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Commentary

Role of eicosanoids on intestinal epithelial homeostasis

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ABSTRACT

The intestinal epithelium is a highly dynamic system that is continuously renewed by a process involving cell proliferation and differentiation. Moreover, it is the main interface with the external environment, and maintenance and regulation of the epithelial structure and epithelial barrier function are key determinants of digestive health and host well being. The tight junction, a multiprotein complex composed of transmembrane proteins associated with the cytoskeletal peri-junctional ring of actin and myosin, is an essential component of this barrier that is strictly regulated in a spatio-temporal manner by a complex signaling network. Defects in the intestinal epithelial barrier function have been observed in inflammatory bowel disease, and a classic example of the connection between inflammation and cancer is the increased risk of colorectal cancer in patients with inflammatory bowel disease. In recent years, several molecules have emerged as critical players contributing to inflammation-associated colorectal cancer. For example, eicosanoids derived from arachidonic acid are proposed as mediators involved in the regulation of epithelial structure/function. Interestingly, the tissue concentration of eicosanoids increases during mucosal inflammation and colorectal cancer development. This overview focuses on the physiological and physiopathological roles of eicosanoids in cell growth/cell differentiation/apoptosis and in the paracellular permeability of the intestinal epithelium. A better understanding of these processes will foster new ideas for the development of therapies for these chronic disorders.

1. Introduction: the intestinal epithelium

The whole digestive tract is fenced by an epithelial monolayer composed of different cell types. The surface area of the small intestine is increased by luminal protrusions, termed villi, and invaginations into the mucosa, the crypts of Lieberkühn. In the mucosa of the large intestine there are no villi, only crypts. Three differentiated cell types (enterocytes, enteroendocrine, and goblet cells) populate the villi, whereas Paneth cells reside at the bottom of the crypts together with stem cells that can be considered the crypt/villus progenitors. Stem cells divide every 12–16 h, generating 200 cells per crypt every day. This cell production is compensated by cell shedding at the tip of the villi (small intestine) or at the surface of the epithelium (large intestine). This proliferative process pushes cells to the top of the villus. Thus, the top of the crypts and villi contains terminally differentiated cells, whereas Paneth cells and stem cells escape this movement and consequently remain permanently at the bottom of the crypts [1]. The proliferative state of stem cells must therefore be regulated by extrinsic cues that report the tissue status and thus adjust the rate of tissue renewal. The nature of these signals, how they are regulated, and how they activate stem cells are mostly unknown. However, given their importance for understanding epithelium homeostasis, epithelium repair processes and colorectal cancer, it would be interesting to shed light on these issues.

The intestinal epithelium is thus a highly dynamic system that is continuously renewed by a process involving cell proliferation and differentiation. Moreover, it is the main interface with the external environment, and maintenance and regulation of the epithelial structure and epithelial barrier function are key determinants of digestive health and host well being. For the maintenance of gut homeostasis it is imperative that the permeability characteristics of the epithelium are tightly regulated so that the seemingly paradoxical functions of allowing nutrient uptake, while excluding potentially harmful antigens from entering the mucosa, can coexist. In situations in which enhanced or prolonged leakiness of the epithelial barrier occurs, the result can be the passage of excessive amounts of pathogenic bacteria

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; EETs, epoxyeicosatrienoic acids; EGF, epidermal growth factor; HETEs, hydroxyeicosatetraenoic acids; HODE, hydroxyoctadecadienoic acids; IBD, inflammatory bowel disease; LOX, lipoxygenase; LTs, leukotrienes; PSC, prostaglandin-expressing stromal cells; PGs, prostaglandins; $cPLA_2$, cytosolic phospholipase A_2; $iPLA_2$, calcium-independent phospholipase A_2; $sPLA_2$, secreted phospholipase A_2; PPAR, peroxisome proliferator-activated receptor; NSAIDs, non-steroidal anti-inflammatory drugs; TJ, tight junction; ZO, zonula occludens.

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and toxic luminal antigens including endotoxins [2] into the mucosa, eliciting an immune response that, if not properly controlled, can lead to chronic inflammation [3].

The mechanisms that regulate the intestinal paracellular permeability to luminal substances and preserve its functional integrity are not completely understood. However, crucial for this integrity are cell polarity and the multifaceted dynamic interactions between the cell adhesion complexes and tight junctions (TJ) with the actin cytoskeleton [4]. Thus, paracellular permeability is regulated primarily by the most apical epithelial intercellular junction, the TJ or zonula occludens (ZO). An intact intestinal epithelial TJ barrier is crucial for providing a barrier function. TJ is a complex multiprotein that is composed of both intracellular and membrane-spanning proteins. Four distinct types of membrane protein have been localized to TJ: occludin, claudins, junctional adhesion molecules and the coxsackie virus and adenovirus receptor, whereas the intracellular complex of TJ-associated proteins includes ZO-1, ZO-2, ZO-3, cingulin, 7H6, symplekin, and ZA-1 [5].

