Nuclear localization of leukotriene A₄ hydrolase in type II alveolar epithelial cells in normal and fibrotic lung

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Brock, Thomas G., Young-Jik Lee, Elana Maydanski, Tessa L. Marburger, Ming Luo, Robert Paine III, and Marc Peters-Golden. Nuclear localization of leukotriene A₄ hydrolase in type II alveolar epithelial cells of normal and fibrotic lung. Am J Physiol Lung Cell Mol Physiol 289: L224–L232, 2005. First published April 1, 2005; doi:10.1152/ajplung.00423.2004.—Leukotriene A₄ (LTA₄) hydrolase is found in many cell types. LTA₄ hydrolase, on the other hand, is found in 5-LO− cells, including alveolar epithelial cells (4, 5). In the absence of 5-LO to initiate leukotriene synthesis, these cells cannot, by themselves, generate appreciable LTB₄. However, they can produce LTB₄ through a “transcellular” mechanism, accepting LTA₄ donated by adjacent neutrophils as substrate for LTA₄ hydrolase (25). In this way, the unstable LTA₄ that is secreted by neutrophils is metabolized to LTB₄ before it can break down. Thus LTA₄ hydrolase in cells lacking 5-LO may be important in determining the total amount of LTB₄ produced in tissues containing recruited leukocytes. The enzyme LTA₄ hydrolase has been shown to be a soluble protein (18, 49). Previously, we reported (7) that, in 5-LO−-containing leukocytes, the subcellular distribution of LTA₄ hydrolase was cell type specific, colocalizing with 5-LO in the nucleoplasm of alveolar macrophages and rat basophilic leukemia cells but remaining in the cytoplasm of neutrophils. During that study, we noticed that certain alveolar epithelial cells also appeared to have LTA₄ hydrolase accumulated in the nucleus. Here, we demonstrate that the localization of LTA₄ hydrolase in alveolar epithelial cells depends on the state of differentiation of the cell, being nuclear in type II cells but cytoplasmic in type I cells. The change in localization of LTA₄ hydrolase occurs with differentiation both in vivo and in vitro. Interestingly, the subcellular localization of LTA₄ hydrolase does not appear to affect the capacity of epithelial cells to convert exogenously supplied LTA₄ to secretable LTB₄. However, the proliferation of A549 cells, which have nuclear LTA₄ hydrolase, is inhibited by the aminopeptidase inhibitor bestatin, whereas that of NIH/3T3 cells, which have exclusively cytoplasmic LTA₄ hydrolase, is not.

LTA₄, which is then modified by the epoxide hydrolase activity of LTA₄ hydrolase to generate LTB₄ (27). Independent of its epoxide hydrolase activity, LTA₄ hydrolase also has an aminopeptidase activity (28, 39) and can be inhibited by aminopeptidase inhibitors, including bestatin (44). LTA₄ hydrolase has a high affinity for short peptides (43), including the enkephalins and dynorphins (24, 41). These aspects of LTA₄ hydrolase have been demonstrated in vitro but have not been found in vivo. In fact, comparison of wild-type mice and those deficient in LTA₄ hydrolase revealed no evidence of a role for the aminopeptidase activity of LTA₄ hydrolase in vivo (9).

LTB₄ is produced primarily by leukocytes because the enzyme 5-LO is largely limited in distribution to these cell types. LTA₄ hydrolase, on the other hand, is found in many cell types, including alveolar epithelial cells (4, 5). In the absence of 5-LO to initiate leukotriene synthesis, these cells cannot, by themselves, generate appreciable LTB₄. However, they can produce LTB₄ through a “transcellular” mechanism, accepting LTA₄ donated by adjacent neutrophils as substrate for LTA₄ hydrolase (25). In this way, the unstable LTA₄ that is secreted by neutrophils is metabolized to LTB₄ before it can break down. Thus LTA₄ hydrolase in cells lacking 5-LO may be important in determining the total amount of LTB₄ produced in tissues containing recruited leukocytes.

