Lateral membrane LXA₄ receptors mediate LXA₄’s anti-inflammatory actions on intestinal epithelium

Torsten Kucharzik, Andrew T. Gewirtz, Didier Merlin, James L. Madara, and Ifor R. Williams

Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia 30322

Submitted 22 October 2001; accepted in final form 21 November 2002

Kucharzik, Torsten, Andrew T. Gewirtz, Didier Merlin, James L. Madara, and Ifor R. Williams. Lateral membrane LXA₄ receptors mediate LXA₄’s anti-inflammatory actions on intestinal epithelium. Am J Physiol Cell Physiol 284: C888–C896, 2003. First published November 27, 2002; 10.1152/ajpcell.00507.2001.—Lipoxin A₄ (LXA₄) and its stable analogs downregulate chemokine secretion in polarized epithelia. This anti-inflammatory effect has been suggested to be mediated by the LXA₄ receptor (LXA₄R), a G protein-coupled receptor. To determine whether LXA₄R is expressed on the apical, basolateral, or both poles of intestinal epithelia, an NH₂-terminal c-myc epitope tag was added to the human LXA₄R cDNA and recombinant retroviruses were used to transduce polarized epithelial cells. In polarized T84 intestinal epithelial cells, c-myc-LXA₄R was preferentially expressed on the basolateral surface as indicated by cell surface-selective biotinylation and confocal microscopy. Furthermore, expression of c-myc-LXA₄R and a truncation mutant lacking the cytoplasmic terminus was primarily confined to the lateral subdomain. We also observed that the expression of myc-LXA₄ conferred enhanced downregulation of IL-8 expression in response to LXA₄ analog and that blockade of the CysLT1 receptor by montelukast did not prevent this response to LXA₄ analog. Thus LXA₄ generated in or near the paracellular space via neutrophil-epithelial interactions can rapidly act on epithelial LXA₄R to downregulate epithelial promotion of intestinal inflammation.

G protein-coupled receptor; polarized epithelium; eicosanoid; epitope tag

The epithelial lining of the gastrointestinal tract forms a vital protective barrier that separates luminal antigens and toxins from the underlying tissue compartments. Intestinal epithelial cells are increasingly recognized to play an important role in host defense microorganisms in the intestinal lumen and in inflammatory responses. They contribute to regulation of immune responses by releasing a variety of pro-inflammatory cytokines and chemokines. For example, intestinal epithelial cells release IL-8 after stimulation with TNF-α or a variety of pathogens, thereby facilitating neutrophil migration into the intestinal epithelium. Homeostatic regulation of epithelial inflammation in the intestine involves the concerted action of both proinflammatory and counterregulatory pathways. One of the active counterregulatory pathways involves the action of eicosanoid mediators, including lipoxin A₄ (LXA₄) and 15-epimeric aspirin-triggered lipoxins (4, 16). These mediators are generated at sites of inflammation and exert anti-inflammatory effects on both neutrophils (9) and intestinal epithelial cells, including the downregulation of epithelial secretion of chemokines that direct neutrophil movement.

Intestinal epithelial cells express at least two G protein-coupled receptors for which LXA₄ and its analogs have been demonstrated to have biological activity (14, 15). One of these receptors is the same LXA₄ receptor (LXA₄R) initially cloned from human and mouse myeloid cells on the basis of its high affinity for LXA₄ (8, 31). This receptor also binds the 15-epi-lipoxins, which are synthesized by acetylated COX-2 in aspirin-treated cells (4). An alternate name used for this receptor is N-formyl peptide receptor-like 1 (FPRL1), because it also binds N-formylmethionyl-leucyl-phenylalanine (fMLP) as well as several other peptide ligands with lower affinities. The second known epithelial cell receptor for LXA₄ is the CysLT1 receptor for leukotriene D₄ (15, 22). LXA₄R and its analogs act as partial agonists and/or antagonists at this receptor and block the function of cysteinyl leukotriene agonists. The CysLT1 receptor is also expressed by intestinal epithelial cells (15), suggesting the possibility that the anti-inflammatory effects of LXA₄ could be mediated through this molecule rather than through LXA₄R.

