Lipid mediators in epithelial cell-cell interactions

S. P. Colgan

Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Thorn 704, 75 Francis Street, Boston, Massachusetts 02115 (USA), Fax +1 617 278 6957,
e-mail: colgan@zeus.bwh.harvard.edu

Abstract. Epithelial cells which line mucosal surfaces (e.g. lung, intestine) play a central role in the coordination of the inflammatory response. In both the healthy and diseased mucosa, epithelia lie anatomically positioned in close proximity to a number of other cell types, including leukocytes, fibroblasts, smooth muscle cells and vascular endothelia. This complex architecture supports a unique microenvironment for biochemical cell-cell crosstalk. Our previous studies and work by others have elucidated lipid mediator signaling networks emanating from epithelial cell-cell interactive pathways, and have defined a number of targets for development of effective therapeutics. This short review will focus on recently defined pathways of lipid mediator function in the mucosa, particularly with regard to the role of the epithelium.

Key words. Epithelium; leukocyte; inflammation; chemokine; chloride secretion.

Introduction

Epithelial cells of the mucosa are a dynamic cell population which serve critical and diverse functions. This monolayer of cells provides regulated barrier function and serves as a conduit for vectorial ion movement, the transport event responsible for mucosal hydration [1]. By secreting solutes and actively transporting fluid across the epithelium, epithelia are able to coordinate compositional changes of the luminal compartment. A number of paracrine mediators, including bioactive lipids, hormones, neurotransmitters and cytokines have been shown to directly regulate epithelial responses [2].

In intact mucosal tissues, epithelia lie anatomically positioned adjacent to a number of subepithelial cell types, including leukocytes, fibroblasts, smooth muscle cells and vascular endothelia. These subepithelial cell populations contribute significantly to epithelial function through paracrine crosstalk pathways. Locally generated mediators bind to epithelial surface receptors, and mediate both physiologic and pathophysiologic functional responses. Important in this regard, both epithelial and subepithelial cell populations express enzymes (e.g. lipoxygenases, cyclooxygenases) capable of utilizing arachidonic acid substrates to generate bioactive lipid mediators. Such lipid mediators can signal via autocrine or paracrine pathways (see recent review by Eberhart and Dubois) [3] and, depending on the tissue microenvironment, can convey a pro- or anti-inflammatory message. This review will highlight recent studies defining cellular responses mediated by lipids derived from epithelial interactions with subepithelial populations.

Epithelial-leukocyte interactions

Basic aspects

Neutrophils (polymorphonuclear leukocytes, PMN) have a demonstrated role in mucosal inflammation. At mucosal surfaces, PMN migration into the epithelium is a first line of defense against infectious agents, and defects in such PMN-epithelial interactions contribute to fulminate microbial infections, mucosal ulcerations and delayed tissue healing [4, 5]. The protective aspects of PMN in mucosal disease are objectively exemplified by the clinical observation that patients with primary defects in PMN function support ongoing mucosal infections, including neutropenic patients and patients afflicted with genetic PMN immunopathologies [e.g. leukocyte adhesion deficiency (LAD), Chediak-Higashi syndrome, myeloperoxidase deficiency, and so on] [6–8]. As a

---

* Corresponding author.

corollary, extensive functional defects in PMN have been observed in these patients with chronic mucosal inflammation.

PMN migration across epithelia is a result of an orchestrated series of events, ultimately resulting in PMN accumulation at sites of tissue injury. The recruitment signals, the cell-cell interaction steps and the regulatory pathways for these events have only recently been explored. It is now appreciated that adhesion-based interactions, involving specific cell adhesion epitopes, are the primary means by which PMNs interact with epithelial cells [4, 9]. For example, recent studies have shown that PMN β_2 integrins are required for PMNs to move across oral epithelia, kidney epithelia [10] and intestinal epithelia [11–13]. These integrins, like others, are heterodimeric glycoproteins which exist in four forms on the PMN. Each displays a unique α subunit (CD11a, b, c or d) and an identical β subunit (CD18) [14, 15]. These receptors are best demonstrated in the genetic disorder LAD, in which patients lack normal expression of the CD 18 β subunit and, as a result, show increased susceptibility to infection due to abnormal leukocyte function [14]. These patients manifest severe mucosal disease, characterized primarily by severe bacterial infections. PMNs from LAD patients fail to migrate across intestinal epithelial monolayers [11–13], indicating the dependence of this event on PMN expression of CD11/18 integrins. At several levels, studies have revealed that PMN-epithelial interactions are dependent on CD11b/18, but not CD11a or CD11c/18 [4]. At the present time, the epithelial ligand for CD11b/18 has not been identified. Studies directed at defining specific PMN-epithelial interactive events have unveiled a functionally inhibitory monoclonal antibody (mAb) which blocks PMN transmigration, but not PMN adhesion, to epithelia [16–18]. Subsequent experiments revealed that the antigen recognized by this mAb (C5/D5) represents a membrane glycoprotein of ~60 kDa and is expressed in a polarized fashion (basolateral). Isolation, purification and microsequencing identified this antigen as CD47 (also termed integrin-associated protein), a previously cloned protein with homology to the immunoglobulin supergene family [19]. Similarly, others have demonstrated that CD47 is important in PMN transendothelial migration [20], suggesting some degree of universality for CD47 in leukocyte-mediated interactions.