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Nuclear LTA₄ Hydrolase in Type II Epithelial Cells

**Materials and Methods**

**Animals, tissues, and cells.** Human lung samples were from biopsies of uninvolved regions from patients with lung cancer (control) or involved regions from patients with IPF, as described (57). The experimental protocol was approved by the University of Michigan Medical Center Institutional Review Board for Approval of Research Involving Human Subjects. Mice were specific pathogen-free 129/Sv mice obtained from the Jackson Laboratory (Bar Harbor, ME). F1 male Fischer 344xBN rats at 6 mo of age were obtained from the National Institute on Aging. The rats were housed individually in specific pathogen-free conditions for 2 wk before experimentation. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the American Physiological Society and the University of Michigan Committee on Use and Care of Animals. A549, NIH/3T3, and rat basophilic leukemia (RBL-1) cells were obtained from American Type Culture Collection (Manassas, VA) and grown under 5% CO₂ in DMEM (In Vitrogen Life Technologies, Carlsbad, CA) supplemented with 10% calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin.

**Bleomycin treatment and immunohistochemical staining.** As described (47), mice were anesthetized and subjected to intratracheal injection with 30 μl of 0.025 U bleomycin (Sigma-Aldrich, St. Louis, MO) diluted in saline. As previously described (7), rat and mouse lungs were removed, inflated, and fixed in 1% paraformaldehyde overnight at 4°C and processed for paraffin sections. Tissue sections were dewaxed in Amealic and rehydrated through decreasing concentrations of ethanol. All materials were then quenched of endogenous peroxidase activity by treatment with 0.3% hydrogen peroxide for 30 min, washed, and blocked with Powerblock (Inogenex, San Ramon, CA). Rabbit polyclonal antibody against LTA₄ hydrolase (a generous gift from Dr. J. Evans, Merck Frosst Center for Therapeutic Research, Pointe Claire-Dorval, Quebec, Canada) was prepared in PBS containing 0.1% BSA (titer 1:750) and applied overnight at 4°C. After slides were washed with 0.1% BSA in PBS, slides were probed with secondary antibody (biotinylated goat anti-rabbit, 1:250) for 30 min at 37°C, washed again, and then treated with avidin-biotinylated peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) for 30 min at room temperature. 3,3-Diaminobenzidine was used as peroxidase substrate; preparations were counterstained with Harris’ hematoxylin.

**Indirect immunofluorescence and confocal microscopy.** Cells were cultured on chamber slides, fixed with methanol at −20°C for 30 min, permeabilized with −20°C acetone, air-dried, and then blocked with 1% BSA in PBS containing nonimmune goat serum. Primary antibody (1:500) was prepared in 1% BSA-PBS and applied for 1 h at 37°C. Mounts were washed three times with 1% BSA-PBS, and rhodamine-conjugated goat anti-rabbit secondary (1:250) was added for 30 min at 37°C, washed again, and then treated with avidin-biotinylated peroxidase complex (Vector Elite ABC kit, Vector Laboratories). Fluorescence was visualized with a Nikon Labophot 2 microscope equipped for epifluorescence or with a Bio-Rad MRC-600 laser confocal microscope.

**Subcellular fractionation and immunoblot analysis.** A549 cells were washed with Dulbecco’s balanced salt solution, trypsinized, and resuspended in ice-cold STKM buffer (250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 25 mM KCl, 5 mM MgCl₂, 1 mM EGTA) with protease inhibitors (1 mM each phenylmethylsulfonyl fluoride, dithiothreitol, soybean trypsin inhibitor, and leupeptin) at 2 × 10⁷ cells/ml and subjected to nitrogen cavitation at 400 psi, for 5 min, at 4°C. Maximal breakage of cells was confirmed by lack of trypan blue exclusion. The resulting cavitate was centrifuged at 1,000 g, 10 min at 4°C, to produce crude isolated nuclei and a postnuclear supernatant. The postnuclear supernatant was centrifuged at 100,000 g, 30 min at 4°C, with the resulting supernatant and pellet (resuspended in STKM buffer) designated “cytosol” and “membrane” fractions, respectively. Isolated nuclei were washed and sonicated (30 bursts at 20% duty cycle) and ultracentrifuged, as described above, to produce nuclear-soluble and -pelletable fractions. Aliquots of fractions were sonicated (10 bursts at 20% duty cycle), and protein concentrations were determined by a modified Coomassie dye-binding assay (Pierce), using BSA as standard. Samples were then subjected to immunoblot analyses as described previously (8). Briefly, samples containing 10 μg of protein were separated by SDS-PAGE under reducing conditions and transferred to a nitrocellulose membrane, which was then blocked, washed, and probed with antibody against LTA₄ hydrolase (1:5,000). Detection involved horseradish peroxidase-conjugated goat anti-rabbit secondary (1:5,000) followed by enhanced chemiluminescence (Amersham Biosciences). Multiple exposure times were taken for all blots to ensure that band densities were within the linear range of exposure. The membrane was then stripped and reprobed with anti-β-tubulin (1:200) as a cytoplasmic cytoskeletal marker (32).