The present commentary will focus on the role of arachidonic acid (AA) cascade enzymes as well as mediators derived from this cascade on intestinal epithelial cell proliferation/differentiation, intestinal barrier function, and immune cells that are present in the mucosa, and consequently on intestinal epithelial homeostasis.

2. Eicosanoids and the intestine

AA is an important polyunsaturated fatty acid of cell membrane phospholipids and also a cellular mediator that acts by itself or following its transformation to eicosanoids, its oxidized biologically active products. Under physiological conditions, the amount of free intracellular available AA is quite low. However, AA release from phospholipids occurs through the activation of phospholipases, primarily phospholipase A_2 (PLA_2).

PLA_2 comprises a large superfamily of distinct enzymes that exhibit different substrate specificities, cofactor requirements, and subcellular localizations, and includes secreted PLA_2s (sPLA_2s), cytosolic PLA_2s (cPLA_2s) and calcium-independent PLA_2s (iPLA_2s). Upon release from biomembranes, AA can be metabolized via three enzymatic pathways: the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P-450 pathways. The COX pathway leads to the formation of thromboxanes and prostaglandins (PGs). There are two COX isozymes: COX-1, which is expressed constitutively and COX-2, an inducible enzyme that is expressed in the majority of mammalian cells. Both isozymes catalyze the synthesis of PGH_2 from AA which is metabolized by specific prostaglandin synthases to release PGs. LOXs insert a hydroxyperoxyl group into the AA, which is subsequently reduced to produce hydroxyeicosatetraenoic acids (HETEs). Thus, 5-LOX leads to the synthesis of 5-HETE, whereas 12- and 15-LOX can form 12- and 15-HETE. 5-HETE can be metabolized to synthesize leukotrienes (LTs) such as LTB_4 and the cysteinyl LTs (LTC_4, LTD_4 and LTE_4). Finally, the cytochrome P-450 monooxygenases also metabolize AA by allylic oxidation, ω/ω-1 hydroxylation or epoxydation to synthesize a wide range of eicosanoids such as HETEs (20-HETE) and epoxyeicosatrienoic acids (EETs) [6,7] (Fig. 1).

Eicosanoids have pleitropic effects on cell physiology and signal transduction and exert their actions through binding to specific members of the G-protein-coupled seven transmembrane domain receptor family. The main prostanoid receptors are the DP (DP_1–DP_2) receptors to PGD_2, EP (EP_1–EP_4) receptors to PGE_2, FP receptor to $PGF_{2\alpha}$, IP receptor to PGI_2 and TP receptor to thromboxanes. Two LTB_4 receptors have been characterized: BLT_1 is a high-affinity receptor whereas BLT_2 is a low-affinity receptor; cysteinyl LTs activate at least two receptors referred to as the $CysLT_1$ and $CysLT_2$ receptors (Fig. 1). Although HETEs and EETs were first described several decades ago, relatively little is known about their mechanism of action. The fact that HETE and EET receptors have not been identified may have contributed to this. Finally, we must consider that peroxisome proliferator-activated receptors that are expressed in the intestinal mucosa and involved in the regulation of cell growth/differentiation and intestinal inflammation can be activated by eicosanoids [8,9].

Regarding the characterization of eicosanoid production within the intestinal tract, considerable variability exists among different species as well as different anatomic locations. Thus, important