Regulation of epithelial-PMN interactions by lipoxins

Leukocyte-epithelial interactive pathways are significantly influenced by locally generated lipid mediators. Of particular interest are a group of lipid mediators termed the lipoxins [21]. Lipoxins are tetraene eicosanoids derived from membrane arachidonic acid through the combined action of 5-lipoxygenase (LO) and 12-LO or 15-LO [22] (see fig. 1). A number of recent in vitro and in vivo studies have revealed that lipoxins, and specifically lipoxin A$_4$ (LXA$_4$), function as an innate 'stop signals', acting to control local inflammatory processes [23–26]. At nanomolar concentrations, LXA$_4$ has been demonstrated to inhibit PMN transmigration across confluent epithelia and endothelia [24, 25]. It is likely that the action(s) of LXA$_4$ are on leukocytes and involve the activation of protein kinase C, since original studies revealed that LXA$_4$ inhibition required PMN preexposure and such responses were sensitive to the protein kinase C inhibitor staurosporine [24]. Additional mechanistic studies have revealed that LXA$_4$ inhibit PMN β_2 integrin (CD11/18) expression [27]. Importantly, compared with LXA$_4$ (hydroxyl groups at carbon positions 5S, 6R and 15S), the positional isomer LXB$_4$ (hydroxyls at carbon positions 5S, 14R and 15S) is not active in PMN transepithelial migration [24], but potently inhibits PMN transendothelial migration and potentiates monocyte adhesion to endothelia [28]. Such studies define an important structure-function relationship with these eicosanoids and highlight the significant differences between endothelial and epithelial cells and within leukocyte subpopulations.

Lipoxins are rapidly (within minutes) converted to inactive compounds by myeloid cells [29]. For this reason, stable lipoxin analogs have be synthesized and biochemically and functionally studied in detail [25] (see fig. 1). Strategies to alter in the native LXA$_4$ molecule have primarily utilized substitutions (methoxy, cyclohexyl or phenoxy groups) at the carbon 15 and/or carbon 20 positions. As a general finding, synthetic lipoxin analogs exhibit greater potency for these counter-regulatory actions than the native compound, likely due to decreased metabolism to inactive compounds [25, 28]. A particularly potent LXA$_4$ analog is 15 (R/S)-methyl-LXA$_4$. This synthetic molecule resembles that of 15-epi-LXA$_4$, a native lipoxin generated in vivo in the presence of aspirin [23], which may contribute in part to the antiinflammatory actions of aspirin. 15 (R/S)-methyl-LXA$_4$ inhibits PMN transmigration across epithelia and effectively blocks PMN adhesion to vascular endothelia at concentrations as low as 10 pM [25]. Structure-function analyses have revealed that 16-phenoxy-LXA$_4$ and 15-cyclohexyl-LXA$_4$ (see fig. 1) are also potent inhibitors of PMN transendothelial and transepithelial migration [EC$_{50}$'s (effective concentration for 50% response) 1–5 nM] [25]. The 15-deoxy-LXA$_4$ compound, lacking a position 15 hydroxyl (see fig. 1), is not metabolized by leukocytes and carries no bioactivity [25]. In vivo, both the native compound and analogs to LXA$_4$ have been demonstrated to block PMN diapedesis within the microcirculation of the hamster cheek pouch [30], depress contraction of the guinea pig ileum [31] and inhibit increases in vascular permeability elicited by acute inflammation [32].
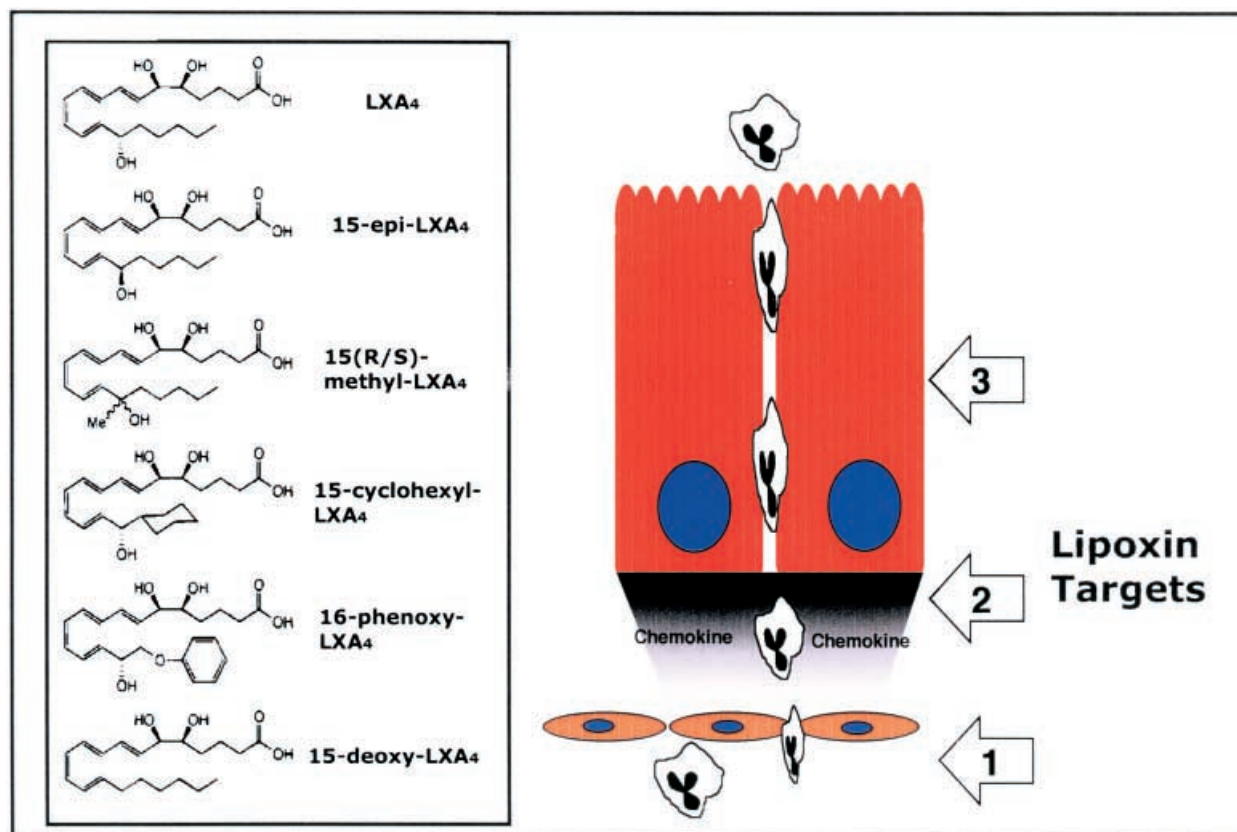
Figure 1. Model of lipoxin targets during active mucosal inflammation. The structure of native lipoxin A_4 (LXA_4) and stable analogs derived from the parent compound [25] are shown in the left panel. The 15-deoxy-LXA_4 compound (bottom structure) is not active. The right panel depicts defined targets for LXA_4 or stable analogs to LXA_4, and include (i) inhibition of PMN-endothelial adhesion and transmigration [25, 73]; (ii) chemokine release from epithelia [26, 41] and (iii) PMN transepithelial migration [24, 25].