**Isolation of rat type II pneumocytes.** Rat type II cells were isolated by elastase digestion and IgG panning as previously described (17). Briefly, rats were anesthetized, the trachea was cannulated, and the pulmonary circulation was perfused free of blood with a balanced salt solution at 4°C. After multiple whole lung lavages with EGTA (1 mM) in a balanced salt solution, porcine pancreatic elastase (4.3 U/ml; Worthington) was instilled via the trachea to release type II cells. Contaminating cells bearing Fc receptors were removed from the cell suspension by panning on plates coated with rat IgG (Sigma-Aldrich). Cells were plated on chamber slides at 2 × 10⁶ cells/cm² in DMEM supplemented with penicillin-streptomycin and 10% newborn calf serum and cultured at 37°C in 5% CO₂. The medium was replaced every 2–3 days.

**Metabolism of LTA₄ to LTB₄.** On days 1 and 7 (after isolation), cells were washed and incubated with various concentrations of LTA₄, freshly hydrolyzed from LTA₄ methyl ester as recommended by supplier (Cayman Chemical, Ann Arbor, MI), in medium. After 30 min at 37°C, the medium was recovered for LTB₄ determination by enzyme immunnoassay according to the supplier’s instructions (Cayman Chemical). The detection limit for LTB₄ was 4 pg/ml; cross-reactivity for AA, 5-HETE, LTA₄, LTC₄, LTD₄, and LTE₄ was <0.01%. Cells were then washed, scraped, and assayed for LTA₄ hydrolase protein by immunoblot analysis.

**Cell proliferation assays.** A549 and NIH/3T3 cells were plated at 4,000 cells/well in 96-well flat-bottomed tissue culture plates in complete media (DMEM, 10% fetal calf serum, and 1% penicillin-streptomycin). After 6 h, cells were quiesced in serum-free media (DMEM and 1% penicillin-streptomycin) for 18 h and then allowed to grow for 24 h in complete medium in the presence of different concentrations (10, 30, 100, 300 μM, adjusted to 0.5% methanol for each well) of betasin (Sigma) plus 10 μCi of [3H]thymidine (Amer sham). As controls, cells were treated with either methanol (0.5%) or 2 μM of the leukotriene biosynthesis inhibitor MK-886 (Bionol, Plymouth Meeting, PA). We harvested cells onto glass fiber filters using a cell harvester and determined incorporated radioactivity by beta-scintillation counting.

**Statistical analysis.** Statistical significance was evaluated by one-way ANOVA, using P < 0.05 as indicative of statistical significance. Pairs of group means were analyzed using the Tukey-Kramer post hoc test.

**Results**

Subcellular distribution of LTA₄ hydrolase in human and mouse alveolar epithelial cells. To evaluate the localization of LTA₄ hydrolase in normal alveolar epithelial cells, sections of tissues from untreated human and mouse lungs were evaluated by immunohistochemistry. Human lung tissue showed strong positive (brown) staining for LTA₄ hydrolase in the nucleus of numerous cells, including cells at junctions of the alveolar walls (Fig. 1A). Human lung tissue stained in parallel with...
nonimmune serum was negative (Fig. 1B). In mouse lung sections, positive staining for LTA₄ hydrolase was found in essentially all cells (Fig. 1C). Blue staining of nuclei by the counterstain hematoxylin was particularly evident in flattened cells, whereas brown staining of nuclei was found in cuboidal cells (Fig. 1D). Positive nuclear staining of cuboidal epithelial cells at alveolar wall junctions suggested the presence of LTA₄ hydrolase in the nuclei of type II pneumocytes. Also, it was observed that the majority of alveolar epithelial cells, particularly those in alveolar walls, had blue nuclei, indicating that LTA₄ hydrolase was not in the nucleus of type I pneumocytes.