Intestinal epithelial cells are polarized cells expressing a distinct set of membrane proteins on their apical and basolateral plasma membranes. The polarized expression of a variety of surface receptors is necessary for epithelial cells to maintain normal structure and function. It is not currently known whether receptors for LXA₄ are polarized in their pattern of expression on intestinal epithelial cells. Further understanding of the mechanism of LXA₄’s anti-inflammatory action in polarized epithelia depends, in part, on identifying the subcellular location at which these receptors normally encounter their ligands. However, such studies have been stymied by the fact that the

Address for reprint requests and other correspondence: I. R. Williams, Dept. of Pathology and Laboratory Medicine, Whitehead Biomedical Research Bldg. 105D, Emory Univ., 615 Michael St., Atlanta, GA 30322 (E-mail: irwilli@emory.edu).

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LXA₄R, like other G protein-coupled receptors, are difficult to raise antibodies to and further that highly polarized intestinal epithelial cell lines are difficult to transfect. In this study, we overcame these technical problems by using retroviral transduction to express an epitope-tagged version of the LXA₄R in several polarized epithelial cell lines. Our results indicate that LXA₄R is preferentially expressed on the basolateral surface and further localized to the lateral membrane domain of such polarized epithelia. We also find that stable overexpression of the LXA₄ receptor in intestinal epithelial cells is associated with enhanced LXA₄-mediated inhibition of TNF-α-induced IL-8 secretion by epithelial cells, thus showing that the counterregulatory effects of LXA₄ and its analogs on intestinal epithelial cells are at least in part mediated through the basolateral LXA₄R.

MATERIALS AND METHODS

Reagents. The stable LXA₄ analog 15-R/S-methyl-LXA₄-methyl ester was kindly provided by Nikos Petassis (University of Southern California, Los Angeles, CA). It was prepared by total organic synthesis, and its structure was confirmed by HPLC, NMR, and mass spectral analysis (29). Daily working stocks of 15-R/S-methyl-LXA₄ (100 μM) were verified by UV spectroscopy using a molar extinction coefficient of 50,000 cm⁻¹·M⁻¹ at 302 nm. These solutions were stored at −70°C in 99% ethanol. Flagellin was purified from Salmonella typhimurium-conditioned medium as described previously (12). Recombinant human TNF-α was purchased from R&D Systems (Minneapolis, MN). A stock solution of montelukast (Merck) was prepared by dissolving tablets containing 10.4 mg of the sodium salt in water.

Epithelial cell lines and culture. Human colonic adenocarcinoma cell lines were grown and passaged with culture conditions previously described for T84 (5) and HT29 clone 19A (HT29cl.19A; Ref. 19) in an atmosphere of 5% CO₂. In brief, T84 cells were cultured in a 1:1 mixture of DMEM and Ham’s F-12 medium supplemented with 14 mM NaHCO₃, 40 mg/ml penicillin, 9 mg/ml streptomycin, 8 mg/ml ampicillin, 5% newborn calf serum, and 15 mM Na⁺ HEPES buffer, pH 7.5. HT29cl.19A cells were cultured in DMEM containing a standard glucose concentration (4.5 g/l) and supplemented with 10.4 mg of the sodium salt in water. Human embryonic lung fibroblasts (16A1, Imgenex, San Diego) to make recombinant retrovirus. A control construct consisting of the coding region of enhanced green fluorescent protein (EGFP) cloned into pLPX was used to assess the efficiency of cell transduction by the recombinant retroviruses. Transfections were done by using a CaPO₄-mediated transfection procedure. Briefly, DNA was mixed with 0.25 M CaCl₂ in a tube and 2×N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES)-buffered saline was added a small amount at a time with mixing. After the CaCl₂-DNA-BES-buffered saline solution was added dropwise, the cells were incubated for 16 h before the medium was changed. After 2 days, the supernatant of the confluent cell line was used to transduce intestinal epithelial cell lines. Briefly, target cells were split 24 h before they were used for transfection at a confluence of ~50%. For infection, fresh retroviral supernatant was added to the target cells on a six-well plate for 15 min at 32°C in the presence of 8 μg/ml Polybrene (Sigma). Cells were then spun at 2,500 rpm for 30 min at 32°C. After the spin the retroviral supernatant was replaced with fresh growth medium. Infection cycles were repeated two more times. Transduction efficiency was ~50% in HT29cl.19A cells and ~15% in T84 cells as detected by flow cytometry with cells transduced with EGFP-encoding retroviruses. Stably transduced HT29cl.19A cells were derived by puromycin selection.