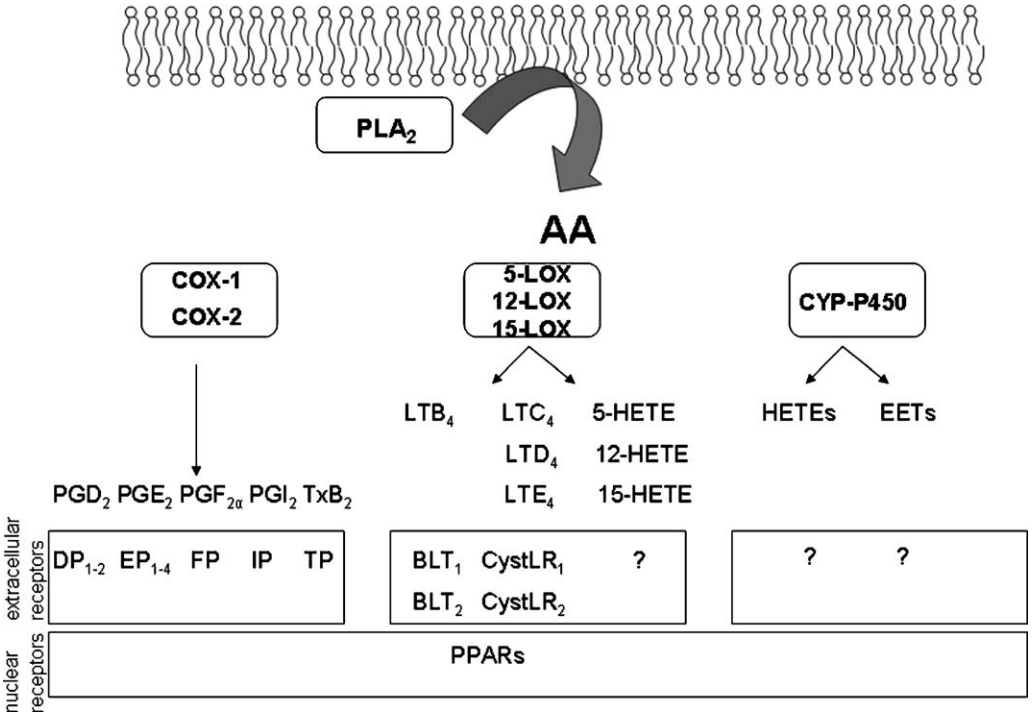

Fig. 1. Scheme of the main enzymes, metabolites and receptors of the arachidonic acid cascade.

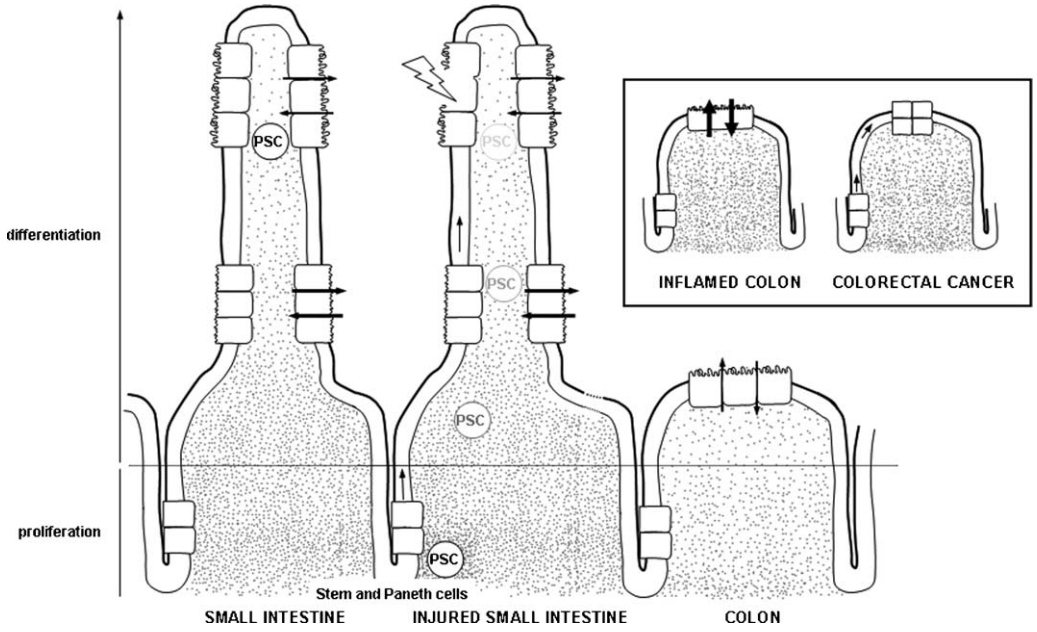

Fig. 2. Structure of the small and large intestine considering epithelial cell proliferation and differentiation along the crypt–villus axis. The asymmetric processes of eicosanoid formation and degradation might lead to an eicosanoid gradient along the crypt–villus (represented by dotted appearance). The elevated eicosanoid concentration in the neighborhood of crypt bottom is involved in the regulation of stem cell proliferation and consequently on cell migration toward the tip villus where the most differentiated epithelial cells were localized. Moreover, in this villus zone the epithelium shows the highest barrier function. The injury of the epithelium induces PSC migration to the crypt bottom, the enhancement of eicosanoid synthesis and consequently the proliferation of stem cells to renew the epithelium. Other conditions in which eicosanoid gradient is modified are IBD characterized by the disruption of epithelial barrier function. Furthermore, the chronicity of these events increases the risk of colorectal cancer development.

regional variations in eicosanoid profiles exist throughout the gut [10]. The cells most likely to be responsible for eicosanoid production include immune cells in the lamina propria and subepithelial mesenchymal cells [10]; however, intestinal epithelial cells have also been shown to be capable of eicosanoid production [10]. In cultured intestinal epithelial cells, prostanoid production has been shown to increase after treatment with growth factors and phorbol esters [11]. In contrast to their production, PG degradation seems to occur in the epithelial layer rather than in the subepithelium [10]. This could contribute to establish a PGs gradient along the cryp/villus axis, although this has yet to be demonstrated (Fig. 2).

