MINIREVIEW

Inflammatory Mediators in Gastrointestinal Defense and Injury (44087)

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Abstract. **Irrespective of where ulceration occurs in the gastrointestinal tract, it is almost always associated with mucosal inflammation. The chemical mediators that coordinate inflammatory responses also have the capability to alter the resistance of the mucosa to injury. Some inflammatory mediators increase the resistance of the mucosa to injury, while others exert "ulcerogenic" effects. In this review, we provide an overview of the major inflammatory mediators that have been shown to exert effects on mucosal defense in the gastrointestinal tract. Among the inflammatory mediators discussed are the eicosanoids (prostaglandins, leukotrienes, thromboxanes), nitric oxide, neuropeptides, and cytokines (IL-1β, TNFα). Several of these mediators are considered potential therapeutic targets for the treatment of ulcerative diseases of the digestive system, and, in some cases, clinical data are available on the efficacy of such approaches.** [P.S.E.B.M. 1997, Vol 214]

The discovery of the association between colonization of the stomach by *Helicobacter pylori* and peptic ulcer disease (1) has led to a re-evaluation of the role of inflammation in the pathogenesis of peptic ulcer disease. It is now clear that inflammation, irrespective of where it occurs in the gastrointestinal tract, profoundly influences mucosal integrity and its ability to resist injury induced by luminal factors. The inflammatory response is largely coordinated by a variety of mediators and cytokines that are liberated from the epithelium and from nerves and immunocytes within the lamina propria in response to injury, infection, or exposure to antigen. Several of these mediators can directly influence mucosal integrity by modulating epithelial and vascular permeability as well as blood flow. It is important to remember that inflammation is a physiological response that is usually self-limiting. However, when inflammation is dysregulated, or 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 influence ulcer development is by impairing one or more of the components of mucosal defense. The term "mucosal defense" refers to the combination of factors that allow the mucosa to withstand exposure to substances spanning a wide range of temperature, pH, and osmolarity, to agents with detergent properties (e.g., bile) and to bacterial products capable of eliciting local and systemic inflammatory reactions (2). Rather than being completely resistant to damage induced by the substances we ingest and the endogenous secretions, the mucosa is likely to be injured regularly. However, repair of such damage can occur very quickly, so that breaches of the epithelial barrier do not necessarily permit potentially harmful substances (e.g., bacterial products, toxins) to enter the systemic circulation.

Mucosal defense has been best characterized in the stomach, where a principal concern is protection from

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the damaging effects of acid. The first level of defense consists of the factors secreted into the lumen, including acid, mucus, bicarbonate, and antibacterial substances (e.g., immunoglobulins, lactoferrin). While acid has the potential to damage the gastroduodenal mucosa, it is important to remember that the principal function of gastric acid is a protective one; that is, to reduce the numbers of ingested bacteria entering the small intestine. The second level of defense is the epithelium, which is remarkably resistant to acid-induced injury (3) and acts as a barrier to passive diffusion of potentially harmful substances. Damage to the epithelium is usually repaired very quickly through a process known as "restitution," which involves migration of healthy epithelial cells over the denuded region. The basal lamina acts as a template for this migration. Restitution is observed in response to injury throughout the gastrointestinal tract.

The microcirculation represents the third level of mucosal defense, and one that is significantly modulated by the nervous system. Diffusion of acid or toxins into the mucosa results in a sensory afferent nerve-mediated elevation of mucosal blood flow which 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 mild irritants that would normally be tolerated. A defect of this type also appears to underly the increased susceptibility to gastric mucosal damage in portal hypertension, at least in experimental models (5, 6).

The fourth level of defense is the mucosal immune system, consisting of various immunocytes resident within the lamina propria that act as "alarm cells." Included in this group of cells are the mast cells and macrophages, which are capable of sensing entry of foreign material (e.g., antigens, endotoxin) into the mucosa and responding by releasing an array of mediators that orchestrate an appropriate inflammatory response.

The final level of mucosal defense is called into play when mucosal damage penetrates the muscularis mucosae. In these circumstances, the mucosa is repaired through growth and redevelopment of gastric glands, renewed innervation by the extrinsic and intrinsic nervous systems, and reestablishment of the microcirculation through angiogenesis.

The resistance of the gastrointestinal 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 have been shown to be influenced by inflammatory mediators. In this paper, we review the contribution of a variety of inflammatory mediators to the modulation of mucosal defense and to the pathogenesis of mucosal injury.

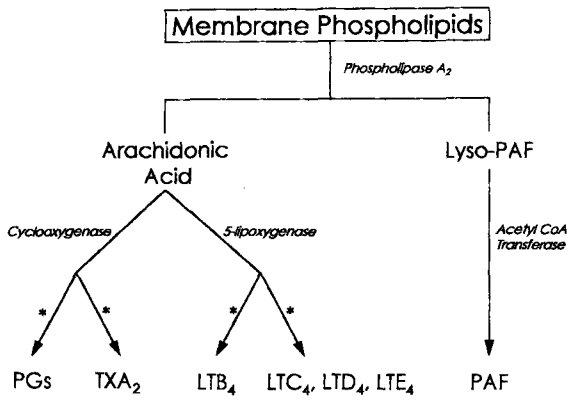

Figure 1. Schematic diagram of the arachidonic acid cascade showing the principal metabolites implicated in the modulation of gastrointestinal mucosal defense and injury. *Enzymes that catalyze the formation of specific subsets of prostaglandins, leukotrienes, and thromboxanes.

