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Review

Leukotriene synthesis by epithelial cells

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Summary. Leukotrienes (LTs) are intercellular signaling molecules that evoke a variety of responses. They are best known as potent promoters of inflammation. Normally, LTs are produced primarily by leukocytes. As a result, current models regarding the production of LTs in the context of disease focus on the leukocytes as the site of production. Structural cells, including epithelial cells, are typically relegated to supportive roles. It is recognized that epithelial cells normally contain all the components necessary for LT synthesis except the enzyme 5-lipoxygenase (5-LO). There is accumulating evidence that some populations of epithelial cells normally express low levels of 5-LO and can synthesize LTs autonomously. Moreover, certain factors, including bacterial and viral infection, can promote the expression of 5-LO in airway, gastrointestinal and skin epithelial cells. The appearance of active 5-LO enzyme in epithelial cells at these sites may contribute to diseases like cancer, colitis and psoriasis. This paper reviews the state of our knowledge regarding the expression of 5-LO in epithelial cells, the factors that modify that expression, and the implications regarding pathogenesis.

Key words: Leukotrienes, 5-lipoxygenase, Epithelial cells, Inflammation

Introduction

Arachidonic acid (AA) metabolism through the 5-lipoxygenase (5-LO) pathway produces the class of lipid mediators known as leukotrienes (LT). These chemical messengers are rapidly secreted immediately after synthesis and move to target cells to orchestrate the immune response (reviewed in Funk, 2001). However, abnormal production of LTs contributes to disease. The overproduction of LTs has been shown to be involved in the pathophysiology of several diseases including inflammatory bowel disease (Stenson, 1990), asthma (Drazen et al., 1994), ulcerative colitis (Cole et al., 1996), psoriasis (Iversen et al., 1997), and cancer (Avis et al., 1996a). The underproduction of LTs can occur with viral infection (Coffey et al., 1996) or sepsis (Morlion et al., 2000) and can impair immune function, manifested as decreased phagocytic and bactericidal action by macrophages with prolonged bacterial infection (Bailie et al., 1996). Thus, mechanisms that regulate the production of LTs are of great interest.

The production of LTs begins with the peroxidation of AA to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE) by the 5-LO enzyme (Fig. 1). This reaction is strongly promoted by the 5-LO activating protein (FLAP). Some of the 5-HPETE product may be reduced by peroxidase to form 5-hydroxyeicosatetraenoic acid (5-HETE) and be secreted in this biologically active form. More typically, 5-LO catalyzes a second enzymatic step, the conversion of 5-HPETE to leukotriene A_4 (LTA_4), an unstable intermediate (Rouzer et al., 1986). LTA_4 can be catalytically converted to leukotriene B_4 (LTB_4) by LTA_4 hydrolase (Haeggstrom et al., 1993) or can be conjugated with glutathione by LTC4 synthase to produce the cysteinyl LT, LTC_4. LTC_4 can be further metabolized to LTD_4 and LTE_4. The 5-LO enzyme is dependent on calcium for activity. As a result, this pathway is only activated following a rise in intracellular calcium, as may occur upon cell stimulation. Because 5-LO initiates LT synthesis, its action represents a key point of regulation in LT synthesis.

Leukotrienes were first discovered in leukocytes. Subsequent studies showed that LTs are primarily made by neutrophils, basophils, eosinophils, monocytes, macrophages, and mast cells. Each cell type expresses the key enzymes, 5-LO and FLAP. However, the expression of the distal enzymes, LTA_4 hydrolase and LTC_4 synthase, varies between cell types, and this determines the types of LT that each cell type can make. Leukotrienes act in biological processes associated with the immune response by targeting and activating specific receptors. Receptor activation on leukocytes promotes adhesion to vascular endothelium and chemotaxis, driving the recruitment of leukocytes. In addition, LTB_4

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activates leukocytes, promoting an array of functions including superoxide generation, degranulation and phagocytosis. Because of its role in attracting and activating both neutrophils and monocytes, LTB_4 plays a central role in promoting inflammation. The cysteinyl LTs bind to the cysteinyl receptors, cysLT1 and cysLT2, which mediate vascular permeability and mucus secretion, vasodilation, bronchoconstriction, edema, and migration of eosinophils. The overproduction of cysteinyl LTs is central to asthma and allergic hyperresponsiveness.