Shoji et al. [12] reported that four PGE_2 receptor (EP_1–EP_4) mRNAs were expressed in human colon tissues. Moreover, they observed EP_1, EP_2 and EP_4 mRNA expression in intestinal epithelial cells. Interestingly, a different regional distribution of PG receptors was reported in intestinal mucosa.

Transformed and non-transformed intestinal epithelial cell lines [13–15] as well as the human intestine [16,17] also produce LOX metabolites such as LTs and HETEs, whereas there is no information about HETE/EET production by cytochrome P-450 in intestinal epithelial cells or the intestine. With respect to the presence of LT receptors in the intestine, the $CysLT_1$ receptor is expressed in intestinal epithelial cells and tumor-derived intestinal cells [15] whereas the $CysLT_2$ receptor is expressed in differentiated intestinal epithelial cells but not in tumor-derived intestinal cell lines [18]. The BLT_1 receptor is also expressed in human colon cancer cell lines and colon cancer tissue but not in normal colon tissue [19].

3. Role of eicosanoids in the control of intestinal epithelial cell growth

Our previous findings demonstrating that AA release by $iPLA_2$ participates in the signaling pathways involved in the control of intestinal epithelial proliferation [20] showed that $iPLA_2$ and COX-2 are involved in PGE_2 release in proliferative epithelial cells, whereas differentiation appears to depress this pathway. These events could also be necessary for the completion of epithelial cell differentiation, or at least for the development of the epithelial barrier function that is characteristic of differentiated epithelium [21]. Defining the molecular mechanisms of action of PGs on cell growth is an area of intense research. Epidermal growth factor (EGF) appears to be a key constituent in the maintenance, growth, repair and barrier integrity of the gastrointestinal mucosa. Recent studies have demonstrated the transactivation of the EGF receptor by PGE_2 in intestinal epithelial cells [22]. Moreover, the Wnt signaling cascade and the activation of PPARδ are involved in the effects of PGs on the proliferation of non-transformed and transformed intestinal epithelial cells. Furthermore, PGE_2 could be involved in the control of the intestinal epithelial cell cycle machinery through the up-regulation of cyclin B_1 and down-regulation of p21 expression independently of p53 [23]. Thus, this proliferative effect of PGE_2 contributes to the development of the epithelium and could be involved in the intestinal epithelial response to injury (Fig. 2). These events could be related to the observation that short-term administration of PGE_2 causes significant stimulation of DNA synthesis and that prolonged PGE_2 treatment markedly increases the weight and DNA content of the intestinal mucosa [24].

Interactions between intestinal epithelial cells and stromal cells, which include fibroblasts, myofibroblasts, endothelial cells and other cell types, may dramatically influence the growth and transformation of the intestinal epithelium [25]. PGs and other eicosanoids are synthesized by these cells [26,27]. Eicosanoids derived from both stromal and epithelial cells may stimulate stromal cells to release growth factors, which, in turn, provide a pro-proliferative and pro-neoplastic environment for the intestinal epithelium. Shao et al. [28] found that exogenous PGE_2 induced the expression and secretion of several pro-proliferative and pro-angiogenic growth factors such as amphiregulin, vascular endothelial growth factors, hepatocyte growth factor and neuregulins

by intestinal subepithelial myofibroblasts, which may mediate intestinal epithelial growth and transformation. Finally, we must consider that activated macrophages also produce eicosanoids [29] that are present near the epithelial progenitor niche [30]. All these cells, in addition to eicosanoids and growth factors produced are strategically located leading an instructive communication with the epithelial stem cells and their descendants.

In agreement with the abovementioned papers, Stenson [31] addressed these effects of PGs, particularly PGE_2, in the intestinal epithelial response to injury in a radiation injury model and in colitis models. Brown et al. [32] reported that epithelial tissue contains cells that express COX-2 at high levels, named prostaglandin-expressing stromal cells (PSCs) (Fig. 2). They observed that the majority of PSCs were located in the lamina propria lining the upper and middle third of the rectal crypt. However, after injury, the number of PSCs at the crypt bottom, adjacent to the intestinal stem cells compartment, increased, and consequently the PGE_2 levels. These authors proposed that local production of PGE_2 by PSCs may be involved in regulating epithelial proliferation after injury. However, other authors proposed that the PGE_2 involved in epithelial tissue repair is due to COX-1 rather than COX-2 [33]. In this way, radiation injury results in increased COX-1 levels in crypt stem cells and their progeny, and that PGE_2 produced through COX-1 promotes crypt stem cell survival and proliferation [34]. Although it is increasingly accepted that the COX pathway is involved in physiological and pathophysiological cell growth, the role of COX isoforms is still a matter of debate.

