HETEs enhance IL-1-mediated COX-2 expression via augmentation of message stability in human colonic myofibroblasts

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HETEs enhance IL-1-mediated COX-2 expression via augmentation of message stability in human colonic myofibroblasts. Am J Physiol Gastrointest Liver Physiol 293: G719–G728, 2007. First published July 19, 2007; doi:10.1152/ajpgi.00117.2007.—Proinflammatory cytokines and eicosanoids are central players in intestinal inflammation. IL-1, a key cytokine associated with intestinal mucosal inflammation, induces COX-2 expression in human colonic myofibroblasts (CMF) and increased prostaglandin E2 secretion is associated with inflammatory bowel disease (IBD) and colorectal cancer (CRC). We have previously demonstrated that IL-1α-induced cyclooxygenase-2 (COX-2) expression is the result of NF-κB- and ERK-mediated transcription, as well as COX-2 message stabilization, which depends on p38, MAPKAPK-2 (MK-2) and human antigen R (HuR) RNA binding protein activation. Lipoxigenase (LOX)-derived hydroxyeicosatetraenoic acids (HETEs) are elevated in IBD and colonic adenomas and “cross talk” has been observed between the COX and LOX pathways. Since COX-2 expression is primarily in CMFs in colonic adenomas, we examined the impact of LOX metabolites, particularly HETEs, on IL-1α-induced COX-2 expression in human CMFs. Although 5(S)-, 12(R)-, and 15(S)-HETEs alone had little to no effect on COX-2 expression, they enhanced IL-1-mediated COX-2 expression 3.6 ± 0.5-fold. Studies utilizing heterogeneous nuclear RNA amplification and 5,6-dichloro-β-d-ribofuranosylbenzimidazole treatment were undertaken to measure COX-2 transcription and message stabilization, respectively. We found that HETEs enhanced IL-1-induced COX-2 mRNA levels in CMF as the result of increased p38, MK-2, and HuR activity, increasing message stability greater than that observed with IL-1 alone. Thus HETEs can act synergistically with IL-1α to induce COX-2 expression in human CMFs. HETEs may play a role in both colonic inflammation and in increasing the risk of CRC in IBD independently and via induction of COX-2-mediated prostaglandin secretion.

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory disease of unknown etiology. The pathophysiology of IBD is believed to occur in two phases: an initial insult that commences tissue damage and then an amplification stage in which tissue destruction continues (41, 85). Several key proinflammatory mediators are involved in the amplification stage, including cytokines, notably TNF-α and IL-1, and eicosanoids such as prostaglandins (PGs), leukotrienes (LTs), and thromboxanes (TXs) (41, 58, 85). Eicosanoids are derived from the metabolism of arachidonic acid (AA) by both cyclooxygenases (COX) and lipoxigenases (LOX). PGs and TXs are produced via COX, whereas the LOX pathway generates LTs and hydroxyeicosatetraenoic acids (HETEs). PGE2, a product of COX activity, and the LOX products 5-HETE and 12-HETE are elevated in the inflamed intestinal mucosa in IBD (25, 65). Also the LOX product LTB4, a potent neutrophil chemoattractant and activator, is significantly elevated in the colonic mucosa in IBD (30, 58, 80, 85).