LTA₄ hydrolase redistribution and activity in rat type II cells in vivo and in vitro. Immunohistochemical staining of normal rat lung tissue indicated that LTA₄ hydrolase was prominent in the nucleus of type II but not type I pneumocytes (Fig. 2A). Both type I and type II cells had positive LTA₄ hydrolase staining in the cytoplasm. Thus, in human, mouse, and rat lungs, nuclear localization of LTA₄ hydrolase was restricted to type II cells, suggesting that LTA₄ hydrolase redistributed from the nucleoplasm to the cytoplasm as type II cells differentiated into type I pneumocytes (45). Isolated type II cells are known to differentiate in culture, developing characteristics of type I pneumocytes over several days (6, 51), so the localization of LTA₄ hydrolase was examined in cultured rat type II cells. Freshly isolated type II cells, stained by immunofluorescence, showed strong nuclear accumulation of LTA₄ hydrolase with some cytosolic enzyme (Fig. 2B). After 1 day in culture, the difference between nuclear and cytoplasmic compartments was reduced, although nuclear staining was still clearly greater (Fig. 2C). After 7 days in culture, nuclei were negative for LTA₄ hydrolase, with fluorescence restricted to the cytoplasm (Fig. 2D). The finding that freshly isolated type II pneumocytes...
uniformly have nuclear accumulation of LTA4 hydrolase supports the immunohistochemical localization in type II cells stained in situ. The redistribution of LTA4 hydrolase in vitro thus appears to be another way in which type II pneumocytes become more type I-like with time in culture.

As noted above, epithelial cells can participate in transcellular metabolism of LTA4, donated by neighboring leukocytes, to produce LTβ4. It seemed plausible that LTA4 hydrolase might convert exogenously added LTA4 to LTβ4 more efficiently when located in the cytoplasm than when deep within the nucleus. Furthermore, this effect might be more pronounced at lower levels of LTA4. To test this, varying doses of LTA4 were added to primary rat alveolar epithelial cells that had LTA4 hydrolase predominantly in the cytoplasm (day 7 cells) or in the nucleus (day 1 cells). LTβ4 synthesis from exogenous LTA4 in day 7 cells did not differ significantly from that of day 1 cells, regardless of the concentration of LTA4 provided (Fig. 3). Immunoblot analysis of cell lysates indicated comparable amounts of total LTA4 hydrolase protein in day 1 and day 7 cells (data not shown). This suggests that, although LTA4 hydrolase appears to be redistributed from the nucleus to the cytoplasm as type II pneumocytes differentiate and become more type I like, their capacity to produce LTβ4 from exogenous LTA4 does not greatly change, at least as judged by extracellular measurements of LTβ4.

Subcellular localization of LTA4 hydrolase in an alveolar epithelial cell line. In addition to nuclear accumulation of LTA4 hydrolase, type II cells differ from type I cells by having a higher capacity to proliferate. If nuclear localization of LTA4 hydrolase correlated with proliferative capacity of epithelial cells, then LTA4 hydrolase should be found in the nucleus of transformed type II cells. The A549 is a continuous cell line derived from a human pulmonary adenocarcinoma that resembles type II alveolar epithelial cells (36). Immunofluorescent staining of proliferating A549 cells for LTA4 hydrolase resulted in bright speckled fluorescence at the nucleus with less bright diffuse fluorescence in the cytoplasm (Fig. 4A). Bright staining for LTA4 hydrolase corresponded to staining for DNA using DAPI (Fig. 4B). For comparison, staining for LTA4 hydrolase in proliferating fibroblast-like NIH/3T3 cells was restricted to the cytoplasm (Fig. 4C), although regions indicated as nuclear by DAPI staining (Fig. 4D) were negative for LTA4 hydrolase. Confocal imaging confirmed that LTA4 hydrolase was abundant within the nucleus (Fig. 4E) as well as the cytoplasm (Fig. 4F) of A549 cells but restricted to the cytoplasm of NIH/3T3 cells (Fig. 4G). Thus nuclear LTA4 hydrolase is present in some proliferating epithelial cells but not in fibroblast-like NIH/3T3 cells.

