MINIREVIEW

Inflammatory Mediators in Gastrointestinal Defense and Injury

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Inflammation of the mucosal layer of the gastrointestinal (GI) tract is not only a feature almost always associated with ulceration of those tissues, but it also plays an important role in both the production and healing of the lesions. The mediators that coordinate inflammatory responses also have the capability to alter the resistance of the mucosa to injury induced by noxious substances, while others render the mucosa more susceptible to injury. In this article, we provide a review of the inflammatory mediators that modulate GI mucosal defense. Among the mediators discussed are nitric oxide, the eicosanoids (prostaglandins, leukotrienes, and thromboxanes), neuropeptides, cytokines, and proteinases. Many of these mediators are considered potential therapeutic targets for the treatment of ulcerative diseases of the digestive tract.

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Inflammation in any part of the gastrointestinal (GI) tract can profoundly influence the function of the mucosal layer that lies closest to the luminal contents. Inflammation can also alter the ability of the mucosa to resist injury induced by luminal factors and the capacity for the mucosa to undergo repair once injury has occurred. The inflammatory response is coordinated to a large extent by an array of chemical mediators that are released from the epithelium and from the immunocytes and nerves within the lamina propria. This release occurs in response to injury, infection, or exposure of these cells to antigen. Although inflammation is a physiological response that is usually self-limiting, in some circumstances, such as when the factor that initiates the inflammatory response persists, inflammation can be unrelenting, leading to excessive tissue injury.

One of the ways in which inflammatory mediators can alter mucosal integrity is by influencing the effectiveness of the various components of "mucosal defense"; that is, the combination of factors that allow the mucosa to withstand exposure to substances with a wide range of pH, temperature, and osmolarity, solutions with detergent properties (e.g., bile), and bacterial products capable of eliciting local and systemic inflammatory reactions (1). When damage to the mucosa does occur, repair of the injury can be achieved very quickly, limiting the possibility of harmful substances (e.g., bacterial products) gaining entry into the systemic circulation.

Mucosal defence has been best characterized in the stomach, which exhibits remarkable resistance to the damaging effects of acid and pepsin. The first level of defence consists of the factors secreted into the lumen, including acid, mucus, bicarbonate, and antibacterial substances (e.g., immunoglobulins and lactoferrin). The principal function of gastric acid is to reduce the numbers of ingested bacteria entering the small intestine. The second level of defence is the epithelium, which is remarkably resistant to acid-induced injury (2). The epithelium also acts as a barrier to the passive diffusion of harmful substances. Damage to the epithelium can be repaired very quickly through a process known as "restitution", which involves migration of healthy epithelial cells from the gastric pits over the denuded region. Restitution is observed in response to injury throughout the GI tract, as well as in other tissues (3).

The microcirculation represents the third level of mucosal defence, and one that is significantly modulated by the

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nervous system and a number of inflammatory mediators. Diffusion of acid or toxins into the mucosa results in a sensory afferent nerve-mediated elevation of mucosal blood flow that is critical for limiting damage and facilitating repair. The blood dilutes and/or neutralizes the acid/toxin and prevents it from accumulating in the mucosal tissue to cytotoxic concentrations. In experimental models in which this reactive hyperemic response is impaired, such as through prior ablation of the sensory afferent nerves with capsaicin (4), the mucosa can be damaged by exposure to irritants that would normally be tolerated (Fig. 1). A defect of this type also appears to underlie the increased susceptibility to gastric mucosal damage in portal hypertension, at least in experimental models (5, 6).

The fourth level of defence is the mucosal immune system, consisting of various immunocytes resident within the lamina propria that act as sentinels. Mast cells and macrophages, for example, can sense the entry of foreign material (e.g., antigens and endotoxin) into the mucosa and can respond by releasing chemical mediators that coordinate an appropriate inflammatory response.

The final level of mucosal defence is called into play when an ulcer has formed—an ulcer being defined as a break in mucosa that extends through the muscularis mucosae. In these circumstances, the ulcer is repaired through growth and re-development of gastric glands, growth of new blood vessels (angiogenesis), and re-innervation of the mucosa by the extrinsic and intrinsic nerves (7).

The resistance of the GI mucosa to injury ultimately depends upon the balance between these defensive factors and the aggressive factors present in the lumen. Several components of mucosal defense can be influenced by inflammatory mediators. In this paper, we review the contribution of some of the major inflammatory mediators to the modulation of mucosal defense and to the pathogenesis of mucosal injury.

Nitric Oxide (NO)

NO has been the subject of extensive studies with respect to its role in GI mucosal defense and the pathogenesis of mucosal injury (8, 9). There remains considerable controversy regarding the predominant role of NO in the GI tract: protective or damaging. The constitutive forms of NO synthase (NOS), neuronal NOS (nNOS) and endothelial NOS (eNOS), are very important in the normal function of the GI tract in that inhibition of these enzymes can result in disturbances of GI motility, blood flow, secretion, etc. On the other hand, the inducible NOS (iNOS), which produces relatively large amounts of NO under certain pathological conditions, contributes to mucosal injury and dysfunction. Suppression of NO synthesis renders the gastric mucosa more susceptible to injury (10), whereas administration of NO donors can protect the stomach from injury (11). Indeed, the latter finding led to the development of a series of NO-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) that do not cause GI damage (12–14) and that accelerate gastric ulcer healing (15).