Although there is some debate as to their specific roles, the involvement of the COX and LOX pathways is clear. PGE2 is well established as a mediator of inflammatory and mitogenic responses and COX-2 mRNA and protein are expressed in the inflamed intestine in IBD (49). LTB4 is considered to be the principal cause of neutrophil infiltration associated with inflammation and relapse in IBD, and inhibition of LT production in both human and animal studies of IBD suggests that lowering LTB4 levels reduces inflammation and expedites healing (30, 58, 80, 85). Although these studies suggest that inhibition of the 5-LOX pathway alone may be therapeutically effective, it is currently believed that COX inhibitors alone are contraindicated in IBD either as a therapy for intestinal inflammation or as a systemic anti-inflammatory to alleviate IBD-associated diseases such as reactive arthritis, sacroiliitis, or ankylosing spondylitis (30, 40, 58, 63, 73, 80, 85). One reason postulated for this is that blocking of the COX pathway is believed to “shunt” AA metabolism into the LOX pathway, although this hypothesis is under debate (14). Since both the COX-2 metabolites (PGE2, PGD2, PGF2α, and TXB2) as well as the LOX metabolites (LTB4, 5-HETE, and 12-HETE) are thought to be involved in the acceleration of inflammation in IBD (41), shunting to either pathway might still result in increased inflammation. Thus blocking COX-2 might increase LTB4 production and therefore worsen inflammation. Alternatively, blocking COX-2 might exacerbate inflammation by blocking PGE2-mediated IL-10 production, a process believed to be important for resolution of inflammation (41). However, recent reports have now challenged the practice of avoidance of COX-2 inhibitors and suggest that the COX-2-specific coxibs may actually be used in treating extraintestinal inflammation (e.g., arthritis) in IBD patients and are not harmful if given for a short time (40, 48, 63, 73). This disparity between reports is felt to lie in the lack of controls or poor design of initial studies (40). Although the use of single pathway inhibitors to treat IBD and its associated ailments is still under debate, it is clear that the current effective strategies for treating IBD follow a “dual-pathway inhibition” in which both COX-2 and 5-LOX [i.e., 5-aminosalicylic acid (5-ASA)] are inhibited or in which phospholipase A2 activity is inhibited (i.e., steroid therapy), thereby preventing the formation of AA (41, 48, 58, 81).

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A serious complication of IBD is the development of colorectal cancer (CRC), which accounts for up to 15% of IBD mortalities (79). Besides their role in inflammation, eicosanoids are known to be important if not central to the development of colonic adenomas and CRC (81). COX-2 expression increases in polyposis and the progression to adenocarcinoma (1, 2, 6, 84) and inhibition of COX-2 has been shown to be an effective treatment to prevent intestinal adenomas and carcinomas in both human and animal models (28, 29, 75). In addition to COX, it is becoming evident that LOX is involved in cancer as well. It has traditionally been believed that 5-LOX was involved in cancer because of its proinflammatory effect (14, 70). However, recent studies demonstrate that, besides playing a role in inflammation, LOX metabolites, specifically 5- and 12-HETEs, have an independent, antiapoptotic effect on breast, prostate, and colon cancer cells and may even induce their proliferation (5, 9, 31, 59). Furthermore, inhibition of LOX activity, when coupled with COX inhibitors, has been shown to reduce tumor growth in murine models of CRC and other cancers (23, 31, 86). This dual inhibition concept has been considered for preventing IBD-associated CRC, and several recent studies demonstrate a beneficial role of 5-ASA in reducing the risk of dysplasia and CRC (61, 62, 78, 79).

We and others have shown that the colonic myofibroblast (CMF) is the principal COX-2-expressing cell in colonic adenomas (3, 67, 84), suggesting that the CMF is the key target cell for the chemoprevention of CRC via COX-2 inhibition. IL-1, whose mucosal levels increase in both acute and chronic intestinal inflammation (22, 87), induces COX-2 expression in CMF. Since LOX levels are elevated in both IBD and adenomas (25, 65, 86) and since leukotrienes (LTD4) have been shown to affect COX transcription (54), we examined the effect of two other LOX-derived AA metabolites, HETEs and lipoxins, on IL-1α-induced COX-2 expression in CMFs. Although we found lipoxins to be without effect, HETEs were found to enhance IL-1-induced COX expression. The ability of HETEs to enhance COX-2 expression was found to be at the level of message stabilization. This novel finding demonstrates that, besides affecting transcriptional control and the effects of shunting, the COX and LOX pathways can interact via regulation of message stability. These data suggest that the benefit of dual inhibition therapy may be in blocking COX-2 activity as well as the production of LOX products that act as enhancers of COX-2 expression.