The discovery of nuclear LTA4 hydrolase in the A549 monoculture allowed further evaluation of localization without contaminating leukocytes, as may be found in isolated type II preparations. To evaluate LTA4 hydrolase subcellular distribution, A549 cells were fractionated into nuclear and nonnuclear fractions, and these were further separated into soluble and pelletable fractions. Immunoblot analyses indicated the presence of LTA4 hydrolase predominantly in the soluble nuclear fraction and the cytoplasmic fraction (Fig. 4H). β-Tubulin, a microtubule protein that does not occur in the nucleus, was absent from the soluble nuclear fraction, demonstrating that this fraction was not contaminated with cytoplasmic proteins. Together, these results indicate that LTA4 hydrolase is soluble and accumulates in the nucleus of proliferating type II-like cells but not fibroblast-like cells.

Because A549 cells, unlike isolated type II pneumocytes, readily proliferate in culture, the importance of LTA4 hydrolase’s aminopeptidase activity on growth could be tested with these cells. Significantly, A549 cells lack detectable 5-LO protein yet express an amount of LTA4 hydrolase that is similar to that found in NIH/3T3 cells (Fig. 5A, rat basophilic leukemia cell lysate as positive control). As expected, MK-886, an inhibitor of LTA4 synthesis (16), had no effect on serum-induced growth of either A549 (Fig. 5B) or NIH/3T3 cells (Fig. 5C). In contrast, the aminopeptidase inhibitor bestatin significantly (~22%) inhibited the growth of A549 cells, which have nuclear LTA4 hydrolase, but not NIH/3T3 cells, which have cytoplasmic LTA4 hydrolase. These results indicate that aminopeptidase activity is responsible for a portion of the growth potential of A549 cells. Because bestatin is known to inhibit the aminopeptidase activity of LTA4 hydrolase (44), it is possible that nuclear LTA4 hydrolase in A549 cells contributes to the growth of those cells through its aminopeptidase activity.

Subcellular localization of LTA4 hydrolase in bleomycin-treated mouse lung tissue. Proliferation of epithelial and fibroblast cells occurs during the repair process of lungs injured by bleomycin. We asked whether nuclear localization of LTA4 hydrolase would be linked to proliferation of epithelial, but not fibroblast, cells in bleomycin-damaged lungs using immunohistochemical staining. Bleomycin injury is typically patchy in distribution; at 14 days postbleomycin, tissue sections stained heavily for LTA4 hydrolase in both uninvolved (Fig. 6A) and fibrotic (Fig. 6B) sites. In regions with relatively normal alveolar architecture, nuclear localization of LTA4 hydrolase was evident in flattened epithelial cells (Fig. 6A, arrowheads) as well as in cuboidal cells, with few cells showing the blue negative nuclear staining. At fibrotic sites, interstitial cells with predominantly cytoplasmic LTA4 hydrolase, reminiscent of the distribution in NIH/3T3 fibroblasts (Fig. 4C), were readily observable (Fig. 6B, arrows). These results suggest that nuclear localization of LTA4 hydrolase is a feature of epithelial cells but not of fibroblast-like cells, associated...
with rapid cell proliferation or tissue repair following bleomycin injury.

At 21 days after bleomycin treatment, staining for LTA₄ hydrolase remained high in fibrotic sites, with the majority of cells having positive-staining nuclei (Fig. 7A). In contrast, staining was diminished and largely restricted to type II cells in uninvolved areas from the same section (Fig. 7B), similar to staining in untreated mouse lung. By 30 days postbleomycin treatment, fibrotic regions remained strongly positive for LTA₄ hydrolase (Fig. 7C), and hyperplastic type II cells with nuclear LTA₄ hydrolase appeared to be delimiting these regions (Fig. 7D). Lung tissue from the same section having normal architecture had normal staining (i.e., like Fig. 1C) for LTA₄ hydrolase (not shown). These findings suggest that nuclear localization of LTA₄ hydrolase remains a feature of hyperplastic epithelial cells at sites of fibrosis or lung repair.