NO exerts many actions in the GI tract in common with prostaglandins (Fig. 2). It can modulate the activity of mucosal immunocytes such as mast cells, as well as modulating leukocyte-endothelial interactions and intestinal epithelial permeability. NO inhibits recruitment of neutrophils to sites of inflammation. NO reduces neutrophil infiltration into the GI tract mucosa and inhibitors of NO synthesis enhance leukocyte recruitment (16). The anti-adhesive properties of NO may range from inhibiting the production of important pro-inflammatory molecules, to directly altering the ability of neutrophils to adhere, and to inhibiting the expression of various adhesion molecules (16). Interestingly, blockade of NO synthesis resulted in significant changes in intestinal epithelial barrier function (17, 18), which could be inhibited by mast cell stabilizers or receptor antagonists for histamine (H-1) and platelet-activating factor (PAF), both of which can be released by mast cells.

The ability of NO to modulate mast cell reactivity was further demonstrated in *in vitro* studies performed by Hogaboam *et al.* (19). Mast cells spontaneously release NO. When exposed to interleukin (IL)-1β, a profound and rapid increase in NO release was observed. Moreover, the release of NO exerted feedback inhibition of PAF release from the

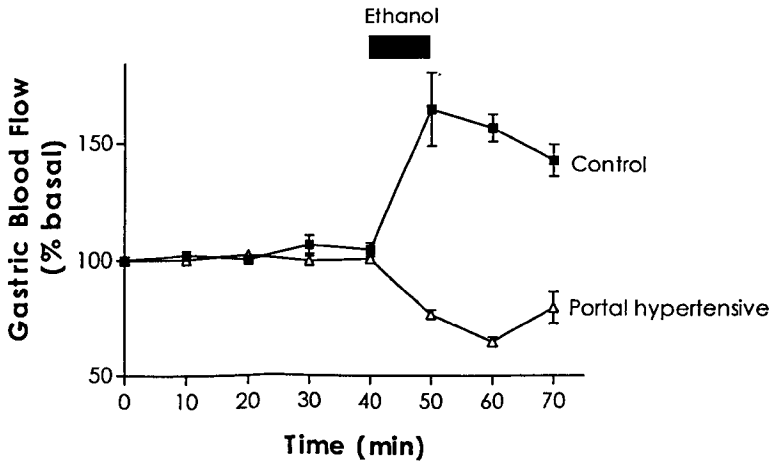

Figure 1. Deficient mucosal hyperemic response in rats with portal hypertension. In normal rats, topical application of an irritant such as 20% ethanol (shown) results in a rapid increase in mucosal blood flow. This protects the mucosa from injury. However, in rats with portal hypertension, the hyperemic response is absent. As a result, extensive mucosal injury is induced by the topical ethanol.

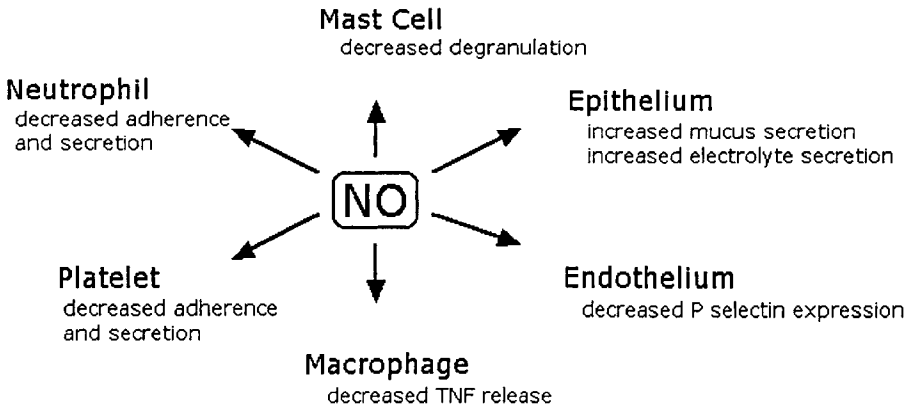

Figure 2. Schematic diagram illustrating a commonly accepted view of the relative roles of COX-1 and -2. There is emerging evidence to suggest that prostaglandins derived from COX-2 make an important contribution to GI mucosal defense, whereas prostaglandins derived from COX-1 play an important role in inflammation and pain.

mast cell, consistent with the findings of Kanwar *et al.* (17) that suggested that blockade of NO synthesis led to release of PAF. Others have demonstrated that exposure of mast cells to exogenous NO leads to a diminution of histamine release (20).