MATERIALS AND METHODS

Cell culture. The commercially available primary human colonic subepithelial myofibroblast isolate (18Co) was obtained from the American Type Culture Collection (CRL-1459) and maintained as described previously (77). Culture media, supplements, and subculturing reagents were purchased from Sigma. Cells were cultured in Eagle’s minimum essential medium supplemented with 10% NuSerum (Becton-Dickinson, Franklin Lakes, NJ) at 37°C in a humidified incubator containing 5% CO2. For experiments, cells were subjected to Western blot analysis as previously described (50).

Northern blot analysis. Total RNA was isolated using the Ultraspec RNA Isolation Reagent (Biotecx Laboratories, Houston, TX), and Northern blotting was performed using a COX-2-specific probe as previously described (50).

Western blot analysis. Protein was isolated from 18Co cells and subjected to Western blot analysis as previously described (50).

Briefly, cells were washed with ice-cold phosphate-buffered saline and lysed in Laemmli sample buffer; 10 μg of protein were separated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA). The membranes were saturated with 5% fat-free dry milk in Tris-buffered saline (50 mM Tris, pH 7.5, 150 mM NaCl) with 0.05% Tween 20 (TBS-T) for 1 h at room temperature, incubated overnight with the appropriate primary antibody, and diluted in 5% bovine serum albumin TBS-T. After washing with TBS-T solution, blots were further incubated with the appropriate peroxidase-conjugated secondary antibody for chemiluminescent detection. Chemiluminescent detection was performed using the Enhanced Chemiluminescence Detection Kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to the supplier’s recommendations.

COX-2 transcription rate analysis. The COX-2 transcription rate was measured by the hnRNA amplification method described by Mifflin et al. (51). On the basis of the fact that nascent, unspliced nuclear RNA (heterogeneous nuclear RNA, hnRNA) is rapidly spliced to generate mature mRNA, quantitation of unspliced COX-2 transcripts is used as an indicator of the COX-2 transcriptional rate. Total RNA samples were treated with DNase (Promega, Madison, WI) and reverse transcribed by use of an oligonucleotide primer specific within intron 3 of the human COX-2 gene to generate heterogeneous nuclear complementary DNA (hncDNA). Samples were then subjected to amplification by PCR using intron-specific primer pairs to generate products traversing COX-2 exons. For each reverse transcription reaction, a mock reaction was carried out in which the reverse transcriptase was left out to control for potential contamination by genomic DNA. The reverse transcription and PCR primers used were as follows (numbering based upon GenBank accession number D28235): Amplification across exons 2 and 3: COX2GENL3422 5'-GGCTGAGTATGGCACCC-3'; 289-nt transcript was comprised of a portion of intron 1, exon 2, intron 2, exon 3, and part of intron 3, whereas in the 289-nt transcription of intron 2 is complete. It was empirically determined that 25 cycles of amplification were within the linear quantitative range for this assay and this number was used throughout. We and others have shown that the colonic myofibroblast (CMF) is the principal COX-2-expressing cell in colonic adenomas (3, 67, 84), suggesting that the CMF is the key target cell for the chemoprevention of CRC via COX-2 inhibition. IL-1, whose mucosal levels increase in both acute and chronic intestinal inflammation (22, 87), induces COX-2 expression in CMF. Since LOX levels are elevated in both IBD and adenomas (25, 65, 86) and since leukotrienes (LTD4) have been shown to affect COX transcription (54), we examined the effect of two other LOX-derived AA metabolites, HETEs and lipoxins, on IL-1α-induced COX-2 expression in CMFs. Although we found lipoxins to be without effect, HETEs were found to enhance IL-1-induced COX expression. The ability of HETEs to enhance COX-2 expression was found to be at the level of message stabilization. This novel finding demonstrates that, besides affecting transcriptional control and the effects of shunting, the COX and LOX pathways can interact via regulation of message stability. These data suggest that the benefit of dual inhibition therapy may be in blocking COX-2 activity as well as the production of LOX products that act as enhancers of COX-2 expression.

mRNA stability assays. Message stability was performed as previously described (51). Confluent 18Co cultures were incubated for 4 h in the presence of IL-1 (500 pg/ml) or IL-1 plus 5-(S)-HETE (10.0 μM). Cells were then placed in serum-free medium containing the RNA polymerase II transcriptional inhibitor DRB (5,6-dichloro-D-ribofuranosylbenzimidazole) (50 μM), DRB plus IL-1, or DRB plus IL-1 and 5-(S)-HETE or IL-1 plus the p38 inhibitor SB-203580 (20 μM). Cultures were then frozen at the indicated times (in hours) for determination of COX-2 mRNA levels by Northern blotting.