**Nuclear localization of LTA₄ hydrolase in human IPF.** IPF is characterized by epithelial cell injury and dysfunction (50) as well as LTB₄ overproduction (56). Bleomycin-injured mouse lung is used to model human IPF; therefore, we asked whether the nuclear LTA₄ hydrolase observed in hyperplastic epithelial cells in the mouse model were also found in human disease. Immunohistochemical analysis of lung sections from patients with IPF showed strong nuclear staining of the hyperplastic type II epithelial cells lining the surface of fibrotic areas (Fig. 8). Staining within the fibrotic region itself was faint and diffuse. Strong nuclear staining for LTA₄ hydrolase in type II cells was observed in sections from all eight patients examined. Thus nuclear localization of LTA₄ hydrolase is found in normal type II epithelial cells in humans, mice, and rats, and this subcellular distribution is associated with proliferation in A549 cells, bleomycin-injured mouse lung, and human fibrotic disease.
In the present study, we have, for the first time, examined the distribution of LTA4 hydrolase in alveolar epithelial cells. We found that LTA4 hydrolase was accumulated in the nucleus of normal type II alveolar epithelial cells in human, mouse, and rat lung, in the type II-like A549 cell line, in lung epithelial cells following bleomycin injury, and in type II alveolar epithelial cells associated with fibrotic regions of lung in patients with IPF. In contrast, LTA4 hydrolase was predominantly cytosolic in normal type I alveolar epithelial cells in situ, in isolated type II cells cultured to become type I-like (45), as well as in fibroblasts. As we found for leukocytes (7), the nuclear import-export of LTA4 hydrolase appears to be a regulated process. In alveolar epithelial cells, the aminopeptidase activity of nuclear LTA4 hydrolase may promote growth, and nuclear export of this activity may be linked to transition from a type II to type I cell phenotype.

The functional significance of positioning LTA4 hydrolase within the nucleus is unclear. The demonstrated biological function for this enzyme in epithelial cells is the synthesis of LTβ4 from donated LTA4. Freshly isolated type II epithelial cells will readily produce LTβ4 from LTA4 secreted by either macrophages (48) or neutrophils (25, 61). LTβ4 levels are increased in bronchoalveolar lavage (55) and lung tissue homogenates (56) from patients with IPF, correlating increased LTβ4 production with prominent nuclear LTA4 hydrolase in type II cells. For this reason, we speculated that nuclear LTA4 hydrolase might convert exogenous LTA4 to LTβ4 better than cytoplasmic enzyme, supporting a contribution by alveolar epithelial cells to lung LTβ4 levels in this disease. However, type II cells at day 7, with cytoplasmic LTA4 hydrolase, secreted as much LTβ4 as type II cells at day 1, when LTA4 hydrolase was accumulated in the nucleus. This indicates that the subcellular distribution of LTA4 hydrolase did not significantly affect the capacity for transcellular metabolism of LTA4. Consistent with this, the level of LTβ4 in lung lavage does not increase in mice after treatment with bleomycin (47), although the subcellular distribution of LTA4 hydrolase does change.

The nuclear localization of LTA4 hydrolase may affect AA metabolism in other ways. For example, the export of LTβ4 is carrier mediated (33), although the carrier has not been identified. Movement of LTA4 hydrolase might change its position relative to the carrier and affect LTβ4 export. Also, epithelial cells may themselves produce small levels of LTA4 (reviewed in Ref. 37). The positioning of LTA4 relative to 5-LO may be important in determining processing of endogenously produced LTA4, as in leukocytes (7). Finally, the lipid substrate of LTA4 hydrolase, LTA4, is recognized to be an unstable inter-

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**DISCUSSION**

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Fig. 5. Cell proliferation of A549, but not 3T3 cells, was inhibited by bestatin. A: immunoblot of cell lysates from RBL-1, 3T3, and A549 cells, probed for LTA4 hydrolase or 5-lipoxygenase (5-LO). Each lane was loaded with 10 µg of total protein. LTA4H, LTA4 hydrolase. B: A549 cells were plated on 96-well plates and treated with vehicle, MK-866, or bestatin at the indicated concentrations. The incorporation of [3H]thymidine was determined by beta-scintillation counting. Results are means (+SE) of 8 wells from a representative experiment. *P < 0.05. C: incorporation of [3H]thymidine in 3T3 cells, evaluated as described in B.