As alluded to above, the role of NO in mucosal defense in situations of mucosal inflammation is complicated, with some studies suggesting that NO contributes to tissue injury, and others suggesting, as in the studies outlined above, that NO primarily acts in a protective manner. The role of NO as a contributor to mucosal injury has been extensively characterized in experimental models of colitis. NO has been suggested to react with superoxide anion, produced by activated neutrophils, to form another potent oxidant, peroxynitrite. Administration of peroxynitrite into the colon produces widespread injury and inflammation somewhat similar to that seen in several experimental models of inflammatory bowel disease (IBD) (21). Furthermore, the colocalization of tyrosine nitration with iNOS immunoreactivity in inflamed colon seems to suggest that iNOS may be responsible for tissue injury via the formation of NO-dependent nitrating species, particularly peroxynitrite (22). However, detection of 3-nitrotyrosine as an indicator of peroxynitrite formation *in vivo* has been questioned recently, because it has been demonstrated that 3-nitrotyrosine could also be formed from nitrite (NO_2^-) in the presence of hypochlorous acid or human polymorphonuclear leukocytes (a source of hypochlorous acid). Other investigators have also been able to reproduce the observations implying that neutrophil infiltration will generate 3-nitrotyrosine irrespective of the presence of peroxynitrite. Although the hypothesis that NO contributes to tissue injury in colitis is supported by several studies demonstrating that administration of NOS inhibitors reduces the severity of colonic damage and inflammation (22–24), such evidence must be weighed against other data suggesting that NO does not cause mucosal injury, even when the NO is administered in very large amounts (25). Indeed, there is recent evidence that agents that release NO in small amounts over a prolonged period of time can greatly reduce inflammation and can accelerate healing in experimental colitis (26).

Eicosanoids

Prostaglandins. Prostaglandins are 20-carbon fatty acids produced from arachidonic acid via the enzyme cyclooxygenase (COX; Fig. 3). Like the other eicosanoids (i.e., leukotrienes and thromboxanes), prostaglandins generally have short half-lives (seconds to minutes) and act in an autocrine or paracrine manner. The discovery of the ability of prostaglandins to reduce or prevent GI injury induced by topical irritants (cytoprotection) by Robert and colleagues (27) resulted in an enormous growth of research

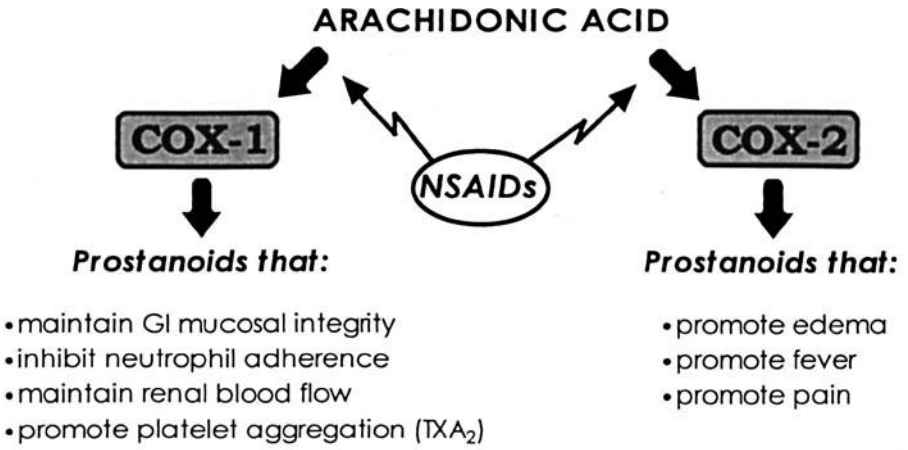

Figure 3. NO contributes to mucosal defense in a number of different ways. This schematic diagram lists some of the major ways in which NO can downregulate inflammatory responses and reduce mucosal injury. Prostaglandins produced by the GI mucosa exert many of the same actions.

into the physiological role of these fatty acids in mucosal defense. It is now well established that suppression of prostaglandin synthesis in the stomach, through inhibition of COX, is a key component of the mechanism underlying ulceration in the upper GI tract associated with the use of NSAIDs (28, 29), and the ability of these drugs to exacerbate mucosal injury in the lower digestive system (30, 31). Prostaglandins appear to exert their cytoprotective actions by stimulating mucus and bicarbonate secretion, maintaining mucosal blood flow, and by enhancing the resistance of epithelial cells to injury induced by cytotoxins (32). Prostaglandins inhibit leukocyte recruitment (33, 34), which could contribute to the beneficial effects of these substances in situations in which the GI mucosa is inflamed. Indeed, it is likely that prostaglandins are one of the molecules that are generated in increased amounts during inflammation that act to downregulate the inflammatory response. This hypothesis is supported by the observations of exacerbation of mucosal inflammation in animal models of colitis when the animals are given NSAIDs (31, 35).

As outlined above, the mucosa and submucosa of the normal GI tract contain a significant number of immunocytes, including mast cells, B and T lymphocytes, macrophages, neutrophils, and eosinophils. The numbers of these cells varies considerably along the length of the GI tract, to some extent reflecting the luminal bacterial load in each region. Some of these immunocytes (e.g., mast cells and macrophages) play an important role in signaling the entry into the lamina propria of foreign material or antigen. These cells release soluble mediators and cytokines that initiate an inflammatory response aimed at preventing the entry of the foreign matter into the systemic circulation. Some mediators act on the vascular endothelium to increase permeability (permitting plasma exudation and facilitating movement of antibodies into the interstitium) and/or to increase expression of adhesion molecules (to recruit leukocytes). Many inflammatory mediators are chemotaxins; leukocytes will migrate up a concentration gradient of these chemicals, toward the source of their release. Some inflammatory mediators are also capable of priming or stimulating leukocytes to release reactive oxygen metabolites, proteases, or other inflammatory mediators, or of stimulating the proliferation and/or differentiation of lymphocytes and other immunocytes.