PGE2 measurements. Confluent 18Co monolayers were incubated for 24 h in the presence of IL-1 (500 pg/ml) or IL-1 plus 5-(S)-HETE (10.0 μM). Cells were then placed in serum-free medium containing the RNA polymerase II transcriptional inhibitor DRB (5,6-dichloro-D-ribofuranosylbenzimidazole) (50 μM), DRB plus IL-1, or DRB plus IL-1 and 5-(S)-HETE or IL-1 plus the p38 inhibitor SB-203580 (20 μM). Cultures were then frozen at the indicated times (in hours) for determination of COX-2 mRNA levels by Northern blotting.

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5(S)-HETE as described above. Cells are harvested at various time points as indicated and placed on ice for 5 min. Cells are then collected in cold PBS by scraping and centrifuged at 150 g for 5 min. The cells are then resuspended in ice-cold EBKL buffer (25 mM HEPES pH 7.6, 5 mM MgCl2, 1.5 mM KCl, 2 mM DTT, 1 mM PMSF, 4 µg/ml each of aprotinin and leupeptin, and 0.1% NP-40). The cells are then lysed by 20 strokes in a Dounce (tight pestle) and the nuclei are removed by two 3-min spins at 600 g. The supernatant is then spun at 10,000 g for 10 min and this supernatant constitutes the cytoplasmic extract. Cytosolic extracts are then subjected to Western blot analysis using denaturing SDS-PAGE as described above and probed with an anti-HuR antibody (Santa Cruz Biotechnologies, Santa Cruz, CA).

Statistical analysis. A two-tailed Student’s t-test was performed on the data obtained for the hnRNA studies. For the time course studies used to examine phosphorylation levels of p38 and MK-2, an ANOVA was performed on the groups and a single-tailed Student’s t-test was performed to compare the IL-1- and IL-1 + HETE-treated groups.

RESULTS

Effect of LOX products on IL-1α-induced COX-2 protein levels. Confluent monolayers of 18Co cells were treated with various LOX products in the presence and absence of IL-1α as indicated in Fig. 1A. We have previously reported that IL-1α induces COX-2 expression in 18Co cells (20, 21, 50). Since eicosanoid production is known to increase during IBD (24, 41, 57) and since LOX products increase during AA metabolism (11, 34, 38, 64) and are produced by neutrophils during inflammation (70, 71), we tested the effect of HETEs and lipoxins on COX-2 protein expression in 18Co. Of the HETEs tested, only 15(S) alone increased COX-2 expression above that in control cells. This effect on COX-2 expression could be seen upon longer exposure of the Western blot (data not shown). However, all of the HETEs tested significantly increased the level of IL-1α-induced COX-2 expression (3.6 ± 0.5-fold compared with control). Cotreatment with lipoxins A4 and B4 had no significant effect on IL-1α-mediated COX-2 induction.

Effect of HETEs on IL-1α-mediated COX-2 mRNA induction. To determine whether HETEs enhanced IL-1α-mediated COX-2 expression at the mRNA as well as the protein level, Northern blot analysis was performed on RNA isolated 24 h after treatment (Fig. 1B). As we have previously shown, IL-1α increased COX-2 mRNA expression 16-fold over control cells (n = 4) (50). As was observed at the protein level (Fig. 1A), only 15(S)-HETEs affected COX-2 mRNA expression on their own, and this induction was detectable but slight. However, the combination of IL-1α + HETEs increased COX-2 mRNA expression three- to fivefold higher than IL-1α alone. Thus the patterns of COX-2 mRNA expression in intestinal myofibroblasts (IMF) treated with IL-1α and HETEs, alone or in combination, reflect the pattern of COX-2 protein expression.