Lipid Mediators

Prostaglandins. Prostaglandins are 20-carbon fatty acids produced from arachidonic acid, after liberation from membrane phospholipids, through the actions of the enzyme cyclo-oxygenase (Fig. 1). Like the other eicosanoids (i.e., leukotrienes, thromboxanes), prostaglandins generally have short half-lives (seconds to minutes) and act in an autocrine or paracrine manner. The recognition of the ability of prostaglandins to reduce or prevent gastrointestinal injury induced by topical irritants, or "cytoprotection" (7), resulted in an enormous growth of research into the physiological role of these fatty acids in mucosal defense. It is now well established that suppression of prostaglandin synthesis, through inhibition of cyclo-oxygenase, is a key component of the mechanism underlying gastric ulceration caused by nonsteroidal anti-inflammatory drugs (NSAIDs) (8, 9), as well as the ability of these drugs to exacerbate mucosal injury in more distal parts of the digestive system (10, 11). While the mechanism through which prostaglandins exert their cytoprotective actions has never been firmly established, it is known that these substances can stimulate mucus and bicarbonate secretion, maintain mucosal blood flow, and, through mechanisms that are not yet fully understood, enhance the resistance of epithelial cells to injury induced by cytotoxins (12). Prostaglandins can also exert anti-inflammatory effects, such as through inhibition of leukocyte recruitment (13, 14), which could contribute to the beneficial effects of these substances in situations in which the gastrointestinal mucosa is inflamed. Indeed, it is likely that prostaglandins are one of the molecules that are generated in increased amounts during inflammation and which 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 inhibitors of prostaglandin synthesis (NSAIDs) (11, 15).

As outlined above, the mucosa of the gastrointestinal tract normally contains a substantial number of immunocytes, including mast cells, macrophages, neutrophils, and esosinophils. The numbers of these cells vary considerably along the length of the gastrointestinal tract, and to some extent reflect the luminal bacterial load in each region. As in other external mucosae (e.g., skin, lungs, urogenital tract), some of these immunocytes play an important role in signaling the entry into the lamina propria of foreign material or antigen. These cells typically release soluble mediators and cytokines that initiate an inflammatory response aimed at limiting the entry of the foreign matter into the systemic circulation. These mediators act in a number of ways. Some mediators act on the vascular endothelium to increase permeability (permitting plasma exudation, 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, towards 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.

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, effects of prostaglandins or prostaglandin synthesis inhibitors on tumor necrosis factor (TNF-α) release from macrophages have been very well characterized. Prostaglandin E_2 (PGE_2) has been shown to be a potent suppressor of TNF-α release from macrophages (16), and also reduces expression of the gene for TNF-α in these cells (17). NSAIDs, on the other hand, increase the release of TNF-α from macrophages and other cells (18–20). For example, administration of indomethacin at doses that caused gastric mucosal injury in rats resulted in marked increases in serum TNF-α levels (18, 21). In humans given bacterial endotoxin, prior administration of an NSAID significantly elevated the release of TNF-α into the systemic circulation (20). Prostaglandins also regulate the release of other cytokines, such as interleukin-1, from macrophages (22, 23).

Prostaglandins can also inhibit the release of TNF-α and other mediators from mast cells. For example, Raud *et al.* demonstrated that prostaglandins could partially suppress acute mast cell–dependent inflammation (24), while Hogaboam *et al.* (25), using isolated mast cells from both the peritoneum and the intestinal mucosa, demonstrated that prostaglandin E_2 dose-dependently inhibited the release of platelet-activating factor (PAF), histamine, and TNF-α. These effects were observed at very low concentrations. For example, with the PGE_1 analog, misoprostol, PAF release from peritoneal mast cells was inhibited at concentrations as low as 10^{-10} *M*, while PGE_2 suppressed TNF-α release from peritoneal mast cells at concentrations as low as 10^{-11} *M*.

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. The majority of the research, in this regard, has focused on effects of prostaglandins on neutrophil extravasation. Neutrophils have been implicated as culprits in the damage associated with various disorders of the gastrointestinal tract, including ischemia-reperfusion (26), NSAID gastropathy (27), and colitis (28, 29). As mentioned above, neutrophils are recruited to a site of injury by the chemotaxins released from immunocytes within the lamina propria. The inflammatory response can also be amplified by the infiltrating neutrophils, since these cells have the capacity to release chemotaxins such as leukotriene B_4. Once again, prostaglandins serve an important modulatory role by downregulating several neutrophil functions that contribute to inflammation and injury. For example, prostaglandins can suppress the generation of reactive oxygen metabolites (which account for much of the tissue injury caused by neutrophils) (30, 31) and the release of the chemotaxins, LTB_4, and interleukin-8 (32–34). The observation that NSAIDs increase the numbers of neutrophils adhering to the vascular endothelium, and that this can be prevented by administering exogenous prostaglandins (13, 14), suggests that prostaglandins are important physiological regulators of neutrophil adherence.