Expression of a truncated LXA₄R mutant in intestinal epithelial cells. A mutant myc-LXA₄R construct was engineered in which the CAA codon encoding the glutamine present immediately following the seventh transmembrane segment (31) was converted into a premature termination codon (TAA). This point mutation (abbreviated as Q307X; amino acid designation based on the wild-type LXA4Rs epitope) results in a truncated LXA₄R protein lacking the final 45 amino acids of the protein that make up the COOH-terminal cytoplasmic tail. The myc-LXA₄R-Q307X construct was cloned into pLPX for preparation of recombinant retrovirus with the same approach as for the wild-type myc-LXA₄R construct.

Intracellular Ca²⁺ measurement in fMLP-stimulated cells. Intracellular Ca²⁺ recordings in monolayers of HT29cl.19A cells were performed as described previously (11) with minor modifications. Briefly, HT29cl.19A cells were plated on 0.4-μm polyester filters coated with collagen that were mounted on the window of a polycarbonate holder that fit diagonally into a standard fluorescence cuvette. After 7 days of cell growth, the filters were washed, loaded with fura 2-acetoxyethyl ester (Molecular Probes), and placed into a Hitachi (Sunnyvale, CA) F-4500 spectrofluorometer thermostated to 37°C. The bottom and side edges of the polycarbon-
ate holder were not coated with vacuum grease for these experiments, so diffusion of added compounds between the apical and basolateral sides was possible. Fluorescence emission was read at 530 nm, whereas the excitation wavelength was changed between 340 and 380 nm four times per second with Intracellular Cation software (Hitachi). After baseline fluorescence was read for 3–5 min, FMLP (10−9 M) was added to the basolateral side of the cuvette. Carbicol (100 μM) was subsequently added to the basolateral side of the cuvette to verify that the cells could respond to a strong agonist with an increase in Ca2+.

**Immunofluorescence microscopy.** Matrix-coated 0.4-μm filters on which retrovirally transduced T84 cells were cultured were cut out and washed three times with HBSS. Fixation was done with 3% paraformaldehyde (10 min at room temperature [RT]) followed by permeabilization with 1% Triton X-100 for 20 min at RT. After being washed in HBSS, filters were then blocked for 1 h with 5% goat serum in a moist chamber. After being washed three times with HBSS, filters were incubated for 1 h with c-myc MAb (2 μg/ml). Sections were washed three times with HBSS again and incubated for 1 h with FITC-conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch). After another wash, the sections were incubated with 5 U/ml rhodamine-phalloidin (Molecular Probes) and washed again. Filters were then mounted on glass slides with p-phenylenediamine. Mounted sections were viewed with a scanning laser confocal microscope (Zeiss, Jena, Germany).

**Immunoblotting of c-myc-tagged LXA4R in intestinal epithelial cells.** Expression of the c-myc-tagged LXA4R by transfected and transduced cells was detected by immunoblotting. Tissue samples were lysed in 500 μl of HBSS containing 1% Triton X-100 (Sigma) and a proteinase inhibitor cocktail. Protein concentration in the lysate was determined by using Pierce bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). After sample buffer was added, 10 μg of total protein of each sample was separated under reducing conditions on a 12% SDS-polyacrylamide gel. Protein transfer to Hybond-C nitrocellulose membranes (Amersham Pharmacia) was performed at 200 mA for 90 min or overnight with a Trans-Blot SemiDry Electrophoretic Transfer Cell (Bio-Rad Laboratories, Richmond, CA). Membranes were blocked with 5% fat-free milk powder in TBS buffer (0.01% Tween 20, 0.05 M Tris-HCl, 0.15 M NaCl, pH 7.5) for 60 min at RT and then incubated for 1 h with c-myc MAb (1:100 dilution; Clontech). Membranes were washed three times with TTBS and incubated with a peroxidase-conjugated goat anti-mouse IgG (1:5,000 dilution; Jackson ImmunoResearch) for 1 h. After extensive washing with TTBS and Tris-buffered saline (TBS), the reaction was developed by enhanced chemiluminescence (Amersham).