The human colon is also able to produce lipoxygenase metabolites [16] and these AA-derived metabolites have also been implicated in the control of intestinal epithelial cell growth. Thus, endogenous production of LTD_4 mediates autocrine survival and proliferation via the nuclear- and membrane-located $CysLT_1$ receptor, triggering a proliferative ERK1/2 signal in non-transformed and transformed intestinal epithelial cells [15,35].

There is little information about HETE/EET production by intestinal epithelial cells or the effect of these eicosanoids on intestinal epithelial growth, although many recent papers have focused on the emerging effects of HETEs on cell signaling and physiological/pathological cell growth [36]. Given that 5-LOX [14], 12-LOX [13] and 15-LOX [37] are expressed by intestinal epithelial cells and that there is differentiated expression of these enzymes during epithelial cell differentiation, these eicosanoids may also have an important role in the physiology of the epithelium. Thus, Kamitani et al. [13] suggested that 12-LOX, and/or the lipid products synthesized by this enzyme are involved in intestinal epithelial cell differentiation and apoptosis. Recently, Collins et al. [17] reported that 12-LOX was increased by up to >100-fold throughout the entire length of the intestine of iron-deficient animals, inducing a strong increase in 12-HETE and 13-hydroxyoctadecadienoic acid (13-HODE) intestinal levels that was correlated with an increase in the number of mitotic cells. Interestingly, these changes in the 12-LOX pathway induce a series of structural changes in the intestine such as increased villus height, width and crypt depth. This elegant experimental model leads to related changes in the expression of AA cascade enzymes and subsequent eicosanoid production, with epithelial cell growth and significant morphological changes in the intestinal mucosa.

Colon cancer is the third most common cancer and the second leading cause of cancer-related deaths. The incidence of colon cancer doubles with each decade of life after the age of 50. Although a great deal of effort has been made toward developing screening strategies, aggressive surgical therapy, and other therapies, there has been little improvement in the outcome for patients with advanced disease.

Today, the causal relationship between inflammation, innate immunity and cancer is more widely accepted; nevertheless, many of the molecular and cellular mechanisms mediating this relationship remain unresolved. However, there is now evidence that inflammatory mediators have a powerful effect on tumor development. Early in the neoplastic process, eicosanoids could be powerful tumor promoters, producing an attractive environment for tumor growth and promoting angiogenesis. Thus, studies in the early 1980s indicated that non-steroidal anti-inflammatory drugs (NSAIDs) were chemopreventive in animal models of colon cancer [38]. In 1991, Thun et al. [39] reported that aspirin use reduce the relative risk of colon cancer and colon cancer mortality among 600,000 individuals, whereas acetaminophen, which does not affect COX activity, did not provide protective effect. Even more relevant for the clinician were subsequent studies that demonstrated that NSAID therapy can cause the regression of adenoma in patients with familial adenomatous polyposis [40]. Furthermore, COX-2 is elevated in colorectal cancers [41], with 50% of adenomas and 80–90% of adenocarcinomas exhibiting increased COX-2 expression, and elevated PGE_2 and 6-keto $PGF_{1\alpha}$ levels [42,43]. However, conflicting data exist regarding the localization of COX-2 in the epithelium or stromal components of colorectal tumors. Using an elegant experimental model, Oshima et al. [44] reported that COX-2 is located in the stromal component and may promote tumor growth by producing bioactive PGs that affect tumor growth in a paracrine fashion. This is consistent with the model postulated by Kinzler and Vogelstein [45], which states that COX-2 affects tumor growth by acting as a landscaping tumor promoter in the stromal component of the adenoma.