Role of chemokines in PMN-epithelial interactions

During both acute and chronic inflammatory processes, mucosal epithelial cells orchestrate the active recruitment of leukocytes, particularly PMN. Epithelia are demonstrated sources of PMN chemotactic cytokines (chemokines), including interleukin-8 (IL-8), GRO-α, GRO-γ and ENA-78 [17, 33, 34]. Epithelia produce and release chemokines in response to multiple activation stimuli, including cytokines [35, 36], infectious agents [34, 37, 38] and cellular hypoxia [17]. Detailed studies have revealed that chemokine release occurs predominantly through the physiologically relevant basolateral surface [17, 39]. Such polarized chemokine targeting 'imprints' the epithelial matrix [39] and selectively recruits PMN to the basolateral epithelial surface [17, 39]. Indeed, selective inhibition of hypoxia-induced IL-8 using antisense oligonucleotides specifically inhibits PMN migration into, but not across, the epithelium [17]. Levels of these chemokines positively correlate with mucosal disease status [40], and for this reason much recent attention has been paid to defining strategies of 'dampening' chemokine generation at mucosal sites. Lipoxins have

been studied in this regard. Previous studies have demonstrated that quiescent epithelia lack a functional LXA_4 receptor (e.g. with regard to ion transport and barrier functional responses) [24]. However, recently it was shown that lipoxins [and the stable lipoxin analog 15 (R/S)-methyl-LXA_4] act directly on epithelia to inhibit cytokine-induced release of IL-8 [41]. Expression of a functional LXA_4 receptor on epithelia required preexposure to cytokines such as interferon-γ and IL-13 [41], of which epithelia express well-characterized receptors. Such findings reveal important counter-regulatory roles for LO-derived lipid mediators in the mucosa.

While LO-derived lipid mediators serve as downregulatory signals for epithelial chemokine release, it appears that cyclooxygenase-derived prostaglandins may enhance chemokine release. For example, PGE_2 induces colonic epithelial IL-8 release through a posttranscriptional mechanism involving elevated cyclic AMP (cAMP) (similar responses were observed with other cAMP agonists) [42]. Induction of IL-8 by PGE_2 paralleled increased messenger RNA (mRNA) stability, and this effect mapped to a cis-acting PGE_2 responsive ele-

ment in the 3′ untranslated region of the IL-8 gene [42]. We have similarly demonstrated a cAMP-dependent induction of IL-8 by hypoxia [43, 44]. Whether such responses are similarly regulated by lipid mediators are not known at the present time.