**Apical and basolateral cell surface biotinylation.** Biotinylation of the apical or basolateral surfaces of filter-grown T84 cells (4 filters for each condition) was done as described previously (23). Briefly, the apical or basolateral sides of the monolayers were incubated for 30 min at RT with a 1 mg/ml solution of freshly prepared sulfo-NHS-biotin (Pierce Chemical) diluted in PBS with 1 mM CaCl2 and 1 mM MgCl2. The reaction was quenched with 50 mM NH4Cl, and cells were lysed with a solution of 1% (wt/vol) Triton X-100 in 20 mM Tris, pH 8.0, 50 mM NaCl, 5 mM EDTA, and 0.2% (wt/vol) bovine serum albumin supplemented with protease inhibitors. The protein solution was diluted with 1 ml of lysis buffer and then incubated with streptavidin-agarose (Pierce) for 24 h at 4°C to bind biotinylated proteins. The bound proteins were separated by SDS-PAGE and blotted to nitrocellulose membranes. The blots were sequentially incubated with anti-c-myc MAb (1:100 dilution) or 1 μg/ml of anti-β1-integrin MAb (clone P4C10; Life Technologies) followed by peroxidase-conjugated goat anti-mouse IgG (1:10,000 dilution; Jackson ImmunoResearch) and developed with the ECL chemiluminescence system (Amersham). LXA4 expression was quantified by densitometry using ImageJ software. Values are expressed as the mean ± SD. Results were analyzed with Student’s t-test. Differences were considered significant if P < 0.05.

**RESULTS**

**Epithelial surface expression of functional epitope-tagged LXA4R by retroviral transduction.** An epitope-tagged version of the human LXA4R was constructed by subcloning the wild-type human LXA4R cDNA sequence into a mammalian expression vector (pCMV-Myc) that added the 13-amino acid c-myc epitope tag to the N-terminal tail of the LXA4R protein. To achieve increased efficiency of transduction in human intestinal epithelial cell lines (HT29cl.19A and T84), the c-myc-tagged construct was subcloned into the pLPCX retroviral expression and transfectants were selected in the presence of puromycin. We verified by immunoblotting that stable transfectants of HT29cl.19A indeed expressed c-myc-LXA4R (Fig. 1A) and that this protein was the expected size. Furthermore, flow cytometric analysis of these transfectants indicated that this epitope-tagged LXA4R was expressed on the cell surface (Fig. 1B). We next sought to examine whether this epitope-tagged receptor expressed in this manner retained previously demonstrated signaling ability. One known signal transduced by LXA4R is elevation of intracellular Ca2+ in response to high concentrations of fMLP (30). Thus we compared cytosolic Ca2+ concentrations ([Ca2+]i) of wild-type HT29cl.19A cells and myc-LXA4R-transfected cells after stimulation with 10−7 M fMLP. HT29cl.19A transfected expressing the myc-LXA4R demonstrated a gradual increase in intracellular [Ca2+]i after fMLP stimulation that was not detected in non transfected HT29cl.19A cells (Fig. 2). This result confirms that the myc-tagged LXA4R retains the capacity to signal through G proteins and indicates that transfected cells likely express more total (i.e., endogenous plus transfected) functional LXA4R than their parental HT29cl.19A cells.
LXA₄R is localized to basolateral surface and most prominent on lateral membrane of polarized epithelia. The polarity of expression cell surface receptors can be a very important aspect of their biology. Attempts to raise antibodies to LXA₄R that could determine the polarity of the endogenous receptor have failed, probably as a consequence of the combination of low levels of the endogenous LXA₄R in these cells and the lack of sufficient specificity of the affinity-purified anti-peptide antibodies that were used (data not shown). Thus we transiently transfected T84 cells with recombinant retroviruses encoding myc-LXA₄R or EGFP (as a control) without antibiotic (i.e., puromycin) selection as an alternative approach to expression of the myc-tagged LXA₄R in these cells. Using three infection cycles with EGFP-encoding retroviruses, we were able to detect expression by ~15% of T84 cells (data not shown). T84 cells were transfected with supernatant containing myc-LXA₄R retroviruses with the same conditions as for the EGFP retroviruses and plated on collagen-coated semipermeable supports for 7–10 days, allowing them to form confluent polarized epithelial monolayers. We then used domain-selective biotinylation of just the apical or basolateral membrane of these polarized model epithelia to determine the polarity of specific cell surface proteins as described previously (23, 26). Briefly, the apical or basolateral surface was biotinylated and quenched and biotinylated proteins were collected with streptavidin-coated agarose beads and immunoblotted with anti-myc. Basolaterally biotinylated surfaces contained much more c-myc-LXA₄R (migrating as a 40-kDa band) than did similarly treated apical surfaces (Fig. 3A). As a control, we verified with the same lysates that the β₁-integrin molecule previously shown to be basolaterally polarized (23) was predominantly detected in the basolaterally biotinylated cells. Thus LXA₄R appears to be preferentially expressed on the basolateral surface.

