensively and coverslipped. Fluorescence was visualized with a Zeiss Axioplan microscope equipped for epifluorescence, or imaged by confocal microscopy using a Bio-Rad MRC-600 laser confocal microscope (25). During confocal microscopy, the rhodamine signal was imaged using a 560 nm long pass filter followed by a 585-nm bandpass filter to minimize the contribution due to autofluorescence.

Enzyme immunoassay (EIA) of eicosanoids
Immunoreactive eicosanoids (LTC4, PGE2, thromboxane B2) in conditioned media were quantitated by EIA (Cytochrome C, Chemical), according to the supplier’s instructions. Means of data for duplicate wells for each condition were counted as single data points in analyses of multiple experiments.

Prelabeling with [3H]AA and stimulation of [3H]AA release
In selected experiments, freshly isolated EOS were prelabelled with [3H]AA (60–100 Ci/mmol; DuPont/NEN, Wilmington, DE) by incubating with 0.5 μCi [3H]AA per 5 × 10^6 cells/ml in serum-free RPMI 1640 for 90 min at 37°C. Cells were then washed, resuspended in HBSS + 2% FCS, and plated in fibronectin-precoated 24-well plates at 5 × 10^5 cells/ml/well. After incubation to allow adherence for 5–120 min, EOS were stimulated by the addition of 2 μM A23187. After 10 min, cultures were acidified to pH 3.0 with 1 N HCl, and media plus cells were extracted with chloroform: methanol as described previously (26). Free [3H]AA was separated by thin layer chromatography (26) and quantitated by liquid scintillation spectrometry. Data are expressed as a percent of the total [3H]AA radioactivity incorporated by EOS, which was determined from parallel unstimulated wells in each experiment.

Whole cell LTC4 synthase assay
EOS (10^6 cells in 200 μl) were either maintained in suspension in siliconized tubes or adhered to fibronectin-precoated 96-well plates in HBSS + 2% FCS and incubated at 37°C in 5% CO2/95% air for 5 or 120 min. LTC4 synthase was assayed in intact cells by a slight modification of the method of Ali et al. (27) as follows. Before the reaction, 2.5 mM acivicin (Sigma), an inhibitor of γ-glutamyl transpeptidase, was added to cells. LTA4 was obtained by hydrolysis of LTA4 methyl ester (Cytochrome C) with NaOH under argon according to the supplier’s instructions immediately before use at each time point. The reaction was started by addition of LTA4 at a final concentration of 40 μM and allowed to proceed at 37°C. After 4 min, media were removed from adherent cultures and immediately microcentrifuged along with suspension cultures. Supernatants were removed and stored at −85°C until assay for LTC4 by EIA.

Stimulation and analysis of superoxide production
Freshly purified EOS were adhered to fibronectin-precoated 96-well plates for 5 or 120 min, then stimulated with either 1 μM PMA (Sigma) or 2 μM A23187 in the presence or absence of 2 μM DPI. Superoxide was determined by monitoring the superoxide dismutase inhabitable reduction of ferricytochrome C (Sigma) at 37°C in a THERMOMax microplate reader (Molecular Devices, Menlo Park, CA), as described (28). Duplicate wells were assayed for each condition and the results were averaged.

Evaluation of translocation of nuclear 5-LO
Cells stained for 5-LO were visualized by confocal microscopy, captured on disk using CoMoS software (Bio-Rad Life Sciences, Hercules, CA), and black and white images were adjusted to full gray scale range (0–255) that defines black as 0 and white as 255. Image files were analyzed using NIH Image software: the central area within lobes of nuclei of individual cells, −625 pixels2 was densitometrically quantitated. Using the value of 150 or less (on a scale of 0–255) as a conservative estimation of a darkened field, nuclei with mean nuclear gray scale values of <150 were defined as demonstrating translocation of 5-LO from the interior of the nucleus to the nuclear envelope. Cells from 10 separate fields (>20 cells per field) were scored per slide per condition.