The epithelium and leukotrienes

The epithelial surface, covering the skin, respiratory and gastrointestinal systems, represents the initial barrier to infection. Epithelial cells (EC) are typically the first cells exposed to pathogens and thus are an important site for regulating the inflammatory response. Resident leukocytes, capable of generating LTs upon stimulation, can be found in and around the epithelium. The release of chemoattractants like LTB_4, by EC or resident leukocytes, attracts leukocytes into the tissue, causing inflammation. These recruited leukocytes, in turn, may make more chemoattractants, further amplifying the inflammatory response. Chronic inflammation at the epithelial surface, accompanied by persistent overproduction of LTs, is a key feature of diseases like asthma and inflammatory bowel disease.

There are three accepted models for LT generation at the epithelial surface. First, resident leukocytes can be stimulated to synthesize and secrete LTs. Thus, application of an allergen to the skin of a sensitized individual will stimulate resident mast cells to secrete LTC_4, leading to the edema associated with the flare and wheal response. Second, recruited leukocytes can secrete LTs. For example, neutrophils recruited into a site of bacterial infection will be activated and release a combination of LTs, including LTB_4, LTA_4 and 5-HETE.

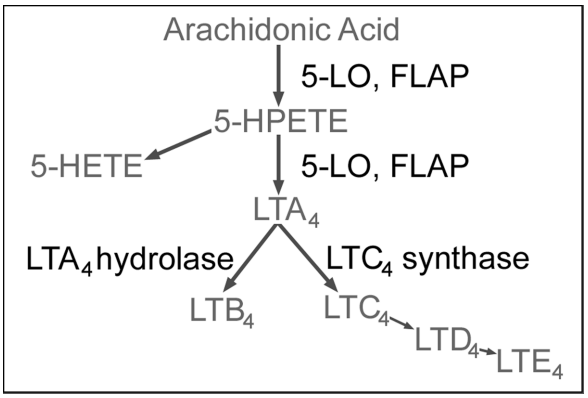

Fig. 1. Enzymes involved in the synthesis of leukotrienes from arachidonic acid. 5-LO: 5-lipoxygenase; FLAP: 5-lipoxygenase activating protein; LT: leukotriene.

These mediators will promote the further recruitment and activation of other neutrophils. The third way that LTs can be produced in epithelial tissue is through transcellular metabolism. It has been amply demonstrated that EC contain the enzyme LTA4 hydrolase. As a result, EC can make LTB4 if provided with LTA_4. Neutrophils are particularly good donors of LTA_4. In addition, leukocytes can use AA secreted from EC as substrate for the leukocyte 5-LO pathway. As a result, it is likely that much of the LT produced at sites of inflammation comes from interactive metabolism between EC and leukocytes.

It is clear that EC themselves contain and release AA, and that they express active LTA_4 hydrolase enzyme. However, it is not clear whether there are populations of EC that normally contain active 5-LO protein, or whether EC can be induced to express 5-LO under certain conditions. Ultimately, the key question will be whether EC, either normally or following induction, make LTs autonomously. The purpose of this review is to examine the available evidence of 5-LO expression and LT synthesis by ECs.

5-LO expression and LT synthesis by airway ECs

The enzyme LTA_4 hydrolase, which converts the 5-LO product LTA_4 to LTB_4, is functionally expressed in human bronchial epithelial cells (Bigby et al., 1989, 1994). It is now well recognized that human airway epithelial cells can generate 5-LO metabolites through transcellular metabolism by which the 5-LO enzymatic product LTA_4 released from the infiltrated leukocytes can further be metabolized to LTB_4 in airway epithelial cells (Grimminger et al., 1992; Zhou et al., 1995).