Effect of HETEs on PGE2 synthesis. We measured PGE2 concentrations in the media to determine whether COX-2 enzymatic activity reflected the changes seen in COX-2 expression (Fig. 1C). Confluent monolayers were treated with IL-1α and HETEs alone or in combination for 24 h. The culture medium was removed and PGE2 content was measured by radioimmunoassay (32). IL-1α and 15(S)-HETE were found to increase PGE2 levels above control on their own (709 ± 13.28 and 114.58 ± 3.68 vs. 13.64 ± 1.88 pg/0.1 ml

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for IL-1, 15(S)-HETE, and untreated wells, respectively; P < 0.01). 5(S) and 12(R)-HETEs alone had no appreciable effect on PGE2 synthesis (24.76 + 0.3 and 25.16 + 2.7 pg/0.1 ml, respectively). However, in combination with IL-1α, all of the HETEs tested dramatically increased COX-2 enzymatic activity four- to fivefold higher than IL-1α alone (2.541 + 249, 1.812 + 21 and 2.785 + 153 pg/0.1 ml for IL-1 plus 5(S), 12(R) and 15(S)-HETE, respectively, P < 0.01). Coupled with the results above, the data demonstrate that, despite having little to no effect on their own, HETEs significantly increase IL-1α-mediated COX-2 production and activity in IMF.

**HETEs alter COX-2 mRNA Stability.** The above results demonstrate that although HETEs cannot induce COX-2 expression on their own, they do enhance IL-1α-mediated expression. hnRNA analysis was performed to determine whether this increase in expression was due to enhanced transcription. hncDNA were generated using primers to intron 3 of the unspliced COX-2 mRNA as described in MATERIALS AND METHODS. hnRNA levels were measured by densitometry and were expressed in arbitrary units. The results demonstrate that 5(S)-HETE alone had no significant effect on COX-2 transcription compared with control cells, with both groups displaying 5(S)-HETE alone had no significant effect on COX-2 transcription rate of COX-2 mRNA of IL-1α treatment alone [1,152,852 + 2,613 vs. 1,160,921 + 13,367 units (P = 0.3), IL-1 + 5(S)-HETE vs. IL-1, respectively, Fig. 2A]. Control experiments in which no reverse transcriptase was added resulted in no detectable PCR products, demonstrating specificity of the products (data not shown). Therefore, experiments were performed to determine whether the increase in COX-2 mRNA levels was due to changes in message stability (Fig. 2B). IMF were treated with IL-1α ± HETE for 4 h and then placed in serum-free media containing 50 μM DRB. RNA was isolated at 1, 2, and 4 h and subject to Northern blot analysis and densitometry as described in MATERIALS AND METHODS. Cotreatment with HETE dramatically increased COX-2 mRNA half-life compared with IL-1α alone (from 1.75 h for IL-1α alone to greater than 5 h for IL-1 + HETE). These data suggest that the increase in COX-2 expression seen with HETE is due to increased mRNA stability.

**Effect of HETEs on p38 activation.** We have previously shown that IL-1α induces MAPK/SAPK activity in IMF (50). IL-1-induced COX-2 expression is dependent on ERK and p38 activation. Although ERK is primarily involved in COX-2 transcription, p38 is primarily involved in COX-2 mRNA stability and inhibition of p38 leads to a dramatic decrease in COX-2 message stability (51). Since 5(S)-HETE affected message stability and not transcription rate, we examined the effect of HETE on p38 activity (Fig. 3A). Differences were found to exist between treatments as determined by ANOVA (P < 0.001), and a single-tailed Student’s t-test was used to compare p38 levels between IL-1 and IL-1 + HETE at each time point. 5(S)-HETE was found to induce p38 activation by itself. In addition 5(S)-HETE increased and prolonged the level of p38 phosphorylation seen with IL-1 alone (Fig. 3A). HETE elevated p38 phosphorylation at 0.5, 1, and 2 h, respectively (Fig. 3A, graph). This increased activation of p38 is likely to contribute to the ability of HETEs to augment IL-1-induced COX-2 expression.