Epithelial-parenchymal cell interactions

In the healthy mucosa, subepithelial cell populations consist of parenchymal and stromal cells intercalated to provide a structural matrix to the tissue. These cell populations are also active in liberation of potent bioactive substances, including lipid mediators. To this end, some studies have suggested that prostaglandin synthesis in the intestine is derived almost exclusively from these subepithelial populations [45]. Prostaglandins are derived from free arachidonic acid through the cyclooxygenases (COX-1 and COX-2), enzymes which bear both cyclooxygenase and peroxidase activity [46]. The role of cyclooxygenase-derived lipid mediators has been widely studied in mucosal tissue, given their clinical relevance to nonsteroidal antiinflammatory therapy [3]. In particular, epithelial cells bear surface receptors for a number of prostaglandins [47]. These receptors are G-protein-coupled, seven-transmembrane-spanning proteins linked to a number of different signaling pathways [48]. The complexity of the intestine and other mucosal tissues complicates identification of individual cell types responsible for such prostaglandin synthesis. Thus, cells grown as cocultures incorporating two distinct cell types have been an effective strategy to define the generation of prostaglandins and elucidate signaling pathways. For instance, studies utilizing intestinal epithelial-fibroblast cocultures have revealed that fibroblast-derived prostanoids, especially PGE_2, promote agonist-stimulated epithelial Cl^- secretion [49], the ion transport event responsible for mucosal hydration [2]. Such enhanced responses to prosecretory agonists were effectively blocked with COX inhibitors. Further studies using cocultures of epithelia with intestinal myofibroblasts revealed that such responses were specific for Ca^{2+} agonists, paralleled COX-2 activation and were fully explained by myofibroblast generation of PGE_2 [50]. These results define a paracrine function for the intestinal fibroblastic sheath and identify this subepithelial layer as an immunophysiologic regulator of the inflammatory response.
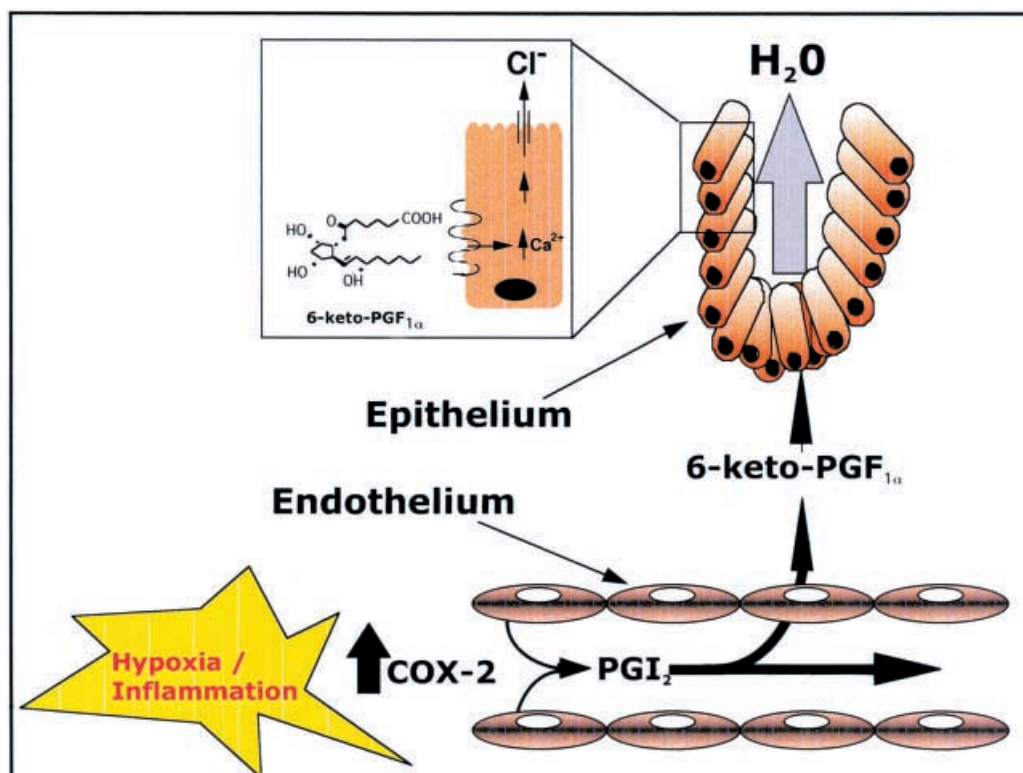

Figure 2. Model of vascular endothelial-mucosal epithelial biochemical crosstalk during inflammation [54]. Proinflammatory conditions which induce endothelial COX-2 (e.g. cytokines, endotoxin, hypoxia) activate liberation of prostacyclin (PGI_2). Prostacyclin is unstable and rapidly hydrolyzes to 6-keto-$PGF_{1\alpha}$. Epithelia bear basolaterally localized receptors for 6-keto-$PGF_{1\alpha}$, the ligation of which activates Ca^{2+}-dependent electrogenic chloride secretion (see inset), the transport event responsible for mucosal hydration.