In addition to the transcellular LT synthesis mechanism, an intriguing possibility is that airway epithelial cells may be a direct source of LTs. Initial evidence for this possibility came from the observation that purified canine tracheal epithelial cells converted exogenous AA to LTB_4 as determined by reverse phase HPLC and radioimmunoassay (Holtzman et al., 1983). Studies provided by Eling et al. (1986) confirmed that freshly isolated canine tracheal epithelial cells metabolized endogenous AA to significant quantities of LTB4 and cysteinyl LTs (1-2 ng/ 10^6 cells). Formation of 5-LO metabolites (5-HETE, LTB4, LTC4) by freshly isolated guinea pig tracheal epithelial cells was also reported when the cells were incubated with exogenous [^{14}C] AA (Oosthuizen et al., 1990). Nahori et al. (1991) studied the morphological features and AA metabolism of primary cultured guinea pig tracheal epithelial cells and found that specific ultrastructure of epithelial cells was maintained and the cells produced eicosanoid metabolites, mainly LTB_4 and PGE_2 spontaneously and upon cell stimulation. Immunohistological studies of porcine lung tissue have also revealed that besides eosinophils and mast cells found in the interstitium, certain bronchial epithelial cells were clearly immunostained by 5-LO antibodies (Komatsu et al.,

1991). Further studies of AA metabolism in respiratory epithelial cells of a variety of species revealed significantly different LO products. Holtzman et al. (1988) examined the capacities of epithelial cells isolated from human, dog and sheep airways to metabolize AA. Both dog and sheep cells converted exogenous and endogenous AA to 5-LO products including LTB_4 without significant 15-LO activity, while human cells converted AA predominantly to the 15-LO product 15-HETE. Two other studies also indicate that human epithelial cells produce mainly 15-HETE and minor components of 5- and 12-HETE and some diHETEs (Hunter et al., 1985; Henke et al., 1988). Although the LO pathway varies in different species, certain airway epithelia may express functional 5-LO, and epithelial-derived LTB_4 may play a more active role than it is normally assumed.

By virtue of their location, the tracheo-bronchial epithelial cells are the first cells in the respiratory tract to encounter inhaled pathogens, allergens and environmental chemicals. There are numerous reports that a variety of inflammatory stimuli lead to induced or augmented synthesis of 5-LO metabolites in airway epithelial cells. Endotoxin is one of the primary agents in organic dust that causes airway inflammation and airflow obstruction (Michel et al., 1991, 1992; Herbert et al., 1992; Schwartz et al., 1994, 1995). Endotoxin produced during bacterial infection has been demonstrated to stimulate cells to release neutrophil chemotactic activity (NCA) and cause cellular toxicity (Rinaldo et al., 1984; Meyrick and Brigham, 1986). Bovine bronchial epithelial cells exposed to endotoxin release both NCA (Koyama et al., 1991b) and monocyte chemotactic activity (MCA; Koyama et al., 1991a). This effect can be blocked by the lipoxygenase inhibitors NDGA and diethylcarbamazine (DEC), suggesting that LTB_4 may be a component of NCA and MCA. Further analysis of [^{3}H]-AA metabolism of endotoxin-treated bovine bronchial epithelial cells by reverse phase HPLC revealed that LTB_4 is the major LO product. Clementsen et al. (1989) reported that Staphylococcus aureus and influenza A virus stimulated human bronchoalveolar cells to release histamine and LTB4. Raz et al. (1993) reported that infection of bovine bronchial epithelial cells with bovine herpes virus-1 (BHV-1) induced increased NCA. Inhibitors that impair LTB_4 synthesis, including NDGA, hydrocortisone and DEC, markedly reduced NCA released after BHV-1 inoculation. Using reverse phase HPLC analysis of the AA metabolites in the cell supernatants, they also confirmed that one of the chemoattractants released was 5-LO metabolite LTB_4 (Raz et al., 1993). Respiratory syncytial virus (RSV) infection is a major risk factor for children to develop and exacerbates asthma (Hall et al., 1984). RSV infection up-regulates the expression of 5-LO mRNA and protein in two human bronchial epithelial cell lines, as detected by RT-PCR and western blot respectively (Behera et al., 1998). As expected, the levels of LTB_4 and cysteinyl-LT (LTC_4, LTD_4, LTE_4) released from RSV-infected HEp-2 and BEAS-2B cells were also significantly increased when measured by enzyme immunoassay. It is conceivable that induced or augmented expression of 5-LO in airway epithelial cells may account for the pathogenesis of a variety of hyperactive airway diseases.