Fig. 6. LTA4 hydrolase staining of lung tissue from mice 14 days after bleomycin treatment. Representative uninvolved (A) and fibrotic (B) regions are from a single lung section of a mouse treated with bleomycin for 14 days (n = 2). Arrowheads in A point to flattened cells with nuclear staining. Arrows in B indicate cells with dense cytoplasmic staining for LTA4 hydrolase. Bars indicate 20 µm.
Fig. 7. Localization of LTA₄ hydrolase in lung tissue from mice treated with bleomycin for 21 days (A and B) or 30 days (C and D). Representative fibrotic (A) and uninvolved (B) regions are from a single lung section of a mouse treated with bleomycin for 21 days (n = 2). Detail (D) from indicated (asterisk) area of representative fibrotic region (C) of 30-day treatment highlights cells lining air spaces having nuclear staining for LTA₄ hydrolase (arrowheads).

Fig. 8. Nuclear localization of LTA₄ hydrolase in alveolar epithelial cells in lung tissue from patients with idiopathic pulmonary fibrosis. Brown staining indicates positive immunohistochemical staining for LTA₄ hydrolase; blue indicates nuclei counterstained with hematoxylin. Images on right (B, D, and F) are magnifications of field indicated by arrows in images on left (A, C, and E). Results are from 3 different patients and are representative of 8 different patients.
mediate in the leukotriene biosynthetic pathway. However, \( \text{LTA}_4 \) is stabilized by fatty acid binding proteins, including the epithelial fatty acid binding protein (15), which can move lipids from the cytoplasm into the nucleus (34). Fatty acid binding proteins can interact with transcription factors (58), as can leukotrienes (14). Furthermore, \( \text{LTA}_4 \) can covalently bind to DNA and RNA, both in vitro and in cells (29). Thus \( \text{LTA}_4 \) can persist within cells, move into the nucleus, and interact with transcription factors and nucleic acids. The localization of \( \text{LTA}_4 \) hydrolase within the nucleus may serve to remove \( \text{LTA}_4 \) by metabolizing it to the rapidly secreted \( \text{LTB}_4 \), in this way changing its effects on cell function.

One cell function apparently associated with the nuclear localization of \( \text{LTA}_4 \) hydrolase in epithelial cells is proliferation: the enzyme is strongly nuclear in hyperplastic type II cells in IPF and also in growing A549 cells and leaves the nucleus as type II cells differentiate to become more type I-like, and nonproliferative, in culture. The overexpression of \( \text{LTA}_4 \) hydrolase in epithelial cells has been linked with tumorigenesis in esophageal adenocarcinoma, and bestatin, an aminopeptidase inhibitor, significantly reduced tumor incidence (10). Interestingly, aminopeptidase inhibitors like bestatin have been shown to suppress DNA synthesis in keratinocytes (52), inhibit proliferation of myeloma cells by intracellular actions (26), and promote the differentiation of U937 histiocytes (40) and lymphocytes (54). Furthermore, enkephalins, which have been shown to be substrates for \( \text{LTA}_4 \) hydrolase in vitro (24), can be found, with their receptors, in the nuclei and perinuclear regions of epithelial cells (59). Enkephalins inhibit epithelial cell proliferation (60), suggesting that their degradation within the nucleus by a peptidase would allow proliferation. Through either its hydrolase or its aminopeptidase activity, \( \text{LTA}_4 \) hydrolase appears to promote epithelial cell proliferation when localized within the nucleus, whereas differentiation correlates with its redistribution into the cytoplasm.

In summary, the enzyme \( \text{LTA}_4 \) hydrolase, a soluble enzyme known to have multiple activities, can be in either the cytoplasm or the nucleoplasm of leukocytes and alveolar epithelial cells. It is likely that redistribution of \( \text{LTA}_4 \) hydrolase between these two compartments is a regulated process and that it occurs in other cell types as well. Contrary to our expectations, our results indicate that nuclear import of \( \text{LTA}_4 \) hydrolase does not affect the ability of the cell to convert exogenous \( \text{LTA}_4 \) to \( \text{LTB}_4 \). However, bestatin, known as both an aminopeptidase inhibitor and direct \( \text{LTA}_4 \) hydrolase inhibitor, impairs growth in A549 cells, which have nuclear \( \text{LTA}_4 \) hydrolase. Together, these results suggest that the aminopeptidase activity of \( \text{LTA}_4 \) hydrolase plays a role in regulating the growth of type II epithelial cells. Moreover, it may be that the nuclear localization of this activity promotes growth in these cells.

GRANTS

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