Epithelial-vascular endothelial interactions

The vascular endothelium functions as more than a passive conduit for blood components, and synthesizes many compounds which precisely regulate blood vessel tone, vascular composition and leukocyte movement [51–53]. Endothelial cells themselves respond to a variety of pro-inflammatory stimuli, including cytokines, endotoxin and hypoxia and in turn release inflammatory mediators such as cytokines and lipid mediators (see fig. 2 model) [52]. The vital role of the endothelium in coordinating inflammation and the proximity of the vasculature to the epithelium provides a potential paracrine crosstalk pathway between these two cell types. In coculture experiments, it was demonstrated that activated endothelia (exposed to endotoxin, cytokines or hypoxia) liberate a small (< 500 Da), stable factor which activates epithelial electrogenic Cl⁻ secretion and concomitant fluid transport (see fig. 2) [54]. Further experiments identified this secretagogue as 6-keto-PGF$_{1\alpha}$, a stable hydrolysis product of prostacyclin (PGI$_2$). Results obtained with synthetic prostanoids indicate that 6-keto-PGF$_{1\alpha}$, but not 2,3-dinor-6-keto-PGF$_{1\alpha}$, activates a basolaterally polarized, Ca^{2+}-coupled epithelial receptor (see fig. 2). While not well characterized, several lines of evidence indicate that 6-keto-PGF$_{1\alpha}$ activates a PGI$_2$ receptor (likely the IP receptor), a recently cloned seven transmembrane-spanning protein [55, 56]. First, epithelial preexposure to the prostacyclin analog carbaprostacyclin resulted in receptor desensitization to subsequent activation by 6-keto-PGF$_{1\alpha}$. Second, while iloprost has also been demonstrated to activate the PGE$_2$ receptor (specifically the EP$_1$ receptor) [57], PGE$_2$ did not desensitize subsequent activation by 6-keto-PGF$_{1\alpha}$ (see 'Results'). Of note, it is possible that epithelial PGI$_2$ (IP) and PGE (EP$_1$) receptors share a common signaling pathway since, for example, oocytes expressing EP$_1$ receptors display a Ca^{2+}-mediated Cl⁻ current, similar to our findings here [58, 59]. Third, while most evidence indicates that the PGI$_2$ receptor signals through cAMP [57], the cloned mouse PGI$_2$ receptor [58] as well as the rabbit cortical collecting duct [60] also signal through intracellular phosphatidyl inositol hydrolysis and elevations in intracellular Ca^{2+}. In addition, prostaglandin responses of porcine intestinal epithelia implicated a role for increased intracellular Ca^{2+} [61]. Thus, these findings of an intestinal epithelial PGI$_2$ receptor signaling through elevation in intracellular Ca^{2+} are not unprecedented in the literature. Taken together, these results reveal a novel action for the prostacyclin hydrolysis product 6-keto-PGF$_{1\alpha}$ and provide a potential endothelial-epithelial crosstalk pathway in intestinal tissue.

Pathophysiology of epithelial cell-cell interactions: Role of COX-2 activation

Under pathological conditions, the influx of inflammatory cells and the liberation of soluble factors can transform mucosal tissue into a phenotypically distinct entity. Similarly, such a phenotype switch can occur at the cellular level. For several reasons, such phenotypic transformations are predominated by activation of COX-2, and pathophysiology parallels COX-2 activation. First, epithelial cells themselves express COX-2 and liberate lipids which mediate activation of autocrine and paracrine pathaways. Such COX-2 expression in epithelia is driven by preexposure to proinflammatory agonists such as transforming growth factor-α in the rat [62] and tumor necrosis factor-α/IL-1 in human epithelia [41]. Specific overexpression of COX-2 in epithelia results in alterations in apoptosis, adhesion and adaptive responses [62–65]. Second, alterations in COX-2 expression manifest at the level of the epithelium. COX-2 null animals [66] have revealed a phenotype of severe renal epithelial defects [66], and a number of epithelial-related abnormalities in female reproduction [67]. Third, as alluded to above (see fig. 2), the COX-2 gene can be regulated by a number of pathways relevant to mucosal disease. The COX-2 promoter is well characterized and bears consensus motifs for nuclear factor kappa B (NF-κB), cAMP response element binding protein (CREB), nuclear factor of IL-6 (NF-IL-6), PEA-3, substance P and TPA [68]. Moreover, generalized cellular hypoxia is a potent activator of COX-2 [54, 69], and the cytokines IL-4 and IL-13, of which epithelia express functional receptors [70, 71], have been shown to downregulate COX-2 [72]. Thus, given the complex nature of tissues lined by epithelia and the multiple inflammatory targets within the subepithelium, activation of existing pathways rapidly and efficiently regulates COX-2 expression in one or more compartments. Ongoing studies to more clearly delineate the role of lipid mediators in mucosal healthy and disease will define future directions toward targeted therapeutics.

1 Powell D. W. (1987) Intestinal water and electrolyte transport. In: Physiology of the Gastrointestinal Tract, pp. 1267–1291, Johnson L. R. (ed), Raven Press, New York

2 Powell D. W. (1991) Immunophysiology of intestinal electrolyte transport. In: Handbook of Physiology, pp. 591–641, Field M. and Frizzell (eds), American Physiological Society

3 Eberhart C. E. and Dubois R. N. (1995) Eicosanoids and the gastrointestinal tract. Gastroenterology **109:** 285–301

4 Parkos C. A., Colgan S. P. and Madara J. L. (1994) Interactions of neutrophils with epithelial cells: lessons from the intestine. J. Am. Soc. Nephrol. **5:** 1–15