We next sought to further define the localization of LXA₄R expression via confocal microscopy. Polarized c-myc-LXA₄R-expressing T84 cells were fixed, perme-
acids of the myc-tagged LXA4R would interfere with the COOH-terminal cytoplasmic tail, as localized to the COOH-terminal intracellular domain. An X-Z image of anti-myc staining by confocal microscopy showed that the myc-tagged LXA4R was clearly located below the perijunctional actin ring. However, c-myc-LXA4R was not uniformly distributed along the basolateral membrane but rather showed specific areas along the lateral membrane of high expression, whereas, in contrast, staining was not observed along the basolateral membrane. Immunostaining of confluent monolayers of T84 cells grown on a semipermeable membrane showed the same pattern of basolateral membrane staining as obtained with the nonmutated myc-LXA4R construct (Fig. 3C). Thus the cytoplasmic tail is not necessary to bring about the basolateral targeting of the LXA4R in polarized epithelial cells.

Overexpression of LXA4R in intestinal epithelial cells enhances inhibitory effect of LXA4 on IL-8 secretion. Polarized intestinal epithelial cell lines constitutively secrete a low basal level of IL-8 (6, 10) that is markedly upregulated in response to stimulation with pathogens or proinflammatory cytokines such as TNF-α. We previously showed (10, 14) that stable analogs of LXA4 partially attenuate the IL-8 secretion induced in T84 cells by S. typhimurium or subsaturating concentrations of TNF-α. Such attenuation of epithelial IL-8 secretion was suggested to be mediated via the LXA4 receptor but was not directly investigated. Because, as shown above, the c-myc-tagged LXA4R we expressed in epithelial cells was capable of signaling in response to ligand, we investigated whether the increased expression of LXA4R in our retrovirally transfected cells might lead to a greater inhibitory effect of an LXA4 analog on IL-8 secretion. Specifically, we measured the effect of 100 nM 15-R/S-methyl-LXA4 on IL-8 secretion on both LXA4R-transfected and nontransfected HT29cl.19A cells. We observed that addition of 15-R/S-methyl LXA4 suppressed TNF-α-induced IL-8 release to a greater extent in the LXA4R-transfected cells (Fig. 4A). Inhibition of induced IL-8 release was depen-
dent on the concentration of TNF-α that was used. IL-8 secretion induced by TNF-α concentrations between 100 and 1,000 pg/ml could be inhibited by 15-R/S-methyl-LXA₄, with progressive loss of the inhibitory effect at higher concentrations of TNF-α (data not shown), consistent with previous studies (14). Salmonella-induced IL-8 secretion by model intestinal epithelia is largely the result of the interaction of Salmonella flagellin with Toll-like receptor 5 (TLR5) (12). Thus we also tested whether the myc-LXA₄R-overexpressing cells were more sensitive to the effects of an LXA₄ analog on IL-8 secretion elicited after TLR5 interaction with Salmonella flagellin (Fig. 4B). 15-R/S-methyl-LXA₄ inhibited flagellin-stimulated IL-8 secretion to a greater extent in LXA₄R-transfected cells than in nontransfected cells. In this experiment, addition of the LXA₄ analog reduced TNF-α-induced IL-8 secretion by 60% (*P < 0.05). B: addition of LXA₄ analog inhibited flagellin-induced IL-8 secretion in myc-LXA₄R-transfected cells stimulated with 5 ng/ml flagellin (57% inhibition; *P < 0.05) or 20 ng/ml flagellin (43% inhibition; *P < 0.05). LXA₄ analog inhibited IL-8 production by EGFP-transfected cells stimulated with 5 ng/ml flagellin (35% inhibition; *P < 0.05) but not by cells stimulated with 20 ng/ml flagellin.