Besides bacterial and viral infection, certain environmental chemicals also appear to up-regulate the synthesis of 5-LO metabolites in tracheo-bronchial epithelia. Ozone (O_3) is an important photochemical oxidant found in both indoor and outdoor air pollutants. It can induce airway epithelial damage, inflammation, hypersecretion and bronchoconstriction and products of AA metabolism from the epithelium have been suggested to mediate the observed physiologic responses (for review, see Lippman, 1989). Indeed, it has been demonstrated that neutrophil infiltration was localized predominantly to the epithelial layer of the airway wall in dogs breathing ozone, suggesting that critical inflammatory mediators were released from the epithelial cells (Holtzman et al., 1983). Leikauf et al. (1988) first reported that ozone exposure to cultured bovine tracheal epithelial cells augmented the eicosanoid metabolism by significantly increasing the release of PGE_2, $PGF_{2\alpha}$, 6-keto $F_{1\alpha}$ and LTB_4. McKinnon et al. (1993) showed that in vitro ozone exposure of a human bronchial epithelial cell line, BEAS-6, increased the release of AA products both from the 5-LO pathway (LTB_4, LTC_4, LTD_4, LTE_4) and the cyclooxygenase pathway (thromboxane B_2, prostaglandin E_2). Shirali et al. (1994) reported that nickel subsulfide (Ni3S2) exposure induced significant increases of 5-HETE and 15-HETE from human embryonic pulmonary epithelial cells (L132), suggesting both 5- and 15-lipoxygenase pathways are up-regulated by nickel subsulfide. Chronic exposure to cigarette smoke induces an influx of inflammatory cells into the low respiratory tract (Hunninghake and Crystal, 1983). Masubuchi et al. (1998) showed that smoke extract stimulated human lung epithelial cells (A549) to release both NCA and MCA in a dose- and time-dependent manner. Measurement of LTB_4 by radio immunoassay (RIA) revealed that A549 cells stimulated with smoke extract at the concentration of 5% for 72 hours induced a significant increase of LTB_4 while LO inhibitors NDGA and DEC, 5-LO specific inhibitor AA-861 as well as LTB4 receptor antagonist ONO 4057 all significantly inhibited the release of NCA and MCA. These observations again suggest that the 5-LO pathway may be induced or up-regulated in airway epithelial cells by environmental chemicals.

Other agents also modify LTB_4 production by epithelial cells. Histamine, released from mast cells in the lung, is a chemical with proinflammatory and bronchoconstrictive property (Barnes, 1997). Aoki et al. (1998) showed that histamine stimulated human bronchial epithelial cells (16HBE 14o-) to produce LTB_4. This effect was inhibited by the 5-LO inhibitor zileuton and by the FLAP inhibitors MK-886 and L-

655238. Bleomycin, an anticancer agent, often causes diffused interstitial lung fibrosis in humans (Balikian et al., 1982; Bauer et al., 1983). Sato et al. (1999) reported that bleomycin stimulated human lung epithelial cells to release neutrophil and monocyte chemotactic activities. Application of bleomycin at a concentration of 10 mg/ml for 72 h induced a significant increase in LTB_4 release from A549 cells while NDGA, DEC, AA-861 and LTB_4 receptor antagonist ONO 4057 inhibited the release of NCA and MCA from A549 cells.