5 Parkos C. A. (1997) Molecular events in neutrophil transepithelial migration. BioEssays **19:** 865–873

6 Arnaout M. A., Pitt J., Cohen H. J., Melamed J., Rosen F. S. and Colten H. R. (1982) Defiency of a granulocyte-membrane gly-

coprotein (gp150) in a boy with recurrent bacterial infections. N. Engl. J. Med. **306:** 693–699

7 Blume R. S., Bennett J. M. and Yankee R. A. (1968) Defective granulocyte regulation in Chediak-Higashi syndrome. N. Engl. J. Med. **279:** 1009–1013

8 Salmon S. E., Cline M. J., Schultz J. and Lehrer R. I. (1970) Myeloperoxidase deficiency. Immunologic study of a genetic leukocyte defect. N. Engl. J. Med. **282:** 250–253

9 Jaye D. L. and Parkos C. A. (2000) Neutrophil migration across intestinal epithelium. Ann. N. Y. Acad. Sci. **915:** 151–161

10 Casale T. B. and Abbas M. K. (1990) Comparison of leukotriene B4-induced neutrophil migration through different cellular barriers. Am. J. Physiol. **258:** C639–647

11 Colgan S. P., Parkos C. A., Delp C., Arnaout M. A. and Madara J. L. (1993) Neutrophil migration across cultured intestinal epithelial monolayers is modulated by epithelial exposure to interferon-gamma in a highly polarized fashion. J. Cell. Biol. **120:** 785–795

12 Parkos C. A., Delp C., Arnaout M. A. and Madara J. L. (1991) Neutrophil migration across a cultured intestinal epithelium: dependence on a CD11b/CD18-mediated event and enhanced efficiency in the physiologic direction. J. Clin. Invest. **88:** 1605–1612

13 Parkos C. A., Colgan S. P., Delp C., Arnaout M. A. and Madara J. L. (1992) Neutrophil migration across a cultured epithelial monolayer elicits a biphasic resistance response representing sequential effects on transcellular and paracellular pathways. J. Cell. Biol. **117:** 757–764

14 Springer T. A. (1990) Adhesion receptors of the immune system. Nature **346:** 425–430

15 Van der Vieren M., Le Trong H., Wood C. L., Moore P. F., John T. St, Staunton D. E. and Gallatin W. M. (1995) A novel leukointegrin, alpha d beta 2, binds preferentially to ICAM-3. Immunity **3:** 683–690

16 Parkos C. A., Colgan S. P., Liang A., Nusrat A., Bacarra A. E., Carnes D. K. et al. (1996) CD 47 mediates post-adhesive events required for neutrophil migration across polarized intestinal epithelia. J. Cell Biol. **132:** 437–450

17 Colgan S. P., Dzus A. L. and Parkos C. A. (1996) Epithelial exposure to hypoxia modulates neutrophil transepithelial migration. J. Exp. Med. **184:** 1003–1015

18 Friedman G. B., Taylor C. T., Parkos C. A. and Colgan S. P. (1998) Epithelial permeability induced by neutrophil transmigration is potentiated by hypoxia: role of intracellular cAMP. J. Cell. Physiol. **176:** 76–84

19 Cambell I. G., Freemont P. S., Foulkes W. and Trowsdale J. (1992) An ovarian tumor marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. Cancer Res. **52:** 5416–5420

20 Cooper D., Lindberg F. P., Gamble J. R., Brown E. J. and Vadas M. A. (1995) Transendothelial migration of neutrophils involves integrin-associated protein (CD47). Proc. Nat. Acad. Sci. USA **92:** 3978–3982

21 Serhan C. N., Haeggstrom J. Z. and Leslie C. C. (1996) Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J. **10:** 1147–1158

22 Serhan C. N. (1994) Lipoxin biosynthesis and its impact in inflammatory and vascular events. Biochim. Biophys. Acta **1212:** 1–25

23 Claria J., Lee M. H. and Serhan C. N. (1996) Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. Mol. Med. **2:** 583–596

24 Colgan S. P., Serhan C. N, Parkos C. A., Delp-Archer C. and Madara J. L. (1993) Lipoxin A₄ modulates transmigration of human neutrophils across intestinal epithelial monolayers. J. Clin. Invest. **92:** 75–82

25 Serhan C. N., Maddox J. F., Petasis N., Papayianni A., Brady H. R., Colgan S. P. et al. (1995) Design of lipoxin A₄ stable analogs that block human neutrophil transmigration and adhesion. Biochemistry **34:** 14609–14615

26 Gewirtz A. T., McCormick B. A., Neisch A. S., Petasis N. A., Gronert K., Serhan C. N. et al. (1998) Pathogen-induced chemokine secretion from model intestinal epithelium is inhibited by lipoxin A₄ analogs. J. Clin. Invest. **101:** 1860–1869

27 Fiore S. and Serhan C. N. (1995) Lipoxin A₄ receptor activation is distinct from that of the formyl peptide receptor in myeloid cells: inhibition of CD11/18 expression by lipoxin A₄-lipoxin A₄ receptor interaction. Biochemistry **34:** 16678–16686