**HETE induces MAPKAPK-2 phosphorylation and HuR translocation.** Stabilization of COX-2 message is dependent on the activation of MAPK-activated protein kinase 2 (MAPKAPK-2) and binding of human antigen R (HuR) to the 3’ untranslated region of the COX-2 message (16, 42, 52, 72). Activation of MAPKAPK-2 was determined by Western blot analysis using an anti-phospho MAPKAPK-2 antibody. As for phospho-p38 levels, differences were noted between treatment groups for MAPKAPK-2 phosphorylation (ANOVA, P < 0.001). IL-1-induced MAPKAPK-2 activation from 0.5 to 4 h (Fig. 3B). HETE alone induced MAPKAPK-2 phosphorylation at 1 h. The fold induction is not calculated since MAPKAPK-2 phosphorylation in the control lanes was below the sensitivity of the assay. The combination of HETE and IL-1 increased...
MAPKAPK-2 phosphorylation above IL-1 alone (Fig. 3B, graph).

We then examined the effect of HETE on HuR translocation into the cytosol from the nucleus. 18Co cells were treated with IL-1, 5(S)-HETE, or IL-1 and 5(S)-HETE; fixed; and nuclear DNA stained with propidium iodide (red); immunofluorescence was performed with an anti-HuR antibody (green) as described. HuR was localized almost exclusively in the nuclei of control cells (Fig. 4A) seen as yellow owing to the colocalization of red and green. Treatment with IL-1 or HETE resulted in nearly identical condensation of nuclear HuR and migration of HuR into the cytosol. The combination of IL-1 and HETE worked additively to induce HuR translocation with a high percentage of HuR being detected in the cytosol. To confirm the immunofluorescence results, cytosolic fractions were isolated from cells and Western blot analysis was performed using a HuR-specific antibody. Representative Western blots of cytosolic fractions isolated 2 and 6 h after treatment with IL-1, 5(S)-HETE, or IL-1 + 5(S)-HETE are shown in Fig. 4B, and a graphic representation of the 6-h data is given. IL-1 treatment resulted in a 3.78 ± 0.2-fold increase in cytosolic HuR above control. HETE alone increased cytosolic HuR levels 4.82 ± 0.5-fold above control and the combination of IL-1 + HETE increased this level to 5.81 ± 0.2-fold at 6 h. Taken together, the immunofluorescence and Western blot analysis demonstrate that both IL-1 and HETE induce HuR translocation and that they act additively in inducing HuR.

Since HuR translocation is reported to require p38 activity, we examined the effect of the p38 inhibitor SB203580 (Calbiochem) on HETE-mediated HuR translocation into the cytosol (Fig. 4C). 18Co cells were pretreated with 20 µM SB203580 for 1 h prior to the addition of HETE or vehicle alone. Samples were treated with 5(S)-HETE as before and cytosolic protein was isolated 4 h after the addition of HETE. Cytosolic HuR was determined by Western blot analysis followed by densitometry, and the ratio of HuR to vimentin was determined to correct for loading. The results are presented as a percent of control HuR levels. Treatment with SB203580 resulted in a decrease in cytosolic HuR (77 ± 17.4% of control). Treatment with HETE alone increased cytosolic HuR levels to 186% of control and this increase was prevented by pretreatment with the p38 inhibitor (99 + 33.4% vs. 186 + 14.3% of control, for the SB203580 + HETE treated cells vs. HETE treatment alone, respectively, *P = 0.04).