One of the mechanisms through which prostaglandins can downregulate inflammatory responses, and in doing so, reduce the severity of mucosal injury, is through modulation of the activity of immunocytes within the mucosa. For example, prostaglandin E_2 (PGE_2) has been shown to be a potent suppressor of TNF-α release from macrophages (36), and it also reduces expression of the gene for TNF-α in these cells (37). NSAIDs, on the other hand, increase the release of TNF-α from macrophages and other cells (38–40). In humans given bacterial endotoxin, prior administration of an NSAID significantly elevated the release of TNF-α into the systemic circulation (40). Prostaglandins also regulate the release of other cytokines such as IL-1 from macrophages (41, 42), and they reduce the production of the potent chemotaxin, leukotriene B_4, from neutrophils (43–45).

The mast cell is another target for the inhibitory effects of prostaglandins. Raud *et al.* (46) demonstrated that prostaglandins could partially suppress acute mast cell-dependent inflammation, whereas Hogaboam *et al.* (47) demonstrated that PGE_2 dose-dependently inhibited the release of PAF, histamine, and TNF-α from peritoneal and intestinal mucosal mast cells. These effects were observed at extremely low prostaglandin concentrations. For example, PAF release from peritoneal mast cells was inhibited by a PGE_1 analogue at concentrations as low as 10^{-10} M, whereas PGE_2 suppressed TNF-α release from peritoneal mast cells at concentrations as low as 10^{-11} M (47).

In addition to acting on immunocytes that are resident within the lamina propria and thereby decreasing the intensity of an inflammatory response, prostaglandins can inhibit the recruitment of leukocytes from the vasculature. Neutrophils have been implicated as culprits in the damage associated with various disorders of the GI tract, including ischemia-reperfusion (48), NSAID gastropathy (49), and colitis (50, 51). Prostaglandins can dampen the contribution of neutrophils to tissue injury by downregulating several neutrophil functions that contribute to inflammation and injury. For example, prostaglandins suppress the generation of reactive oxygen metabolites by neutrophils (which account for much of the tissue injury caused by these cells) (52, 53). The observation that NSAIDs increase the numbers of neutrophils adhering to the vascular endothelium, and that this can be prevented by administering exogenous prostaglandins (33, 34), suggests that prostaglandins are important physiological regulators of neutrophil adherence and extravasation.

The discovery in the early 1990s of a second isoform of COX (54) has led to a complete reappraisal of the role of this enzyme in producing prostaglandins in various circumstances. It is now widely believed that the prostaglandins produced under normal circumstances, which play such an important role in modulating blood flow and such mucosal defense factors as mucus secretion, are derived from the constitutively expressed isoform of COX, COX-1. On the other hand, prostaglandins produced in the context of inflammation are largely derived from the inducible isoform of COX, COX-2 (55). This theory has been somewhat oversimplified to the following: COX-1 produces prostaglandins that perform beneficial functions, whereas COX-2 produces prostaglandins that exert detrimental (i.e., pro-inflammatory) effects (Fig. 3). This hypothesis underlies the considerable resources that have been invested in the development of highly selective inhibitors of COX-2, which have been suggested to have anti-inflammatory and analgesic effects, but to lack ulcerogenic effects. Although the recently marketed COX-2 inhibitors have been a tremendous com-

mercial success, there remain questions about the central tenets of the "COX-2 hypothesis" (56).

First, there is considerable evidence that prostaglandins derived from COX-1 contribute to the generation of the features of inflammation, particularly pain. Several selective inhibitors of COX-2 were found to significantly reduce carrageenan-induced inflammation in the rat only when given at doses at which the drugs also suppressed COX-1 (57). Moreover, a significant portion of inflammatory prostaglandin synthesis in some models is attributable to COX-1 (57, 58). Mice lacking COX-2 mount an inflammatory response to some stimuli that is indistinguishable from that seen in wild-type controls (59). Indeed, COX-2-deficient mice have been shown to have an impaired ability to downregulate inflammatory responses (57), and COX-2 has been suggested, based on studies performed in a rat pleurisy model, to be important in turning off inflammatory responses (60). The second area where the COX-2 hypothesis has been challenged is with respect to the contribution of COX-2 to mucosal defense. Although it was initially thought that COX-2 was not expressed in the GI tract (61), there is now considerable evidence that COX-2 is very rapidly upregulated in response to quite subtle stimulation of the mucosa. For example, COX-2 is upregulated within 1 hr of administration of aspirin (62), administration of a chemical irritant (63), or of induction of ischemia-reperfusion (64). In such circumstances, COX-2 is performing an important function because administration of a selective COX-2 inhibitor leads to a significant elevation of mucosal injury (63, 64). COX-2-derived prostaglandins also play a very important role in ulcer healing in the stomach and the colon. Induction of ulceration results in a marked increase in COX-2 expression in both tissues (65, 66). Administration of a selective COX-2 inhibitor to rats with gastric ulcers or with experimental colitis results in a profound inhibition of healing of the lesions, and an exacerbation of the inflammation (65, 66). The third area in which the COX-2 theory has been challenged is on the claim that inhibition of COX-2 will not result in the detrimental effects observed with conventional NSAIDs. Although the use of COX-2 inhibitors by patients with arthritis is associated with less ulcer complications, these agents still produce ulcers and cause ulcer bleeding and perforation (67, 68). However, concomitant use of low-dose aspirin with a selective COX-2 inhibitor abrogates the benefit, in terms of reduced GI toxicity, gained by use of a selective COX-2 inhibitor instead of a conventional NSAID (67). Moreover, the use of selective COX-2 inhibitors is associated with significant renal toxicity, possibly more frequent than seen with conventional NSAIDs. In one large clinical study there was a 5-fold higher incidence of myocardial infarction in patients taking the selective COX-2 inhibitor than in patients taking a conventional NSAIDs (68). Thus, although selective COX-2 inhibitors represent an advance over older NSAIDs, the magnitude of the advance does not appear to be anywhere near what had been predicted for these agents (55), and there remain significant questions about both the efficacy and safety of this class of drugs.