In the upper airway, mucosa-associated lymphoid tissues (MALT) including the tonsils and adenoids are organized tissues constantly subjected to viral and bacterial infection and other environmental stimuli. A recent study of LT synthesis in human MALT provided by Brock and Lesperance (2000) presented the first evidence that the stratified epithelium of both palatine and pharyngeal tonsils contains the enzymes 5-LO, FLAP and LTA_4 hydrolase and can synthesize LTB_4 and 5-HETE. Interestingly, the amounts of the 5-LO protein and the extent of epithelium stratification in tonsils vary significantly between tissue samples from different human donors. These results suggest that MALT epithelium may be a source of secreted LTs and LT generation in MALT epithelium may be subjected to modulation.

5-LO expression and LT synthesis by gastrointestinal ECs

The metabolites of the 5-LO pathway, in particular LTB_4 and 5-HETE, have long been identified as important mediators of the inflammatory process in the gastrointestinal tract. In inflammatory bowel disease (IBD), these two molecules have been suggested as important mediators in the pathogenesis of intestinal inflammation (for review, see Stenson 1990). Early in 1983, Boughton-Smith et al. observed increased synthesis of HETEs in human IBD mucosa incubated with [^{14}C]-labeled AA. Sharon and Stenson (1984) first reported that colonic mucosa from human patients suffering from ulcerative colitis released significantly high amounts of LTB_4 (average of 254 ng LTB_4/gram mucosa tissue), whereas normal mucosa produced little if any (less than 5 ng/gram), suggesting a role for LTB4 in the pathogenesis of IBD. Thereafter, there have been a number of reports of increased LT products in animal models of intestinal inflammation (Sharon and Stenson, 1985; Zipser et al., 1987; Boughton-Smith et al., 1988). Since infiltration of the colonic mucosa with neutrophils is an important histologic feature of IBD, it has been assumed that the elevated LTB_4 and 5-HETE synthesis found in diseased mucosa is largely due to the infiltrating leukocytes. However, the factors that initiate and drive chronic leukocyte recruitment are unrecognized. Is it possible that epithelial cells attract and activate leukocytes by secreting LTs?

To answer this question, focus has shifted to establish if isolated intestinal epithelial cells possess LT synthesis capacity. Dias et al. (1992) first reported that a human colonocyte epithelial cell line CaCo-2 stimulated with calcium ionophore A23187 was capable of producing LTB_4 in a dose- and time-dependent way. In addition, LTB_4 synthesis by CaCo-2 cells was found to be increased 6.1 fold above control levels when cells were stimulated with exogenous AA. Treatment with MK-886, a specific inhibitor of the 5-LO pathway, inhibited LTB_4 synthesis by 79% to 94% in the various groups. In another report, Dias et al. (1994) further showed that bile salt stimulated LTB_4 production in CaCo-2 cells and that dexamethasone inhibited bile salt-induced LTB_4 synthesis. These studies suggested that intestinal epithelial cells have an intrinsic capacity to produce leukotrienes via the 5-LO pathway. Sjolander et al. (1993) found that an epithelial cell line, intestine 407, isolated from the jejunum and ileum of a human embryo, produced 5-HETE (0.2-0.5 nmol per 10^7 cells) and LTB_4 (0.6-2.0 nmol per 10^7 cells) when the cells were stimulated with calcium ionophore. More recently, Cortese et al. (1995) screened the human intestinal epithelial cell lines CaCo-2 and HT29 (including two clonal derivatives HT29-18, HT29-18C1) for evidence of LT-associated enzyme transcripts and LT synthesis. They showed that LTA_4 hydrolase was highly expressed at both cell lines while 5-LO transcripts were detected only in HT29 cells as examined by Northern blot. CaCo cells failed to show 5-LO messages even with poly A^+-mRNA, although the transcript could be amplified with RT-PCR. Message for FLAP, the 5-lipoxygenase-activating protein, was detectable in both cell lines, but only with poly A^+-mRNA by Northern blot. In a sonicated cell preparation, HT29, but not CaCo-2, revealed detectable levels of 5-HETE and LTB_4 while NDGA treatment abolished the formation of the two metabolites. Using a different clonal derivative of the HT29 cell line, HT29C1-19A, Battu et al. (1997) also showed that HT29C1-19A cells express both 5-LO and LTA_4 hydrolase, but no FLAP mRNA as found by RT-PCR. When freshly trypsinized cells were incubated with [^{14}C]-labeled AA and calcium ionophore for 15 min at 37 °C, the metabolic profile demonstrated that 5-HETE, LTB4 and its isomers were the major products of the 5-LO pathway, corresponding to 9% of the total radioactivity detected. In the presence of 10^{-4} M NDGA, 5-HETE, LTB_4 and isomers were markedly decreased. These results suggest that intestinal epithelial cells may possess a limited capacity to generate 5-LO metabolites. However, this limited capacity may be up-regulated in certain circumstances.