The above data demonstrate that HETE increase the activation of p38 and MAPKAPK-2 and the translocation of HuR into the cytosol and that the translocation of HuR is dependent on p38 activation. Since p38, MAPKAPK-2, and HuR activation are known to contribute to COX-2 mRNA stabilization and given that HETEs do not increase COX-2 transcription but do increase COX-2 mRNA half-life, the data suggest that
HETEs enhance IL-1-mediated COX-2 expression via increased message stabilization.

**DISCUSSION**

Both COX-2 and 5-LOX are elevated in the inflamed colon as well as in colonic adenomas and adenocarcinomas (33, 53, 54, 86). PGE2, produced from AA by COX-2, is a key mediator of both inflammation and CRC (15, 26, 37, 60, 68, 82, 83). PGE2 levels are elevated in malignant tissue and PGE2 is the predominant prostaglandin found in tumors in both animal and human models of CRC (15, 26, 37, 60, 83). Studies demonstrate that COX-2-derived PGE2 leads to increased proliferation and invasiveness of colon cancer cells and that COX-2 expression increases the risk of adenoma and adenocarcinoma formation in familial adenomatous polyposis patients and animal models (15). Therefore inhibition of COX-2 has been a focus in the chemoprevention of CRC (47, 62, 81). In contrast, both COX-2 and 5-LOX have been targets in the treatment of IBD (8, 14). It is believed that the increased expression of COX-2 and the resulting overproduction of PGE2 is an underlying mechanism by which IBD increases the risk of CRC (49, 54).

Although COX-2 and 5-LOX represent two distinct metabolic pathways for AA, it is becoming clear that they do not function independently. Inhibition of COX-2 activity has been shown to shunt AA metabolism toward the LOX side of the pathway, in particular toward 5-LOX with the production of leukotrienes and 5-HETE (27, 35, 39, 76). However, inhibition of 5-LOX was found to have no effect on PGE2 synthesis, suggesting that the shunt is unidirectional (86). Recent evidence, however, suggests that 5-LOX products do have a regulatory effect on COX-2 activity. The 5-LOX product leukotriene D4 binds to its receptor (cysLT1R) and leads to the upregulation of COX-2 transcription, a process demonstrated in intestinal epithelial cells and in colorectal adeno-

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**Fig. 4.** HETE-induced cytosolic translocation of human antigen R (HuR). A: immunofluorescence microscopy demonstrating IL-1- and 5(S)-HETE-induced cytosolic translocation of HuR. Subconfluent 18Co were treated with IL-1 +/− 5(S)-HETE for 1 h. The cells were then fixed and permeabilized and stained with an anti-HuR antibody (primary) and then an Alexa 633 antibody (secondary) (green). Nuclei were stained with propidium iodide (red). HuR was found to be predominantly localized in the nuclei in control cells [seen as yellow owing to the colocalization of HuR (green) and propidium iodide (red)]. Both IL-1 and 5(S)-HETE appeared to act equally to induce a punctuate appearance of HuR in the nuclei as well as mobilization of HuR into the cytosol. The combination of IL-1 and 5(S)-HETE had an additive effect in which translocation of HuR to the cytosol was greater than treatment with either agent alone. B: Western blot analysis of HuR content in the cytosolic fraction of 18Co cells treated with IL-1 and 5(S)-HETE. Confluent monolayers were treated as above and cytosolic fractions isolated (MATERIALS AND METHODS) 2 and 6 h after treatment. Membranes were probed for HuR and vimentin (loading control), densitometry was performed, and the results were shown as cytosolic HuR as a percent of control. Data is shown as the averages and standard errors of 3 experiments.
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Fig. 5. Model for the regulation of COX-2 expression by IL-1 and HETEs. IL-1 binds to its receptor and activates the MAPK and PKCζ pathways as well as NF-κB. Transcription of COX-2 mRNA is dependent on ERK and p38 activation as well as the generation of reactive oxygen species (ROS) to drive NF-κB-mediated transcription at the COX-2 promoter. In addition to its effect of transcription, p38 contributes to COX-2 mRNA stabilization via activating MAPKAPK-2 and possibly HuR. HETEs, by binding to their receptor or through receptor-independent mechanisms, activate p38, MK-2, and HuR and thus enhance COX-2 expression by increasing message stability. HETEs, by themselves, were found to have no effect on COX-2 transcription nor did they increase IL-1-induced COX-2 transcription. The mechanism by which both IL-1 and HETEs activate HuR may or may not involve p38 acting directly on HuR.