Despite the very strong evidence of a causative role of prostaglandins in general, and PGE_2 in particular, in intestinal cancer, the underlying molecular mechanisms have remained obscure until recently. However, studies by several research groups are now clarifying this point. Dietary manipulation of AA content in APC^{min} animals suggests that AA is involved in tumorigenesis [46]. Using APC^{min}-induced tumorigenesis and $cPLA_2^{-/-}$ mice, Hong et al. [47] demonstrated the pivotal role of enzymes involved in AA release in small intestine polyp formation whereas they observed a trend in the opposite direction in the small intestine, probably, by limiting proapoptotic signals [48]. Furthermore, a number of animal studies involving APC-mutant mice are converging on a central pathway involving the COX pathway. In addition, a recent study by Castellone et al. [49] demonstrated that PGE_2 increases the activation of the Tcf/Lef transcription factor and activated components of the canonical Wnt signaling cascade. Thus, PGE_2 EP receptor interaction results in the displacement of APC and the loss of phosphorylation of β-catenin. In the absence of phosphorylation, β-catenin is not degraded, but instead translocates to the nucleus and activates Tcf/Lef. A possible alternative mechanism by which PGs may induce cell growth is the EGF pathway. EGF receptor (EGFR) signaling is more relevant to early APC-dependent tumorigenesis, and PGE_2 can transactivate EGFR in intestinal epithelial cells and consequently the EGFR–PI3K–Akt cell survival/proliferative pathway in vivo, as reported recently by Moran et al. [43]. These findings support the original hypothesis that the anti-tumorigenesis effects of NSAIDs work through decreased PG production. However, these explanations have lacked molecular detail, in large part because of a poor understanding of which PG receptors are involved. In the last decade, important findings were obtained with respect to this point. Targeted deletion of the EP_1 receptor reduced colonic lesions by 60% in a colon cancer experimental model and APC^{min}-induced polyp formation was also reduced by 57% following treatment with a specific EP_1 antagonist [50]. Considering all together, we now have a complete picture of the main elements involved in the effects of PGs such as PGE_2 on epithelial cell growth in physiological and pathophysiological conditions: AA is released by PLA_2, then metabolized by COX-1/COX-2 to produce bioactive

eicosanoids such as PGE_2, which interact with specific receptors (EP_1 and EP_4) and activate the cell signaling involved in the control of the cell cycle.

Research on PPARs has revealed that they play a fundamental role in cellular proliferation/differentiation processes [51]. In human colon cancer cell lines and tumor cells, PGE_2 transactivates PPARδ through the PI3 kinase/Akt pathway. PGE_2 treatment of APC^{min} mice increased the intestinal adenoma burden; however, PGE_2 had no effect on the adenoma burden in APC^{min} mice deficient in PPARδ. Other findings indicate that the proliferative effects of PGE_2 are also mediated through PPARδ [52].

Cianchi et al. [53] reported that the two major metabolic pathways of the AA cascade, COX and 5-LOX, are simultaneously up-regulated in human colorectal cancer. Interestingly, inhibition of either COX or 5-LOX alone resulted in activation of the other pathway, and consequently, combined treatment with COX and 5-LOX modulators produced greater inhibition of tumor cell proliferation. High expression of the BLT_1 receptor [19] and the $CystLT_1$ receptor was detected in human colon cancer tissues, whereas $CysLT_2$ receptor expression was reduced in colon cancer and was associated with poor prognosis, due to its capacity to induce differentiation and growth inhibition [18]. Thus, the $CysLT_2$ receptor could have more anti-tumorigenic activity than the $CysLT_1$ receptor in intestinal epithelial cells, and the balance between the two receptors is important for the outcome of tumor progression. These findings indicate that AA cascade enzyme expression and eicosanoid production are important, but that the balance between eicosanoid receptors is also important for tumor progression and disease outcome.

Dietary fiber (non-starch polysaccharides) bypasses digestion in the stomach and small intestine, as do oligosaccharides and some resistant starches. In the large intestine, symbiotic bacteria ferment these carbohydrates releasing butyrate, a short-chain fatty acid, as a by-product. At physiological concentrations, butyrate has been shown to induce growth inhibition, differentiation and apoptosis in colorectal tumor cells *in vitro* [54]. These observations may explain, in part, the correlation between a high fiber diet and a low incidence of colorectal cancer. Crew et al. [55] observed that COX-2 inhibitors sensitize the cell to growth inhibition induced by butyrate in colorectal carcinoma cells expressing COX-2 protein. These findings may mean that high fiber diet content would enable lower doses of COX-2 inhibitors to be used in chemoprevention.

Despite progress in knowledge of the effect of PGs and LTs on intestinal epithelial cell growth, we are a long way from fully understanding the role of the AA cascade in intestinal epithelial cell growth/differentiation/apoptosis, which will hopefully lead to a greater understanding of colorectal cancer in terms of the molecular pathways involved, and thus provide potential applications for the clinical setting.