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FIGURE 1. Subcellular localization of 5-LO in human EOS. A, freshly purified cells maintained in suspension for 5 min, fixed, mounted, and then stained for 5-LO. Bar = 10 μM for A and E, B–D, Cells adhered to fibronectin for 5 min before fixation and stained for 5-LO. B, Red signal from green excitation (rhodamine indicating 5-LO). Bar = 10 μM for B–D. C, Green signal from blue excitation (autofluorescence). D, Overlay of A and B. The overlay of emission signals in this false color presentation appears yellow. E, Control cells maintained in suspension for 120 min, then fixed, mounted, and stained for 5-LO. F–H, Cells adhered to fibronectin for 120 min. F, Red signal from green excitation (rhodamine indicating 5-LO). Bar = 10 μm for F–H. G, Green signal from blue excitation (autofluorescence). H, Overlay of F and H. Results are representative of seven independent experiments.

Statistical analysis
In most cases, experiments were repeated at least three times, using different human donors. Values were expressed as means ± SEM. Statistical significance was evaluated by Analysis of Variance (ANOVA), using p < 0.05 as indicative of statistical significance.

Results
Subcellular localization of 5-LO in human EOS
Previous studies have indicated that adherence of EOS to fibronectin can alter the regulation of AA metabolism in these cells (29, 30). For this reason, we examined freshly purified human EOS that were either maintained in suspension in silicon-treated tubes or adhered to fibronectin-coated slides before treatment. In cells that were maintained in suspension and then fixed and stained for 5-LO, fluorescence was greatest throughout the cytosolic compartment and notably absent from the bi-lobed nucleus (Fig. 1A). Punctate fluorescence was also evident in many cells, suggesting the association of 5-LO with lipid bodies, as has been reported previously (31). A similar pattern with the nuclei demonstrating a lack of staining, was observed in EOS that adhered to fibronectin for only 5 min (Fig. 1B). Preliminary experiments showed that the granules within fixed EOS displayed significant autofluorescence when excited with blue light (seen as a green emission signal; Fig. 1C), and this was used to further examine the subcellular localization of 5-LO. In a red-blue-green image analysis, areas of overlap of red and green signals appear as yellow. The overlay of Fig. 1B and C (Fig. 1D) indicated broad colocalization of 5-LO with the granules, as most of each cell appeared yellow and essentially no 5-LO was associated with the nucleus. By this method, it was not possible to ascertain whether 5-LO was directly associated with the granules or merely in the cytoplasm that surrounds the granules. However, these results are consistent with those of Weller and colleagues (31), who reported diffuse cytoplasmic staining for 5-LO obtained by immunocytochemical methods in freshly purified human EOS. Our results further indicated that there was no significant difference in the subcellular localization of 5-LO before and immediately after adherence to fibronectin.

To assess the effect of adherence on 5-LO localization in EOS, cells were examined after either suspension culture or prolonged adherence to fibronectin-coated slides. When EOS were maintained in suspension for 120 min, then fixed and stained for 5-LO, the enzyme was found solely in the cytoplasmic compartment (Fig. 1E), as observed in freshly purified EOS. However, when EOS were adhered to fibronectin for 120 min and then stained, 5-LO was found predominantly within the nucleus (Fig. 1F). In all cells, the bi-lobed nucleus was heavily stained and brighter in fluorescence than the bulk of the cytosol. In contrast, the autofluorescence of the granules illuminated the cytoplasmic compartment of adherent EOS excited with blue light (Fig. 1G). The overlaid images separated the specific signal of 5-LO again, now in the nucleus from the nonspecific autofluorescent signal: the cytoplasm, which appeared yellow in briefly adhered EOS (Fig. 1D), now remained largely green (Fig. 1H). The redistribution of 5-LO into the nucleus was evident after adherence for 30 min and appeared to be complete by 60 min (data not shown).