5-LO expression and LT synthesis by skin ECs

It is known that LTs can express their inflammatory properties in human skin after local injections (Camp et al., 1983; Soter et al., 1983). Topical administration of LTB4 to normal human skin resulted in the formation of intra-epidermal microabscesses (Camp et al., 1984; Paulissen et al., 1990), one of earliest morphologic

events in psoriasis. Although LTs have been indicated to play an important role in inflammatory skin diseases such as psoriasis and atopic dermatitis (for review, see Fogh and Kragballe, 2000; Iversen and Kragballe, 2000), the sources of LTs in human skin have not been fully elucidated. Skin epidermis is a highly organized stratified squamous epithelium, consisting predominantly of keratinocytes with several minor cell populations such as Langerhans cells (LCs), melanocytes, Merkel cells and occasionally leukocytes (Murphy, 1995). Human cultured keratinocytes and epidermis have been shown to transform neutrophil-derived LTA_4 into LTB_4 in vitro (Sola et al., 1992; Iversen et al., 1993, 1994b). The key enzyme, LTA_4 hydrolase, has been identified in human skin EC (Ikai et al., 1994; Iversen et al., 1995, 1996). Transcellular synthesis of cysteinyl LTs by human epidermis has also been shown (Iversen et al., 1994a). Thus transcellular metabolism of AA via the interplay of epidermal keratinocytes and the infiltrated leukocytes may contribute to the LT synthesis in the skin.

In addition to the transcellular mechanism, there are a number of reports to indicate that epidermal keratinocytes and even the Langerhans cells (see below) are direct sources of LTs. Ziboh et al. (1984) presented the first evidence of a functional 5-LO pathway in mouse skin. Challenge of [^{14}C]-AA labeled murine keratinocytes with calcium ionophore resulted in formation of small quantities of radioactive metabolites of AA with chromatographic properties of 5-HETE and LTD_4. An extension of the study with subcellular preparations by the same group further established that both 5-LO-derived LTB_4 and 12-LO-derived 12-HETE were produced by murine keratinocytes whereas NDGA decreased the formation of both metabolites (Ziboh et al., 1984). Brain et al. (1982b) first detected a product with chromatographic and chemotactic activities identical to LTB_4 from primary human keratinocytes stimulated with calcium ionophore. Further studies provided by Grabbe et al. (1984, 1985) confirmed that human epidermal keratinocytes produced LTB_4-like arachidonate metabolites. A recent study by Janssen-Timmen et al. (1995) has demonstrated that a nontransformed human keratinocyte cell line, HaCaT, and normal human keratinocytes (NHKs) maintained in an undifferentiated state in vitro had low or undetectable 5-LO gene expression as determined by 5-LO mRNA and protein analysis, cell-free enzyme activity and LT production in intact cells. However, 5-LO expression (both mRNA and protein levels) and 5-LO metabolites (5-HETE, LTB_4 and its isomers, LTC_4) in HaCaT and NHK cells were markedly induced under culture conditions that favor their differentiation such as prolonged incubation in media supplemented with fetal calf serum. Further studies of 5-LO localization in normal human epidermis by in situ hybridization, immunohistochemistry, as well as in purified epidermal cell populations by RT-PCR and western blot have shown that LCs are the major 5-LO expressing cells in normal human epidermis (Spanbroek et al., 1998). The enzymes FLAP and LTA4 hydrolase were also detected in LCs (Spanbroek et al., 1998), suggesting LCs may possess the capacity for autonomous LT synthesis. These different experimental data suggest that 5-LO expression and action may be either intrinsic or inducible in skin EC.