Leukotrienes. Like prostaglandins, leukotrienes are synthesized from arachidonic acid. The rate-limiting step in leukotriene synthesis is the enzyme 5-lipoxygenase (Fig. 1). There are a number of different types of leukotrienes, but they can be conveniently subdivided into two main subclasses: leukotriene B_4 and the peptido-leukotrienes (LTC_4, LTD_4, and LTE_4). As the name suggests, the latter consist of both fatty acid and amino acid moieties. Leukotriene synthesis occurs mainly in immunocytes, epithelial cells, and endothelial cells. In the mucosa, the mast cell is a major source of peptido-leukotrienes, whereas the neutrophil appears to be the predominant source of leukotriene B_4.

LTB_4 is a very potent chemotaxin for neutrophils. It has marginal or no effect on vascular permeability and mucosal blood flow, but can promote leukocyte recruitment of from the vasculature by upregulating expression of the β_2 integrins (CD11/CD18) on those cells. LTB_4 can also stimulate the release of reactive oxygen metabolites from neutrophils, which contributes significantly to the tissue injury associated with mucosal inflammation. LTB_4 has been suggested to contribute to the pathogenesis of NSAID-induced gastric damage through its ability to promote leukocyte adherence to the vascular endothelium (33, 69). This process appears to be critical in the pathogenesis gastric ulceration associated with NSAID administration (49). Receptor antagonists for LTB_4 and inhibitors of 5-lipoxygenase have been shown to attenuate NSAID-induced leukocyte adherence (33) and to reduce the severity of NSAID-induced mucosal damage (70). Elevated production of LTB_4 by the human stomach has been documented in patients taking NSAIDs (69). LTB_4 may play a similar role in the pathogenesis of ulceration associated with *Helicobacter pylori* infection (71). Interestingly, gastric juice LTB_4 levels are significantly higher in patients with gastric *H. pylori* colonization than in those who are *H. pylori* negative (72).

In the 1980s, a significant body of evidence was generated to support a role for LTB_4 in the pathogenesis of IBD. Mucosal production of LTB_4 in human and experimental colitis is markedly increased over that in the normal colon (73–77). The majority of the chemotactic activity that could be extracted from inflamed human colon could be attributed to LTB_4 (78). Intracolonic administration of LTB_4 was found to exacerbate tissue injury in a rat model of colitis (79), wheras 5-LO inhibitors were shown to significantly accelerate healing of experimental colitis (76, 77). However, in other models, elevated LTB4 production appeared to occur as a consequence of inflammation, but not to contribute significantly to the tissue injury of degree of inflammation (80). Despite most of the data supporting a role for LTB_4 in IBD, inhibitors of leukotriene synthesis have failed to significantly modify disease severity or produced only modest effects when tested in clinical trials (81, 82).

In contrast to LTB_4, the peptido-leukotrienes exhibit

little if any chemotactic activity, but are potent stimulators of smooth muscle contraction. Peptido-leukotrienes also increase the permeability of the vascular endothelium, and increase P-selectin expression on these cells (83), thereby promoting the rolling of leukocytes. Intraarterial infusion of peptido-leukotrienes profoundly increases the susceptibility of the rat stomach to injury induced by topical irritants, most likely through a reduction of mucosal blood flow (84).

Antigenic activation of mucosal mast cells can greatly increase the susceptibility to mucosal injury, and this is largely due to the release of peptido-leukotrienes from the mast cells (85, 86). Peptido-leukotrienes have also been suggested to mediate the intestinal damage associated with mucosal mast cell activation (87).

Thromboxane. Thromboxane is the major arachidonic acid metabolite produced by platelet (via COX-1). The platelet accounts for about 95% of the thromboxane detectable in serum, with neutrophils being another source (88). Thromboxane is a very potent vasoconstrictor and agonist for platelet aggregation. As any reduction in mucosal blood flow could potentially render the mucosa more susceptible to injury, thromboxane has been suggested to be an important contributor to ulceration in the GI tract. As thromboxane also has the capacity to stimulate leukotriene B_4 release and the adherence of leukocytes to the vascular endothelium (89), thromboxane may also contribute to mucosal injury through modulation of inflammatory responses.

Whittle and coworkers (90) showed that close arterial administration of arachidonic acid into the dog gastric microcirculation resulted in a profound reduction of gastric blood flow. This was attributable to generation of thromboxane from the arachidonic acid (90). Moreover, similar effects could be elicited with a thromboxane mimetic, which also greatly increased the susceptibility of the gastric mucosa to injury (91).