To verify that 5-LO was in fact moving into the bi-lobed nucleus of EOS, optical sections of individual cells stained for 5-LO were prepared by confocal microscopy (Fig. 2). Serial sections of suspension-cultured EOS stained for 5-LO showed bright cytosolic staining with punctate cytosolic inclusions offset by deep nonstaining pockets corresponding to the nuclear lobes (Fig. 2A–D). On the other hand, serial sections of EOS that adhered for 120 min to fibronectin showed 5-LO within the nucleus in all optical slices (Fig. 2E–H), confirming the intranuclear localization of the enzyme as opposed to association only with the nuclear envelope.
Our previous studies with PMNs indicated that recruitment into sites of inflammation, like adherence, resulted in the import of 5-LO from the cytosol into the nucleus (18). To test whether recruitment promoted a similar response in EOS, leukocytes were recovered from rat peritoneum 4 h after glycogen instillation. Differential counting of recruited leukocytes following Wright-Giemsa staining indicated that 3–6% of all leukocytes were EOS. When unstained samples were prepared for indirect immunofluorescent microscopy, 5-LO was localized to the nucleus of all cells (Fig. 3A). Approximately 5% of these cells, a number corresponding to the percent EOS determined by differential counting, were characterized by the intense autofluorescence under blue light that is typical of EOS (Fig. 3B). Thus, as with PMNs, recruited EOS contained intranuclear 5-LO.

5-LO activation and AA metabolism in adherent human EOS

Recent studies have demonstrated that upon activation cytosolic 5-LO translocates to the nuclear envelope and the associated endoplasmic reticulum (ER) in peritoneal (14) and alveolar (16, 17) macrophages, PMN (18, 24), and rat basophilic leukemia cells (16, 25). When EOS were adhered to fibronectin for 5 min and then stimulated with the chemotactic peptide FMLP, 5-LO was distributed in a perinuclear pattern, presumably at the nuclear envelope and ER (Fig. 4A). 5-LO was also evident in cytosolic inclusions that might correspond to lipid bodies as has been described (31). In response to stimulation with the calcium ionophore A23187, 5-LO was abundantly evident at the nuclear envelope and also within the cell body at a site discrete from the nuclear envelope (Fig. 4B). This extranuclear 5-LO was greatest at the center of the cell body, consistent with localization on a cytoplasmic membrane system, most likely the ER. The discrete inclusions, evident in resting and FMLP-stimulated EOS stained for 5-LO, were absent after stimulation with A23187.

Human EOS isolated by anti-CD16 negative immunomagnetic selection were not stimulated to produce any detectable immunoreactive LTC4 in the absence of agonist stimulation (data not shown). On the other hand, EOS stimulated with calcium ionophore A23187 produced large amounts of LTC4. Notably, after adherence to fibronectin for 5 min, EOS released about twice as much LTC4 as cells maintained in suspension for the same time when stimulated with 2 μM A23187 (Fig. 5A). This is similar to the enhancement of LTC4 synthesis seen in EOS adhered to fibronectin as compared with BSA, reported previously by Anwar et al. (30). Surprisingly, we found that continued incubation on fibronectin resulted in a significant loss in capacity to release LTC4 upon subsequent stimulation: EOS adhered for 120 min released ~10% of that released by cells adhered for only 5 min. In contrast, cells maintained in suspension for 120 min under the same conditions released amounts of LTC4 that were similar to those produced by cells in suspension at 5 min. As for EOS stimulated with A23187 (Fig. 5A), cells adhered for 120 min and then stimulated with FMLP plus cytochalasin B produced about 90% less LTC4 than cells adhered for only 5 min (Fig. 5B). The adherence-associated decline in capacity for synthesis of LTC4 could not be overcome by stimulating EOS with higher concentrations of A23187 (up to 10 μM; data not shown). Decreased LTC4 synthesis in adherent EOS was not due to loss of viability under these culture conditions, because 98% of cells still excluded trypan blue after adherence for 120 min.
Diminished LTC₄ production after adherence could possibly result from decreased activity of phospholipase(s), leading to reduced availability of AA, the substrate for 5-LO. However, adherence did not significantly affect the release of [³H]AA from prelabeled EOS stimulated with A23187 (Fig. 6A). Also, the addition of exogenous AA (10 μM) could not overcome the loss of LTC₄ synthetic capacity observed with 120-min adherence (Fig. 6B). Finally, adherence to fibronectin was not associated with a decline in capacity for synthesis of thromboxane or PGE₂ over time (data not shown). These results indicate that the decline in LTC₄ production in adherent EOS does not result from reduced phospholipase-mediated release of AA.