As observed in inflammatory bowel diseases, activation of the 5-LO pathway has been implicated in skin diseases such as psoriasis (Brain et al., 1982a, 1984, 1985; Fogh et al., 1989) and atopic dermatitis (Talbot et al., 1985; Ruzicka et al., 1986). However, the cellular source of LTs in these skin diseases has yet to be determined. Keratinocytes, which seem to have intrinsic LT synthesis capacity as discussed above, may serve as a direct source of the LTs found in inflammatory skin diseases. In agreement with this hypothesis, a recent study has demonstrated that human keratinocytes produced significant amounts of LTB_4 and 12-HETE after bacterial challenge (Eberhard et al., 2000). Moreover, when stimulated with epithelial supernatants harvested after bacterial infection, neutrophils released significantly higher amount of PGE_2, LTB_4, 12-HETE and 15-HETE. These results suggest that AA metabolism via 5-LO and other LO pathway in skin keratinocytes can be up-regulated by bacterial infection, and these initial AA mediators produced by keratinocytes may act as an early inflammatory signal for the initiation of the immune response in skin inflammation diseases.

5-LO expression and LT synthesis by cancer ECs

It is known that 5-LO metabolites have mitogenic activity and promote the proliferation of normal human cells of different types. For example, LTB_4, LTC_4 and LTD_4 stimulate DNA synthesis in cultured human epidermal keratinocytes (Kragballe et al., 1985). LTB_4 causes proliferation of interleukin 2-dependent T lymphocytes (Atluru and Goodwin, 1986) while LTC_4 binds to human glomerular epithelial cells and promotes their proliferation in vitro (Baud et al., 1985). Cysteinyl LTs have also been demonstrated to increase proliferation of cultured human skin fibroblast when endogenous prostaglandins are suppressed (Baud et al., 1987). Pulmonary fibroblast proliferation is one of the most characterized histological features of idiopathic pulmonary fibrosis (IPF), and constitutive activation of 5-LO in the lungs of patients with IPF has been recently established (Wilborn et al., 1996), suggesting a role for 5-LO products in pulmonary fibroblast proliferation and pathogenesis of IPF.

5-LO products are also capable of modulating the growth of various cancer cells. Bortuzzo et al. (1996) have shown that LTB_4 increases the proliferation rate of two colon epithelial cancer cell lines (HT-29 and HCT-15) in a time- and concentration-dependent manner and the proliferative effect of LTB_4 in HT-29 cells was inhibited by SC-41930, a competitive antagonist of

LTB_4, suggesting that the growth stimulation may be mediated specifically through LTB_4 and its receptor binding in epithelial cells. In two human epithelial cell lines derived from hormone-responsive (LNCaP) and non-responsive prostate cancer (PC3), exogenous AA was found to stimulate cell growth while this effect of AA was specifically inhibited by AA861 and MK-886 (Ghosh and Myers, 1997). Addition of exogenous 5-HETE could reverse the growth inhibitory effect of MK-886, suggesting a mitogenic role of 5-HETE for prostate cancer cells. 5-HETE has also shown to stimulate the growth of human small cell lung cancer (SCLC) and non-SCLC cells (Avis et al., 1996) while exogenously added LTC_4 and LTD_4 was reported to stimulate the growth of human gastric cancer cell line AGS (Shimakura and Boland, 1992).