With the development of inhibitors of thromboxane synthesis (blockers of thromboxane synthetase), it was possible to further define the contribution of thromboxane to mucosal injury in experimental models of gastric injury. Thromboxane synthase inhibitors were shown to reduce the gastric damage induced by bile salts or by ethanol (92, 93). Takahashi *et al.* (94) demonstrated that thromboxane A_2 synthesis in the stomach was significantly elevated in ulcerated tissue, and a thromboxane synthase inhibitor markedly accelerated ulcer healing by promoting regeneration of the mucosa. Very few studies have been performed to evaluate the role of thromboxane in ulcer disease in humans. Hawkey *et al.* (95) reported that there were no changes in thromboxane levels in gastric tissue taken from ulcer patients versus controls, irrespective of the biopsy site (at or removed from the ulcer) or the presence or absence of inflammation at the biopsy site.

Thromboxane may also mediate some forms of damage in the small intestine. Endotoxin-induced jejunal damage was associated with significant increases in thromboxane and PAF production (96). Thromboxane synthase inhibitors markedly reduced thromboxane synthesis and the tissue injury without affecting PAF synthesis.

In the case of IBD, there is considerable interest in the possibility that thromboxane is an important mediator of mucosal injury because of the considerable evidence for altered thrombogenesis in these patients, particularly in Crohn's disease (97). Indeed, impaired mucosal perfusion, as would occur when a vasoconstrictor like thromboxane is overproduced, has been suggested to be a precipitating event in the pathogenesis of Crohn's disease (98). Mucosal thromboxane synthesis has been shown to be markedly elevated in a rat model of colitis (99). Treatment with prednisone or 5-aminosalicylate reduced the severity of mucosal injury and the thromboxane production. Treatment with either or two thromboxane synthetase inhibitors also reduced the severity of mucosal injury (99). Furthermore, Taniguchi and coworkers (100) have shown that intraluminal administration of a thromboxane A_2 receptor antagonist once daily for 7 days significantly decreased the severity of colonic damage in (trinitrobenzene sulfonic acid) TNBS-induced colitis in rats (100). Ridogrel, which is a dual thromboxane synthetase inhibitor and thromboxane receptor antagonist, has been assessed in ulcerative colitis. Once-daily rectal treatment produced a comparable reduction in the severity of colitis to that achieved with prednisolone (101). In the studies by other groups, treatment with ridogrel for 4 (102) or 8 weeks (103) was found to be as effective in reducing the inflammation and symptoms of ulcerative colitis as a standard dose of 5-aminosalicylic acid.

PAF

Like the eicosanoids, PAF is derived from membrane phospholipids through the action of phospholipases (Fig. 1). PAF is produced and released by most types of cells, and it can exert effects on a wide range of target cells and organs (104). Among its more potent actions are the ability to modulate smooth muscle tone, to activate neutrophils, and to act as a chemotaxin for eosinophils. Interest in PAF in the context of mucosal defense was initiated by reports that PAF was an extremely potent ulcerogenic factor (105, 106). At nanomolar doses, PAF caused hemorrhagic lesions in the stomach and intestine (105, 106) and at picomolar doses, it predisposed the gastric mucosa to damage induced by topical irritants (107). PAF was also shown to be responsible for most of the tissue injury observed in the GI tract in experimental endotoxic shock (108), hemorrhagic shock, and ischemia-reperfusion (109–111). There is good evidence that mucosal injury in these models was mediated through the ability of PAF to stimulate leukocyte adherence to the vascular endothelium and to activate granulocytes to release reactive oxygen metabolites (109–112). In addition to upregulating expression of β2 integrins (CD11/CD18) on leukocytes, PAF itself can act as an adhesion molecule when it is expressed on the surface of endothelial cells (113). PAF receptor antagonists ameliorate GI injury in several models of shock-associated damage (108, 109, 114). PAF can also

stimulate the release of other potent ulcerogens such as the peptido-leukotrienes (115) and thromboxane (96).

PAF is produced in the intestine in large amounts during helminth infections (116), with the mast cell being a primary cellular source (117). PAF appears to be an important stimulus for the epithelial secretion that is aimed at clearing helminth and other infections (118). It is also likely that PAF contributes significantly to the marked recruitment of eosinophils into the lamina propria of the infected intestine (116).

PAF has also been implicated in the pathogenesis of disorders of the lower intestine, including neonatal necrotizing enterocolitis (106) and IBD (119–123). PAF is produced in increased amounts in the mucosa of humans (119) and animals (120–122) with colitis, as well as in the stool of IBD patients (123). Several drugs commonly used in the treatment of IBD, including corticosteroids and sulfasalazine, have been shown to inhibit the production of PAF by the colon (119). That PAF actually contributes to the tissue injury in colitis is supported by the demonstrated efficacy of PAF receptor antagonists in reducing mucosal injury in animal models of colitis (120–122). The epithelium has been suggested to be a major source of the production of this mediator in ulcerative colitis (124).