Decreased LTC₄ synthesis could also result from reduced conversion of the 5-LO product LTA₄ to LTC₄ by LTC₄ synthase. However, EOS adhered for 120 min showed an enhanced, rather than diminished, ability to convert exogenous LTA₄ to LTC₄, as compared with cells adhered for only 5 min (Fig. 7). Thus, the diminished LT production could not be due to reduced LTC₄ synthase activity.

Finally, decreased measurable LTC₄ could result from oxidative degradation by EOS of newly synthesized LT. This has been shown to occur by a myeloperoxidase-dependent mechanism after stimulation of the respiratory burst in isolated human blood PMN (32, 33). To assess whether oxidative LTC₄ catabolism might occur in adherent EOS, we determined their capacity for generation of superoxide anion (O₂⁻) after adherence for 5 and 120 min. As shown in Table I, stimulation with PMA resulted in generation of nearly 10-fold less O₂⁻ by EOS adhered for 120 min as compared with those adhered for 5 min, suggesting that the potential for oxidative LT metabolism actually declines with longer adherence time. Table I also shows that, in contrast to PMA, A23187 is only a weak stimulus of O₂⁻ production in EOS. Finally, DPI, an inhibitor of NADPH oxidase (34), did not prevent the decrease in synthesis of immunoreactive LTC₄ from 120 min adherent EOS, despite the fact that it effectively blocked O₂⁻ production by PMA-stimulated EOS. Taken together, these data indicate that the...
agonist did not enhance 5-LO translocation in 120 min-adherent EOS, suggesting partial translocation within these cells. Higher doses of DPI. O2– generation and LTC4 released into culture supernatants were determined in separate wells (in duplicate) as the SOD-inhibitable reduction of ferricytochrome C and by EIA, respectively.

**Effect of adherence on the translocation of 5-LO**

In PMN, the movement of 5-LO into the nucleus was accompanied by a 3- to 5-fold increase in the stimulated LTB4 production in those cells (18); in EOS, the same movement correlated with a 90% decrease in stimulated LTC4 production. The translocation of 5-LO from the nucleoplasm to the nuclear envelope was readily detected following stimulation of recruited PMN with A23187 in our previous study (18). Translocation of cytoplasmic 5-LO was also observable following stimulation of EOS with either FMLP or A23187 after adherence to fibronectin for only 5 min (Fig. 4). However, when EOS were adhered for 120 min and then stimulated, the intranuclear pool 5-LO rarely exhibited translocation to the nuclear envelope. No cells showed translocation of intranuclear 5-LO in response to stimulation with FMLP in the presence of cytochalasin B (Fig. 8A). The vast majority of cells stimulated with the calcium ionophore A23187 also failed to show translocation of intranuclear 5-LO (Fig. 8B), although some darkening of the nuclear compartment that characteristically accompanies translocation was clearly detectable in some cells (Fig. 8C). Quantitatively, only 8.3 ± 3.7% of EOS stimulated with 2 μM A23187 showed pronounced translocation of intranuclear 5-LO. Interestingly, patches of 5-LO fluorescence were evident at the periphery of nuclei in some A23187-stimulated EOS (arrows in Fig. 8B), suggesting partial translocation within these cells. Higher doses of agonist did not enhance 5-LO translocation in 120 min-adherent EOS (data not shown).