The growth stimulatory effect of 5-LO products on different cancer cell lines suggest that cancer cells may produce different 5-LO metabolites as autocrine growth factors. Indeed, there have been increasing reports that 5-LO expression and LT synthesis are present or induced in cancer cell lines derived from a variety of malignant tissues. As we discussed previously, several colon cancer cell lines, such as HT-29 and CaCo-2, can independently produce small amounts of 5-LO products (Dias et al., 1992; Cortese et al., 1995; Battu et al., 1997). In contrast to the relative large amounts of 5-LO products produced from leukocytes that exert proinflammatory effects, the smaller mounts of 5-LO metabolites synthesized by cancer cells may mainly serve as growth regulatory molecules. The AGS cells from human gastric cancer were also shown to produce prominent amounts of LTD_4, LTC_4 and LTB_4 in response to calcium ionophore (Shimakura and Boland, 1992). Both constitutive production of 5-HETE by prostate cancer cell lines of LNCaP and PC3 in serum free medium with no external stimulus (Ghosh and Myers, 1998) and growth factor (insulin-like growth factor 1 and gastrin-releasing peptide) stimulated 5-HETE synthesis by different SCLC cells (Avis et al., 1996a) have been observed as measured by RIA and reverse phase HPLC respectively. Examination of 5-LO expression by RT-PCR in different human lung epithelial cancers (Avis et al., 1996; Moody et al., 1998) and pancreatic cancer cells (Ding et al., 1999) confirmed the presence of 5-LO mRNA in these cancer cells. A recent survey of 20 different epithelial cancer cell lines derived from human colon, lung, prostate, and breast tissues found that mRNAs coding for 5-LO and FLAP were universally expressed in all tested cancer cells (Hong et al., 1999). Expression of 5-LO in primary clinical specimens of lung cancer has been shown by in situ RT-PCR (Avis et al., 1996). A more recent study demonstrated that both 5-LO expression (as measured by mRNA and protein levels) and its enzymatic product 5-HETE (as measured by RIA) were significantly elevated in human prostate adenocarcinoma (Gupta et al., 2001), suggesting a role for 5-LO in prostate carcinogenesis.

There is increasing evidence that inhibition of AA metabolism by 5-LO inhibitors leads to growth inhibition and further activates apoptosis in various cancer cells (for review, see Steele et al., 2000). Avis et al. (1996) showed that inhibition of 5-LO metabolism by selective inhibitors resulted in significant growth reduction in different lung tumor cell lines; further in vivo evaluation demonstrated enhanced levels of apoptosis in mouse autologous tumors treated with NDGA, a general LO inhibitor. Ghosh and Myers (1998) reported that inhibition of LT synthesis by MK-886 completely blocked 5-HETE formation and induced apoptosis in both hormone-responsive (LNCaP) and non-responsive (PC3) prostate cancer cell lines. This study also indicated that 5-HETE may act as a cell survival factor as exogenous 5-HETE protected both cell lines from apoptosis induced by MK-886. Anderson et al. (1998) have also shown that treatment of PC-3 prostate cells with the 5-LO inhibitor SC41661A or the FLAP inhibitor MK-886 reduced cell proliferation and activated non-necrotic, programmed cell death. Recently, Avis et al. (2001) have shown that inhibitors of the 5-LO pathway induced apoptosis in several human breast cancer cell lines in vitro. In a pilot in vivo study, they reported an increased number of apoptotic cells in xenografts from nude mice injected with MCF-7 tumor cells after treatment with 5-LO inhibitor (Avis et al., 2001). Altogether, these studies indicate that activation of the 5-LO pathway may occur in some epithelial cancers and that 5-LO products may play important roles in tumor promotion and progression.

Concluding remarks

Concepts regarding the role of ECs in physiology and disease have moved from considering the epithelium to be simply a passive barrier, with ECs emerging as active participants in tissue and organ function. Numerous studies, including those cited in this review, have demonstrated that epithelial cells can be induced to express the 5-LO enzyme and generate LTs. However, the specific factors that induce 5-LO express, the intracellular signaling pathways and transcriptional activators involved, and the specific types of epithelial cells that can be induced remain to be elucidated. In addition, the role of epithelial cell-derived LTs in pathogenesis of different diseases and the potential benefits of therapeutic intervention are unclear. These topics clearly deserve additional study.

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