28 Maddox J. F., Colgan S. P., Clish C., Petasis N. A., Fokin V. V. and Serhan C. N. (1998) Lipoxin B₄ regulates monocyte and neutrophil adherence and motility: design of stable lipoxin B₄ analogs with increased biologic activity. FASEB J. **12:** 487–494

29 Maddox J. F. and Serhan C. N. (1996) Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. J. Exp. Med. **183:** 137–146

30 Raud J., Palmertz U., Dahlen S.-E. and Hedqvist P. (1991) Lipoxins inhibit microvascular inflammatory actions of leukotriene B₄. In: Cell-Cell Interactions in the Release of Inflammatory Mediators, pp. 185–192, Wong P. Y.-K. and Serhan C. N. (eds), Plenum Press, New York

31 Dahlen S. E., Franzen L., Raud J., Serhan C. N., Westlund P., Wikstrom E. et al. (1988) Actions of lipoxin A4 and related compounds in smooth muscle preparations and on the microcirculation in vivo. Adv. Exp. Med. Biol. **229:** 107–30

32 Takano T., Fiore S., Maddox J. F., Brady H. R., Petasis N. A. and Serhan C. N. (1997) Aspirin-triggered 15-epi-lipoxin A4 (LXA4) and LXA4 stable analogues are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. J. Exp. Med. **185:** 1693–1704

33 Jung H. C., Eckmann L., Yang S.-K., Panja A., Fierer J., Morzycka-Wroblewska E. et al. (1995) A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J. Clin. Invest. **95:** 55–65

34 McCormick B. A., Colgan S. P., Delp-Archer C., Miller S. I. and Madara J. L. (1993) *Salmonella typhimurium* attachment to human intestinal epithelial monolayers: transcellular signaling to subepithelial neutrophils. J. Cell Biol. **123:** 895–907

35 Schurer-Maly C. C., Eckmann L., Kagnoff M. F., Falco M. T. and Maly F.-E. (1994) Colonic epithelial cell lines as a source of interleukin-8: stimulation by inflammatory cytokines and bacterial lipopolysaccharide. Immunology **81:** 85–91

36 Yang S. K., Eckmann L., Panja A. and Kagnoff M. F. (1997) Differential and regulated expression of C-X-C, C-C, and C-chemokines by human colon epithelial cells. Gastroenterology **113:** 1214–1223

37 Eckman L., Kagnoff M. F. and Fierer J. (1993) Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect. Immun. **61:** 4569–4574

38 Laurent F., Eckmann L., Savidge T. C., Morgan G., Theodos C., Naciri M. et al. (1997) *Cryptosporidium parvum* infection of human intestinal epithelial cells induces the polarized secretion of C-X-C chemokines. Infect. Immun. **65:** 5067–5073

39 McCormick B. A., Hofman P. M., Kim J., Carnes D., Miller S. I. and Madara J. L. (1995) Surface attachment of *Salmonella typhimurium* to intestinal epithelia imprints the subepithelial matrix with gradients chemotactic for neutrophils. J. Cell Biol. **131:** 1599–1608

40 van Deventer S. J. (1997) Review article: chemokine production by intestinal epithelial cells: a therapeutic target in inflammatory bowel disease? Aliment. Pharmacol. Ther. **3:** 116–120; discussion 120-121

41 Gronert K., Gewirtz A., Madara J. L. and Serhan C. N. (1998) Identification of a human enterocyte lipoxin A4 receptor that is regulated by interleukin (IL)-13 and inteferon gamma and inhibits tumor necrosis factor alpha-induced IL-8 release. J. Exp. Med. **187:** 1285–1294

42 Yu Y. and Chadee K. (1998) Prostaglandin E2 stimulates IL-8 gene expression in human colonic epithelial cells by a post-transcriptional mechanism. J. Immunol. **161:** 3746–3752

43 Taylor C. T., Dzus A. L. and Colgan S. P. (1998) Autocrine regulation of intestinal epithelial permeability induced by hypoxia: role for basolateral release of tumor necrosis factor-α (TNF-α). Gastroenterology **114:** 657–668

44 Taylor C. T., Fueki N. and Colgan S. P. (1998) Critical role of cAMP response element binding protein (CREB) expression in hypoxia-elicited induction of epithelial TNFα. Gastroenterology **114:** A423

45 Lawson L. D. and Powell D. W. (1987) Bradykinin-stimulated eicosanoid synthesis and secretion of rabbit ileal components. Am. J. Physiol. (Gastrointest. Liver. Physiol.) **252:** G783–G790

46 Dubois R. N., Abramson S. B., Crofford L., Gupta R. A., Simon L. S., Van De Putte L. B. et al. (1998) Cyclooxygenase in biology and disease. FASEB J. **12:** 1063–1073

47 Barrett K. E. (1993) Positive and negative regulation of chloride secretion in T84 cells. Am. J. Physiol. **265:** C859–C868

48 Coleman R. A., Smith W. L. and Narumiya S. (1994) VIII. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharm. Rev. **46:** 205–229

49 Berschneider H. M. and Powell D. W. (1992) Fibroblasts modulate intestinal secretory responses to inflammatory mediators. J. Clin. Invest. **89:** 484–489