We also observed a consistent trend toward increased suppression of basal IL-8 production by 15-R/S-methyl-LXA₄ in the myc-LXA₄R-overexpressing HT29cl.19A cells. Figure 5 summarizes data from a series of five experiments that showed an average of 41% inhibition (±SD of 21%) of basal IL-8 production in transfected cells by 15-R/S-methyl-LXA₄ compared with an average of 15% inhibition (±SD of 22%) in nontransfected cells. The enhanced lipoxin responsiveness of the LXA₄R-transfected cells for both agonist-induced and basal IL-8 production supports the hypothesis that at least part of the inhibitory effect of lipoxin analogs on the IL-8 response of intestinal epithelial cells is mediated through the LXA₄R and is more efficient when a larger number of receptors are available.

Fig. 4. Intestinal epithelial cells overexpressing the LXA₄R show increased sensitivity to the inhibitory effects of LXA₄ on TNF-α- and flagellin-induced IL-8 secretion. Wild-type, myc-LXA₄R overexpressing, or EGFP-transfected HT29cl.19A cells were grown to confluence on semipermeable collagen-coated filters. The cells were pretreated with 100 nM 15-R/S-methyl-LXA₄ for 1 h, and some of the cultures were stimulated basolaterally with human TNF-α (1 ng/ml) or Salmonella flagellin (5 or 20 ng/ml). The supernatant was collected after 5 h of stimulation and assayed for IL-8 by ELISA. A: LXA₄ analog inhibited TNF-α-induced IL-8 secretion to a greater extent in myc-LXA₄R-transfected cells than in nontransfected cells. In this experiment, addition of the LXA₄ analog reduced TNF-α-induced IL-8 secretion by 60% (*P < 0.05). B: addition of LXA₄ analog inhibited flagellin-induced IL-8 secretion in myc-LXA₄R-transfected cells stimulated with 5 ng/ml flagellin (57% inhibition; *P < 0.05) or 20 ng/ml flagellin (43% inhibition; *P < 0.05). LXA₄ analog inhibited IL-8 production by EGFP-transfected cells stimulated with 5 ng/ml flagellin (35% inhibition; *P < 0.05) but not by cells stimulated with 20 ng/ml flagellin.

Fig. 5. Intestinal epithelial cells overexpressing the LXA₄R show increased sensitivity to the inhibitory effects of LXA₄ on basal IL-8 secretion. Wild-type and myc-LXA₄R-overexpressing HT29cl.19A cells were grown to confluence on semipermeable collagen-coated filters. The medium was replaced, and some wells received 100 nM 15-R/S-methyl-LXA₄. The supernatant was collected after 6 h and assayed for IL-8 by ELISA. The graph shows the % decrease in basal IL-8 production by both cell types in the presence of 15-R/S-methyl-LXA₄ in 5 independent experiments. The horizontal bars indicate the mean % decrease in IL-8 production. The difference between the 2 cell types was statistically significant (P = 0.04).
Pharmacological inhibition of CysLT1 receptor by montelukast does not interfere with inhibitory effect of LXA4 on TNF-α-stimulated IL-8 secretion. To determine whether the CysLT1 receptor was involved in the observed inhibitory effects of LXA4 analog on IL-8 secretion, we tested whether the CysLT1 receptor antagonist montelukast influences TNF-α-stimulated IL-8 production in myc-LXA4R-overexpressing HT29cl.19A cells or the inhibition of this response by 15R/S-methyl-LXA4. At a concentration of 100 nM (significantly higher than the reported half-maximal inhibitory concentration for this antagonist at the CysLT1 receptor; Ref. 17), montelukast had no significant effect on TNF-α-elicited IL-8 production or the inhibition of this response by 15R/S-methyl-LXA4 (Fig. 6). We conclude that the effects of LXA4 analog in this system are not inhibited when CysLT1 receptors on HT29cl.19A cells are subjected to pharmacological blockade.