**Discussion**

EOS are the predominant infiltrating inflammatory cells in asthma and other allergic diseases, and cysteiny1 LTs secreted from EOS are important mediators of the bronchospasm, mucus secretion, and edema that are associated with these diseases. As 5-LO catalyzes a rate-limiting step in LT synthesis, understanding its regulation in EOS is important to understanding the basis for asthma and allergic responses. Recent findings by our lab and others have demonstrated that the subcellular distribution of 5-LO among different cell types is heterogeneous. That is, 5-LO is cytosolic in resting monocytes (17) and peritoneal macrophages (14), whereas it is accumulated within the nucleus of alveolar macrophages (17), mast cells (15), and rat basophilic leukemia cells (16). Interestingly, in resting blood PMNs, which like EOS are granulocytes, 5-LO is cytosolic (16), as we have found to be the case in resting blood EOS in this study. More recently, we have reported that the subcellular distribution of 5-LO in resting PMNs is dynamic. Thus, when PMNs are recruited into sites of inflammation or are adhered to surfaces, 5-LO moves from the cytosol to the nucleolus (18). We report here that a similar shift in subcellular compartmentation of 5-LO occurs in unstimulated EOS, either after prolonged adherence or after recruitment into the inflamed rat peritoneum. Finally, 5-LO has recently been localized to cytosolic lipid bodies as well as the cytoplasm of freshly purified blood EOS treated in suspension with platelet-activating factor (31).

An important finding of the current study is that nuclear import of 5-LO, which accompanies prolonged adherence, is associated with a diminished LT synthetic capacity in EOS. This result is particularly surprising in light of the fact that intranuclear compartmentation of 5-LO correlated with enhanced LT synthetic capacity in PMNs after recruitment (18), or in alveolar macrophages (as compared with peritoneal macrophages or blood monocytes) (19). The reduced production of LTC4 in EOS after adherence could not be attributed to changes in the activities of phospholipases (measured as AA release), LTC4 synthase (measured as the conversion of LTA4 to LTC4), or the oxidative metabolism of LTC4. These results highlighted a specific defect in the ability of 5-LO to metabolize AA to LTA4 in adherent EOS.

**Table I. Effect of NADPH oxidase inhibition on O2– generation and LTC4 synthesis in eosinophils adhered for 5 or 120 min**

<table>
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<th>O2– (μmol/min/106 cells)</th>
<th>LTC4 (pg/106 cells)</th>
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<td>5 min</td>
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<td>PMA</td>
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<td>PMA + DPI</td>
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<tr>
<td>A23187</td>
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<tr>
<td>A23187 + DPI</td>
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* EOS (10⁵/well) were adhered to fibronectin-coated plastic for 5 or 120 min, then stimulated with PMA (1 μM) or A23187 (2 μM) in the absence or presence of 2 μM DPI. O2– generation and LTC4 released into culture supernatants were determined in separate wells (in duplicate) as the SOD-inhibitable reduction of ferricytochrome C and by EIA, respectively.

* ND, not done.

**Figure 8.** Subcellular localization of 5-LO in EOS adhered to fibronectin-coated plastic for 60 min, then washed and stimulated with either FMLP plus cytochalasin B (A) or A23187 (B). An example of translocation in response to A23187 stimulation of adherent EOS is also given (C). Arrows indicate sites of patchy accumulation of 5-LO at the edge of the nucleus. Results are representative of three independent experiments for FMLP and eight experiments for A23187.
translocate to the nuclear envelope would dissociate 5-LO from both substrate release and the 5-LO activating action of FLAP at that site. It is also possible that the action of intranuclear 5-LO is poorly coupled to that of LTC\(_4\) synthase, so that the 5-LO product, LTA\(_4\), would be inefficiently converted to LTC\(_4\). We have not yet investigated this possibility.