Neuropeptides

Peptide mediators released from both extrinsic and intrinsic nerves within the GI tract influence virtually every component of the function of this organ. As outlined above, one of the most important components of mucosal defense is the hyperemic response to luminal irritants. This response is mediated via the release of calcitonin gene-related peptide (CGRP) from sensory afferent nerves, which causes the release from the vascular endothelium of NO, which in turn causes dilation of submucosal arterioles. The resulting increase in mucosal blood flow helps to dilute, neutralize, and remove any back-diffusing toxin and acid. Although best characterized in the stomach, the hyperemic response to irritants occurs throughout the GI tract. In situations in which this pathway has been impaired, such as through ablation of sensory afferent nerves with capsaicin, the mucosa does not mount an appropriate hyperemic response. As a result, exposure to mild irritants results in the development of extensive mucosal necrosis (4, 125). Although conventionally regarded as a neural response, there is evidence that mucosal immunocytes such as the mast cell may also play a role in the blood flow changes that occur in response to irritants, and may therefore influence mucosal resistance to injury.

In addition to releasing CGRP, sensory afferent nerves in the GI mucosa also release substance P. These nerves are found in close apposition to mucosal mast cells (126), and there is evidence that substance P can activate mast cells to release histamine (127). Under normal situations, a significant contribution of mast cell-derived histamine to the gastric hyperemic response elicited by topical application of acid or capsaicin cannot be detected (128). However, when rats with mastocytosis were studied, the hyperemic response to the irritants was significantly enhanced over that seen in normal rats, and this was abolished by pretreatment with histamine H_1 receptor antagonists or mucosal mast cell stabilizers (128).

In studies of human tissue, substance P failed to induce mast cell activation in histologically normal mucosa. However, it significantly promoted histamine release from mucosal mast cells in the specimens taken from inflamed or noninflamed tissue from patients with IBD (129). Thus, at least in a circumstance of mucosal mast cell hyperplasia, activation of sensory afferent nerves leads to histamine release from mucosal mast cells, which can then contribute to the generation of the mucosal hyperemic response to luminal irritants.

Neuropeptides may also influence acute inflammatory responses within the GI tract via their effects on mucosal immunocytes. As discussed above, the recruitment of granulocytes into the lamina propria is both a defensive response to infection and a critical step in the repair of damaged tissue. A number of neuropeptides, including neuropeptide Y, have been shown to increase expression of adhesion molecules on the vascular endothelium and therefore promote granulocyte adherence (130). Substance P can also promote neutrophil recruitment into the lamina propria, both by directly upregulating endothelial adhesion molecule expression (131) and through the ability of this neuropeptide to activate mast cells (132).

Cytokines

Cytokines play a central role in the regulation of the mucosal immune system, and therefore are extremely important in mucosal defense. Most of the available information in this regard pertains to the small and large intestine, because of the importance of cytokines in the pathogenesis of IBD. There is considerable evidence suggesting that one of the key factors in the pathogenesis of IBD is a disturbed balance between the production of pro- and anti-inflammatory cytokines. Three cytokines have been the most extensively characterized in this regard: IL-1β and tumor necrosis factor-α, both of which are regarded as pro-inflammatory cytokines, and IL-10, largely regarded as an anti-inflammatory cytokine.

Both IL-1β and TNF-α are cytokines released early in an inflammatory reaction, and both contribute to systemic responses to inflammation or infection such as the acute phase response, effects on appetite, and the generation of fever (133). IL-1 is produced by various types of cells, including monocytes, macrophages, neutrophils, endothelial cells, and fibroblasts (134). An endogenous IL-1 receptor antagonist (IL-1ra) is produced by many of the same cells that produce IL-1, and a recombinant form of this antagonist has been shown to inhibit many of the biological activities of IL-1 *in vitro* and *in vivo* (134). Elevated levels of IL-1 have been demonstrated in plasma and tissue of patients

with IBD, as well as in several experimental models of colitis (see Ref. 135 for a review). Furthermore, the ratio of IL-1:IL-1ra is increased in Crohn's disease and ulcerative colitis.

With respect to the upper GI tract, IL-1 has been shown to increase the resistance to injury. IL-1 has been shown to reduce the severity of gastroduodenal damage in several models (136–139). The mechanism responsible for the protective actions of IL-1 are not fully understood, but in the case of NSAID-induced gastric damage, this cytokine may reduce injury through a paradoxical inhibitory action on leukocyte adherence (137). In addition to being able to inhibit gastric acid secretion (136, 137, 140–142), at least partly through centrally mediated actions (142), IL-1 also can induce COX-2 expression (143) and iNOS expression (144). Thus, IL-1 may reduce gastroduodenal injury through its ability to stimulate prostaglandin and NO release. Furthermore, IL-1 has been shown to inhibit the release of other ulcer-promoting mediators (e.g., PAF and histamine) from mast cells (19, 20).

TNF-α appears to be a key contributor to many forms of gastric mucosal injury, including that associated with *H. pylori* infection and the use of NSAIDs. In the case of the latter, TNF-α release into plasma has been shown to be markedly elevated by NSAIDs. Inhibition of TNF-α synthesis results in attenuation of the damaging effects of NSAIDs in the rat stomach (38, 145). Prostaglandins are potent inhibitors of TNF-α release from both the macrophage (36, 37) and the mast cell (47). It is possible, therefore, that this accounts, at least in part, for the ability of prostaglandins to reduce the severity of NSAID-induced gastric injury.