**DISCUSSION**

LXA4 and its stable analogs downregulate chemokine secretion by polarized intestinal epithelial cell lines and colonic epithelium (13). Epithelial cells express two different high-affinity binding sites for LXA4, namely LXA4R and CysLT1, that have been proposed to mediate the anti-inflammatory effects of this eicosanoid. We show here that overexpression of LXA4R led to a greater reduction in chemokine secretion in the presence of LXA4 analog, indicating that LXA4 attenuation of epithelial chemokine secretion is mediated, at least in part, by the LXA4R. Furthermore, the selective CysLT1 receptor antagonist montelukast (17) did not interfere with the inhibitory effect of LXA4 analog. These results indicate that the effects of LXA4 analog on intestinal epithelial cells are primarily mediated through the LXA4R and do not require availability of the CysLT1 receptor. Thus pharmacological analogs that seek to mimic LXA4’s anti-inflammatory bioaction on epithelial cells should retain the ability to ligate LXA4R.

Polarized intestinal epithelial cells selectively sort a variety of cell surface proteins to their apical and basolateral membranes, and proper sorting can be essential for specific absorptive, secretory, endocrine, and signal transduction functions (2). For this reason, we sought to determine the polarity of the LXA4R. Methods of determining the polarity of a specific protein generally depend on either being able to generate antibodies to the protein of interest or transfection with epitope-tagged or EGFP fusion constructs. Insertion of short epitope tags into proteins generally does not affect their intracellular trafficking or function, although interruption of important protein motifs with resultant effects on protein trafficking and/or function is possible with this approach (32). Lack of antigenicity of the protein of interest and transfection inefficiency of polarized intestinal epithelial cells can provide significant technical obstacles. We circumvented these problems by high-efficiency retroviral transduction of polarized epithelial cells with epitope-tagged LXA4R. This retroviral expression method should be a versatile approach to defining the polarity of other cell surface molecules in polarized cell lines that are inefficiently transfected with standard plasmid DNA constructs. Here, we utilized this approach for three distinct polarized epithelial cell lines and determined that LXA4R is expressed preferentially on the basolateral surface. LXA4R expression was not uniform on this membrane but rather further localized to lateral areas of this membrane domain.

Although, classically, membrane proteins on polarized epithelia have been described as apical or basolateral, it is becoming clear that in fact more precise targeting exists. Localization to the lateral subdomain has been described for several other G protein-coupled receptors including the vasopressin V2 receptor (28), the α2a-adrenergic receptor (27), and the human CTR-2 calcitonin receptor (26). One molecular mechanism contributing to the polarized expression of plasma membrane proteins is the presence of specific “targeting” motifs within the primary amino acid sequence. For example, basolateral targeting motifs have been identified for several G protein-coupled receptors including the α2a-adrenergic receptor (27), the FSH receptor (1), the mGluR7 glutamate receptor (20), and the M3 acetylcholine receptor (25). In some cases, these basolateral tar-
Lipoxin A₄ receptor localizes to lateral membrane

We thank James Hudson for technical assistance and Andrew Kowalczyk for suggesting use of the 293–10A1 packaging cells for production of retroviruses.

This work was supported by National Institutes of Health Grants DK-47662 (to J. L. Madara), DK-09800 (to A. T. Gewirtz), DK-02831 (to D. Merlin), and AR-44268 (to I. R. Williams) and the Crohn’s and Colitis Foundation of America (D. Merlin). T. Kucharzik was supported by a fellowship award (Ku 1328-1) from the Deutsche Forschungsgemeinschaft (DFG).

Present address of T. Kucharzik: Dept. of Medicine B, University of Münster, Albert-Schweitzer-Str. 33, D-48129 Münster, Germany.

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