Why doesn’t intranuclear 5-LO translocate to the nuclear envelope? Translocation of 5-LO is a calcium-dependent process (35) and may also require direct phosphorylation of the enzyme by protein tyrosine kinases (36, 37). A failure in either of these two processes may be sufficient to block translocation. In particular, precedents exist for the transient changes in calcium within the nucleus that would be needed to drive 5-LO translocation to be either tightly linked to changes in cytosolic calcium (38, 39) or regulated independently (40). Stimulation of EOS with FMLP or ionophore, as performed in this study, may have been sufficient to cause a calcium transient in the cytosol but not in the nucleus, and as a result activated cytosolic, but not intranuclear, 5-LO. Alternatively, the directed translocation of 5-LO may require additional cofactors or accessory proteins that have yet to be identified, and these may be lacking in the nucleus of EOS.

The current study presents results regarding 5-LO compartmentation and the regulation of LT synthesis that are provocative and perhaps paradoxical, particularly in the context of asthma and allergy. Many atopic individuals, and in particular aspirin-sensitive asthmatics, have an elevated LTC\(_4\) synthetic capacity (41–43), and airway EOSs are considered to be a major source of LTC\(_4\) in allergic responses and asthma (44). On the other hand, adherence to matrix proteins like fibronectin is necessary for the recruitment of EOSs into the airway, and we report here that this adherence diminishes, rather than elevates, LTC\(_4\) synthetic capacity. This apparent conflict may be reconciled through several considerations. First, our results may reflect the response of recruited EOS in nonallergic conditions. Therefore, a failure to import 5-LO and thus decrease LTC\(_4\) synthesis during cell recruitment may be a key element of allergic responses and some types of asthma. The nuclear import of larger proteins, like 5-LO, requires the presence of a nuclear import sequence on that protein (45, 46), which may be activated or deactivated by phosphorylation at a neighboring site on the same protein (47). A failure to activate the nuclear import sequence on 5-LO, e.g., by blocking phosphorylation-mediated activation, could prevent its import during adherence and recruitment, in effect “locking” the EOS in a high LTC\(_4\)-producing mode. This finding would predict that elicited EOSs in normal individuals would show intranuclear 5-LO, as we found in elicited rat EOSs (Fig. 3), whereas in conditions characterized by overproduction of LTC\(_4\), recruited EOSs would show cytoplasmic 5-LO. This possibility is currently under investigation.

A second consideration regarding our findings is that both nuclear import of 5-LO and diminished LT synthesis may be temporal consequences of adherence. That is, with cell adherence for extended periods of time (i.e., greater than 120 min), 5-LO may return to the cytoplasm, resulting in a recovery of LTC\(_4\) synthetic capacity. Augmented production of LTC\(_4\) may then be driven by extracellular factors, such as platelet-activating factor (31), granulocyte-macrophage CSF (48), IL-3 and IL-5 (49), or TNF (50). As a third consideration, nuclear import of 5-LO may not necessarily result in down-regulation of LTC\(_4\) synthetic capacity in vivo. Although we have shown that EOS recruited into the rat peritoneum show intranuclear 5-LO, we have not determined whether they have a diminished capacity to synthesize LTC\(_4\), as do cells adhered to fibronectin in vitro. This too may depend on extracellular factors that may have the capacity to prime EOS for LTC\(_4\) production. Finally, our results may only apply to our experimental conditions; we already know that they do not necessarily apply to other cell types (e.g., PMNs), and they may differ with alternate matrices or culture conditions. Clearly, further studies, particularly in individuals with asthma or other allergic conditions, are needed to truly assess the relevance of our findings to those conditions.

Acknowledgments

We thank Dr. Jilly Evans for the generous donation of the 5-LO Ab.

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