4. Role of eicosanoids in the control of intestinal barrier function

Inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, is characterized by chronic, relapsing inflammation of the gastrointestinal tract, affecting at least 1 in 1000 people in Western countries [56]. Although the molecular mechanisms underlying the pathogenesis of IBD have not been identified, at least two events in which eicosanoids are involved have been described. The first is defects in the mucosal barrier, which may allow the continuous stimulation of the mucosal immune system due to impairment of the epithelial barrier function. Increased paracellular permeability of the epithelium has been well documented both in acutely and chronically damaged areas of the intestine [57]. Moreover, it is well known that intestinal permeability is regulated directly through alteration of TJ proteins, or indirectly through effects on the cytoskeleton. However, although altered intestinal permeability has been reported in patients with IBD, and the grade of barrier defect related to the onset of symptoms, the mechanism responsible and the mediators have not been completely identified. In fact, TNF-α and IFN-γ have been identified as being responsible, at least in part, for the increase in paracellular permeability [58,59], and the mechanisms underlying the regulation of TJ permeability by TNF-α and IFN-γ either directly through the alteration of TJ proteins, or indirectly through effects on the cytoskeleton have been well documented [60].

The second event that occurs in IBD is an increase in the inflammatory response with an associated increase in cytokine, eicosanoid and free radical production. Eicosanoids play a significant role in IBD as mediator of inflammation. Thus, the levels of prostanoids such as PGE_2, $PGF_{2\alpha}$ and PGD_2, as well as 12-HETE, 15-HETE and LTB_4 are higher in inflamed compared to normal mucosa [61,62]. Moreover, the increase in PGE_2 levels is correlated with disease activity [63]. Furthermore, during remission, PGE_2 and LTB_4 levels return to values comparable with normal colorectal mucosa [61]. The enhancement of PGs is a consequence of the overexpression of COX and prostaglandin synthases in inflamed intestinal mucosa [64]. This supports the role of eicosanoids in IBD. Further evidence that supports the importance of eicosanoids in IBD is that sulfasalazine, mesalamine and glucocorticoids, effective treatments, reduce eicosanoid synthesis [65,66].

Enteroinvasive bacteria that are responsible for diarrheal diseases also activate the expression of genes such as COX-2 in the intestinal epithelium, provoking PGE_2 production and consequently impaired barrier function [67]. On the other hand, we must consider that intestinal epithelial cells have the capacity to recognize and respond to commensal microbiota. Elements of the microbiota inhibit the NF-kB pathway by hijacking the PPAR-γ pathway, and consequently modulate the COX pathway [68]. Activation of PPAR-γ by butyrate has also been shown to reduce colonic permeability, probably through the promotion of intestinal epithelial cell differentiation and the consequent reinforcement of the TJs [69]. The microflora seems to be regulated to ensure protection from injury and induction of repair. An attractive hypothesis is that under normal physiological conditions the coordinated induction of various protective mechanisms helps to control pivotal elements involved in the AA cascade, thus maintaining normal epithelial cell homeostasis and the interplay with commensal bacteria.

In summary it seems that AA release and PGE_2 synthesis by the COX pathway could be involved in the regulation of intestinal paracellular permeability (Fig. 2). Thus, we observed that intestinal epithelial differentiation induces a decrease in both $iPLA_2$ activity and COX-2 expression and, consequently, a decrease in AA release and PGE_2 synthesis in parallel with a reduction in paracellular permeability and consequently the development of a barrier function that can be disrupted by the exogenous addition of PGE_2 [21]. Recently, we observed that this modulation of the epithelial barrier function is mediated by the interaction with PGE_2 receptors EP_1 and EP_4. Events that activate PLC–IP_3–Ca^{2+} and cAMP–PKA pathways that lead to an intracellular calcium concentration and the redistribution of TJ proteins such as occludin and the perijunctional actin ring that may be mediated these effects [70].

Given that NSAIDs, which are COX pathway inhibitors, might exacerbate the extent of IBD as well as epithelial barrier disruption [71], we must consider whether other eicosanoids have any effect on the elements that regulate epithelial barrier function. There is no information about the evolution of LOX expression in IBD, an important step in the AA cascade in this pathophysiological