These data also present a possible mechanism by which HETEs act antiapoptotically in tumor models. Since many cancer cells express COX-2, it is plausible that the exogenous addition of HETEs may result in even further overexpression of COX-2, resulting in PGE2 production, which is known to be antiapoptotic in epithelial cells. Furthermore, HETEs may also contribute to the initiation and progression of colon cancer by its ability to activate HuR. It has recently been reported that in both IB and colon carcinogenesis the HuR gene is overexpressed in the cytosol (7). HuR was overexpressed in colon tissue isolated from ulcerative colitis, colonic adenomas, adenocarcinomas, and metastasis as well as in colon cancer cells. HuR prevents the degradation of many tightly regulated mRNAs including those of cancer-associated gene expression and was found to colocalize with COX-2 overexpression. Overexpression of HuR was found to double the tumor burden in APCMin mice compared with APMcMin mice with normal HuR expression (7). Furthermore, increased HuR binding in colon cancer cells has been shown to enhance many of the signaling pathways that contribute to cancer progression (44, 45). HuR can enhance cell division by increasing the expression of EGF, GM-CSF, Fos, Myc, and cyclins A, B1, and D1, enable tumor growth through angiogenesis by binding to VEGF and HIF-1 transcripts and increase the tumor’s invasive potential by increasing MMP-9, MT1A, and uPA expression.
Our results demonstrate that HETEs can increase the activation of HuR and suggest that HETE may contribute to adenoma and adenocarcinoma formation through HuR activation by increasing COX-2 expression as well as stabilizing the transcripts of other cancer-associated genes. Thus HETEs have the potential to contribute to carcinogenesis via both COX-2-dependent and independent mechanisms. For example, the ability of HETEs to activate p38 suggests that they may play a role in exacerbating intestinal inflammation by driving the expression of proinflammatory cytokines as well as transcriptional mediators involved in both inflammation and cancer (56, 69, 74).

Our novel data demonstrate the ability of HETEs to increase COX-2 expression via message stabilization. This finding adds an additional aspect to the cross talk between the COX and LOX pathways. Thus, in addition to shunting via inhibition of COX activity and LTD4-increased COX-2 transcription (54, 55), we have now demonstrated that LOX metabolites, in this case HETEs, can also increase COX-2 expression by increasing message stability. The ability of LOX products to act as inducers of COX expression at multiple levels suggests a biological need to maintain an equilibrium between these two pathways. This might reflect the involvement in both of these pathways in both initiating and resolving inflammation and may explain why inhibition of only one pathway exacerbates inflammation. For example, studies demonstrate that dual inhibition of COX and LOX reduces intestinal injury associated with COX inhibition alone in both animal models and in the normal human population (13, 14). Thus our data support the “dual inhibitor” approach in treating IBD and might explain why inhibition of COX-2 alone by traditional NSAIDs has been associated with flare-ups, whereas 5-ASA, which inhibits both pathways (46), is considered safe and effective in IBD. Furthermore, recent reports suggest that, besides ameliorating IBD, 5-ASA reduces the risk of developing CRC in IBD patients. Aminosalicylates were found to lower the incidence of CRC and dysplasia as well as to induce apoptosis of tumor, but not normal intestinal mucosal cells (12, 47, 61, 78, 79). 5-ASA is considered to have rare and few side effects. Also, although mesalazine induced apoptosis of tumor cells, it did not adversely affect normal mucosal cells (12), which agrees with the safety findings of dual inhibition (13, 14). Since both COX and LOX pathways are elevated in and contribute to the formation of adenomas and adenocarcinomas, we suggest that 5-ASA may be considered as a safe and effective chemopreventative strategy for inhibiting the development of adenomas and CRC.

GRANTS
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