Over the past few years, a considerable amount of data has been generated to suggest that TNF-α is an extremely important mediator of the tissue injury and inflammation observed in IBD. TNF-α levels in plasma (146) and stool (147) of children with IBD have been shown to be markedly elevated, as are the numbers of TNF-α-positive macrophages in the lamina propria of patients with IBD (148). Moreover, there are very impressive data suggesting beneficial effects of treatment of at least a subset of patients with Crohn's disease with an anti-TNF antibody (149, 150).

IL-10 is mainly produced by Th2 lymphocytes, but it can also be produced by both Th1 cells and monocytes (135). It has central role in downregulating the inflammatory cascade by depressing the production of a number of pro-inflammatory cytokines (151, 152), and enhancing the production of other anti-inflammatory cytokines (153). Individuals genetically predisposed to produce less IL-10 are at a higher risk of developing IBD, in particular ulcerative colitis (154). Akagi and colleagues (155) also demonstrated that overexpression of TNF-α and IFN-γ in Crohn's disease, together with under-production of IL-10, might lead to development of severe Crohn's disease. IL-10 can inhibit the production of TNF-α and IL-1β, and can induce production of IL-1ra (151, 154). IL-10-deficient mice develop chronic enterocolitis (157), which can be prevented by intragastric administration of IL-10-secreting *Lactococcus lactis* (158). Similar result were reported by Barbara and colleagues (159) who demonstrated that gene therapy using an adenovirus-IL-10 construct was successful in preventing but not in reversing experimental colitis in these rats.

Proteinases

Proteinases have well-characterized roles in protein degradation. However, certain proteinases (e.g., thrombin, trypsin, and tryptase) also act as signaling molecules that regulate cells by cleaving proteinase-activated receptors (PARs). PARs are members of a subfamily of G protein-coupled receptors (161, 162). Proteinases cleave PARs within the extracellular N terminus to unmask a tethered ligand domain that binds to the cleaved receptor, thereby causing their activation. Synthetic peptides corresponding to the tethered ligands can also specifically activate certain PARs. Four PARs have been cloned thus far. PAR1 is the classical receptor for thrombin, although thrombin can also activate PAR2 and PAR4. Agonists of PAR2 elicit acute inflammation (162–164), in part via a neurogenic mechanism (165). PAR2 is expressed by primary spinal afferent neurons, and PAR2 agonists stimulate release of substance P and CGRP in peripheral tissues (165). The activation of PARs on neurons in the intestine might be of particular importance for several reasons. Intestinal tissues are exposed to the proteases known to activate PARs (e.g., thrombin, trypsin, and tryptase) in both physiological and pathophysiological conditions. As discussed above, the enteric nervous system plays an essential role in modulating mucosal defense, as well as many other functions. PAR1 and PAR2 have been shown to be expressed on guinea pig myenteric neurons that also express neurotransmitters (e.g., substance P, vasoactive intestinal peptide, and NOS) (166). Considering the fact that PAR2 activation of spinal afferent neurons results in release of substance P and CGRP, it seems likely that activation of PARs on myenteric and submucosal neurons might result in the release of these same neurotransmitters, which are known to participate in the modulation of mucosal defense. Indeed, application of trypsin to the contraluminal side of porcine ileum has recently been shown to induce changes in ion transport by a mechanism dependent on prostaglandin production and neuronal activation (167). PAR2 has also been shown to be expressed both on cholinergic and noncholinergic submucosal neurons in the porcine ileum.

Are the proteinases that are capable of activating PARs released into the intestinal lumen or lamina propria? Although little is known about extrapancreatic trypsin, trypsinogen is released by several cell types (e.g., endothelial and epithelial cells) (168, 169). Tryptase, which constitutes the major protein released during human mast cell degranulation, is able to cleave PAR2 *in vitro* (170) in cells that naturally express the receptor or in cells transfected with the receptor. The observation that tryptase *in vitro* can cleave

and activate PAR2 suggests a role for this receptor in inflammatory states in humans that are associated with mast cell degranulation. As mast cells are closely associated with nerves (171, 172), mast cell proteases, including tryptase, are potential candidates for the activation of PAR2 on neurons. Thrombin, which is released during endothelial damage, could have access to neurons and appears to be the most likely agonist to activate neuronal PAR1 *in vivo*. At the present time, antagonists of the PAR receptors are not widely available. When such pharmacological tools become available, it will be possible to better define the role of PARs in GI mucosal defense.

Conclusions

In the past three decades, there has been a shift in thinking with respect to GI mucosal defense away from a focus on the harmful secretions within the lumen of the GI tract and toward a focus on the tissue responses that constitute what is referred to as "mucosal defense". This has come about partly because of the discovery of an association between infection with *Helicobacter pylori* and peptic ulcer disease, and partially because of the realization of the critical role played by prostaglandins in mucosal defense. There is now a very good appreciation of the various mechanisms involved in mucosal responses to luminal irritants. Much progress has been made in identifying the chemical signals that coordinate the mucosal defense response, as outlined in this paper. These chemical mediators represent potential therapeutic targets. Indeed, targeting of mediators such as TNF-α in recent years has proven to be effective in treating ulcerative/inflammatory diseases of the GI tract.

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