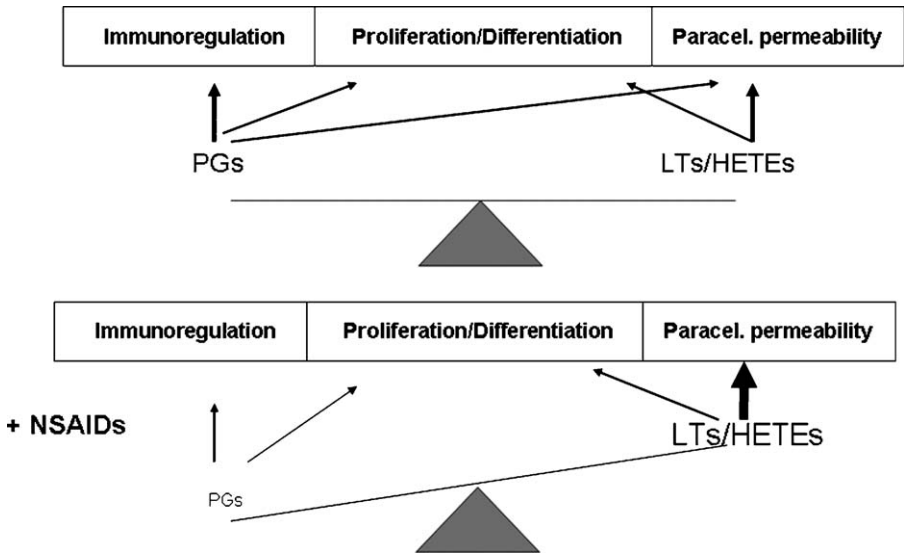

Fig. 3. Effects of eicosanoid balance on immunoregulation, proliferation/differentiation and paracellular permeability. In physiological conditions (upper figure), the balance of eicosanoid levels participates in the control of the abovementioned events, resulting in the maintenance of epithelial homeostasis. The treatment with NSAIDs, may disrupt this balance, reducing PGs levels and thus increasing the production of LTs/HETEs. Consequently, these effects may induce the up-regulation of the immune response and the disruption of epithelial barrier function, that may be involved in the development and exacerbation of IBD.

condition. Moreover, to the date, there is little information on the effects of eicosanoids produced by this pathway on TJ and consequently on barrier function. However, Rodríguez-Lagunas et al. [72] have recently reported that LTD_4 and 5-, 12- and 15-HETE produced by the LOX pathway alter the epithelial barrier function to a greater extent than prostanoids. Thus, the administration of NSAIDs could allow LT and HETE synthesis by the LOX pathway to proceed unchecked while blocking PG formation, and hypothetically could shift the net effect of prevailing eicosanoids toward proinflammation and the impairment of barrier function (Fig. 3). These findings might explain the negative effects of NSAID treatments on IBD evolution.

5. Eicosanoids as immunoregulatory factors in intestinal mucosa

Another important aspect of the actions of eicosanoids in the intestine is their immunoregulatory effect. The close proximity of a wide array of antigens and lymphocytes in the gastrointestinal tract emphasizes the need to maintain immunological homeostasis and avoid an inappropriate immune response to nonpathogenic antigens, with subsequent epithelial alterations. Eicosanoids may also be involved in this process. PGE_2 is known to have immunomodulatory effects: it downregulates the major histocompatibility complex class II and cytokine receptor expression as well as cytokine production [73,74], events that might be involved in controlling an inflammatory immune response to dietary antigens. Consequently, PGs produced by epithelial cells, fibroblasts or lamina propria mononuclear cells could downregulate the intestinal immune system (Fig. 3). The importance of COX-dependent AA metabolites as immunoregulatory factors in the intestinal mucosa is supported by the observation that NSAIDs exacerbate clinical activity in human inflammatory bowel disease [71]. In addition, some COX-$2^{-/-}$ mice show peritonitis and intestinal abscess formation, possibly secondary to inappropriate immune activation in the absence of high levels of PGE_2 [75]. Newberry et al. [76] proposed that 1 nM PGE_2 could have this immunomodulatory effect, such a concentration being reached in the intestinal mucosa. It will be necessary to study the effects of other eicosanoids such as HETEs and EETs on intestinal immunoregulation to clarify their role in this important aspect of intestinal epithelial homeostasis.

6. Conclusions and perspectives

An attractive hypothesis is that under normal physiological conditions the coordinated induction of various protective mechanisms helps to control pivotal elements involved in the AA cascade, but the increase in eicosanoid mucosal levels may contribute to the development of inflammatory processes as well as colorectal cancer. Answers to the question considered during this commentary may help us to determine the role of eicosanoids in the maintenance of intestinal homeostasis as well as to design protocols for using dietary modifications or pharmacological treatments in the prevention or treatment of IBD and colorectal cancer.

Given the different effects of eicosanoids we must consider that pharmacological manipulation of the AA cascade may have contradictory effects. For example, NSAID treatments reduce the risk of colorectal cancer whereas they exacerbate IBD. In the same way, treatment with an EP_4 receptor antagonist protected against the development of colon cancer [77], but exacerbated experimental colitis [78]. The rational design of treatments that allow the impairment of cell growth or barrier dysfunction induced by eicosanoids without up-regulation of the immune response may be a useful approach for IBD and colorectal cancer.

In conclusion, more studies are needed to clarify the pivotal questions that remain unanswered in this exciting field. In particular, additional work is required to explore mechanistic issues involving the effects of LTs and HETEs/EETs on epithelial cell growth/differentiation, barrier function, and on the intestinal immune response.

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