50 Hinterleitner T. A., Saada J. I., Berschneider H. M., Powell D. W. and Valentich J. D. (1996) IL-1 stimulates intestinal myofibroblast COX gene expression and augments activation of Cl$^-$ secretion in T84 cells. Am. J. Physiol. (Cell Physiol) **271:** C1262–C1266

51 Breider M. (1993) Endothelium and inflammation. JAVMA **203:** 300–306

52 Pober J. S. and Cotran R. S. (1990) Overview: the role of endothelial cells in inflammation. Transplantation **50:** 537–541

53 Vane J. R., Anggard E. E. and Botting R. M. (1990) Regulatory functions of the vascular endothelium. N. Engl. J. Med. **323:** 27–36

54 Blume E. D., Taylor C. T., Lennon P. F., Stahl G. L. and Colgan S. P. (1998) Activated endothelial cells elicit paracrine induction of epithelial chloride secretion: 6-keto-PGF$_{1\alpha}$ is an epithelial secretagogue. J. Clin. Invest. **102:** 1161–1172

55 Chadwick V. S., Mellor D. M., Myers D. B., Selden A. C., Keshararzian A., Broom M. F. et al. (1988) Production of peptides inducing chemotaxis and lysosomal enzyme release in human neutrophils by intestinal bacteria in-vitro and in-vivo. Scand. J. Gastro. **23:** 121–128

56 Nash S., Stafford J. and Madara J. L. (1987) Effects of polymorphonuclear leukocyte transmigration on barrier function of cultured intestinal epithelial monolayers. J. Clin. Invest. **80:** 1104–1113

57 Breyer M. D., Jacobson H. R. and Breyer R. M. (1996) Functional and molecular aspects of real prostaglandin receptors. J. Am. Soc. Nephrol. **7:** 8–17

58 Namba T., Oida H., Sugimoto Y., Kakizuka A., Negishi M., Ichikawa A. et al. (1994) cDNA cloning of a mouse prostacy-clin receptor: multiple signalling pathways and expression in thymic medulla. J. Biol. Chem. **269:** 9986–9992

59 Katsuyama M., Sugimoto Y., Namba T., Irie A., Negishi M., Narumiya S. et al. (1994) Cloning and expression of a cDNA for the human prostacyclin receptor. FEBS Lett. **344:** 74–78

60 Hebert R. L., Regnier L. and Peterson L. N. (1995) Rabbit cortical collecting ducts express a novel prostacyclin receptor. Am. J. Physiol. (Renal Fluid Electrolyte Physiol) **268:** F145–154

61 Blikslager A. T., Roberts M. C., Rhoades J. M. and Argenzio R. A. (1997) Prostaglandins I2 and E2 have a synergistic role in rescuing epithelial barrier function in porcine ileum. J. Clin. Invest. **100:** 1928–1933

62 DuBois R. N., Awad J., Morrow J., Roberts L. J. and Bishop P. R. (1994) Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. J. Clin. Invest. **93:** 493–498

63 Tsujii M. and DuBois R. N. (1995) Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell **83:** 493–501

64 Sheng H., Shao J., Morrow J. D., Beauchamp R. D. and DuBois R. N. (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer. Res. **58:** 362–366

65 Ethridge R. T., Hellmich M. R., DuBois R. N. and Evers B. M. (1998) Inhibition of heat-shock protein 70 induction in intestinal cells overexpressing cyclooxygenase 2. Gastroenterology **115:** 1454–1463

66 Dinchuk J. E., Car B. D., Focht R. J., Johnston J. J., Jaffee B. D., Covington M. B. et al. (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature **378:** 406–409

67 Lim H., Paria B. C., Das S. K., Dinchuk J. E., Langenbach R., Trzaskos J. M. et al. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell **91:** 197–208

68 Lukiw W. J., Pelaez R. P., Martinez J. and Bazan N. G. (1998) Budesonide epimer R or dexamethasone selectively inhibit platelet-activating factor-induced or interleukin 1beta-induced DNA binding activity of cis-acting transcription factors and cyclooxygenase-2 gene expression in human epidermal keratinocytes. Proc. Natl. Acad. Sci. USA **95:** 3914–3919

69 Schmedtje J. F. Jr, Ji Y. S., Liu W. L., DuBois R. N. and Runge M. S. (1997) Hypoxia induces cyclooxygenase-2 via the NF-kappaB p65 transcription factor in human vascular endothelial cells. J. Biol. Chem. **272:** 601–608

70 Zünd G., Madara J. L., Dzus A. L., Awtrey C. S. and Colgan S. P. (1996) Interleukin 4 and interleukin 13 differentially regulate epithelial chloride secretion. J. Biol. Chem **271:** 7460–7464

71 Colgan S. P., Resnick M. B., Parkos C. A., Delp-Archer C., Bacarra A. E., Weller P. F. et al. (1994) Interleukin-4 directly modulates function of a model human intestinal epithelium. J. Immunol. **153:** 2122–2129

72 Crofford L. J. (1997) COX-1 and COX-2 tissue expression: implications and predictions. J. Rheumatol. **49:** 15–19

73 Brady H. R., Papayianni A. and Serhan C. N. (1995) Potential vascular roles for lipoxins in the 'stop programs' of host defense and inflammation. Trends Cardiovasc. Med. **5:** 186–192