The discovery 5 years ago of a second isoform of the cyclo-oxygenase enzyme (35), confirming a theory first suggested in 1972 (36), has led to a reevaluation 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 cyclo-oxygenase, COX-1. On the other hand, prostaglandins produced in the context of inflammation are largely, perhaps exclusively, derived from the inducible isoform of cyclo-oxygenase, COX-2 (37). This theory has been somewhat oversimplified to the following: COX-1 produces prostaglandins that perform beneficial functions, while COX-2 produces prostaglandins that exert detrimental (i.e., pro-inflammatory) effects. This hypothesis underlies the considerable resources being 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. However, there is now considerable evidence emerging

for physiological roles for prostaglandins produced from COX-2, as well as some evidence that prostaglandins produced from COX-1 can contribute to inflammatory responses. Given the evidence that prostaglandins play an important role in limiting inflammatory responses in the gastrointestinal mucosa, some important questions include, Is COX-2 induced in such situations? and Are prostaglandins produced by COX-2 exerting beneficial effects in terms of mucosal defense?

Studies performed recently in our laboratory provide evidence for marked upregulation of COX-2 (protein and mRNA) within the colon of the rat following induction of colitis. Moreover, we found that the majority of prostaglandins produced by the inflamed colon were derived from COX-2. That these prostaglandins were performing a vital function in terms of mucosal defense was confirmed by our finding that selective inhibitors of COX-2 exacerbated the colonic damage in this model of colitis. A manuscript describing these studies has been submitted. These studies support the hypothesis that COX-2 can be induced in order to downregulate an inflammatory response in the gastrointestinal mucosa. The prostaglandins produced from this isoform of COX are also likely to contribute to the healing of ulcers. Indeed, elevated COX-2 expression has been documented in experimental ulcer models, and the prostaglandins produced from this isozyme has been suggested by some studies (38), but not others (39), to contribute to ulcer healing.

Leukotrienes. Like prostaglandins, leukotrienes are synthesized from arachidonic acid. However, in the case of the leukotrienes, the rate-limiting step in their synthesis is the enzyme 5-lipoxygenase (Fig. 1). The leukotrienes can be 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 subclass contains amino acid moieties. Leukotrienes are produced mainly by immunocytes, although there is some evidence for their production by epithelial and endothelial cells as well. In the mucosa, the mast cell appears to be the major source of peptido-leukotrienes, while the neutrophil appears to be the predominant source of leukotriene B_4.

LTB_4 is a very potent chemotaxin, particularly for neutrophils. LTB_4 does not appear to affect vascular permeability or mucosal blood flow, but can promote the recruitment of leukocytes from the vasculature by upregulating expression of the β_2 integrins, CD11/CD18, on those cells. LTB_4 can also stimulate neutrophils to release reactive oxygen metabolites, which could contribute to tissue injury associated with mucosal inflammation. While intra-arterial administration of LTB_4 did not alter the susceptibility of the rat stomach to damage induced by an irritant, LTB_4 has been suggested to contribute to the pathogenesis of NSAID-induced gastric damage (13, 40), most likely through its ability to promote leukocyte adherence to the vascular endothelium. This process has been suggested to be a critical event in the pathogenesis of NSAID-induced gastric damage (27). Receptor antagonists for LTB_4 and inhibitors of 5-lipoxygenase have been shown to attenuate NSAID-induced leukocyte adherence (13) and to reduce the severity of NSAID-induced mucosal damage (41). LTB_4 has also been suggested to mediate, in part, the leukocyte adherence that can be induced by water extracts of cultured *H. pylori* (42).

In the 1980s, a significant body of evidence was generated to support a role for LTB_4 in the pathogenesis of inflammatory bowel disease (IBD). Mucosal production of LTB_4 in human and experimental colitis was found to be markedly increased over that in the normal colon (43–47). Lobos *et al.* (48) demonstrated that LTB_4 accounted for the majority of the chemotactic activity that could be extracted from inflamed colon, while intracolonic administration of LTB_4 was found to exacerbate tissue injury in a rat model of colitis (49). Moreover, treatment of rats with colitis with inhibitors of 5-lipoxygenase resulted in a significant acceleration of healing in experimental colitis (46, 47). Despite these data supporting a role for LTB_4 in IBD, clinical trials of inhibitors of leukotriene synthesis have been very disappointing. Inhibitors that produced a profound suppression of leukotriene synthesis failed to significantly modify disease severity, or produced only modest effects (50, 51).

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 have the capacity to increase the expression of P-selectin on these cells (52), thereby promoting the rolling of leukocytes. When infused intra-arterially, the peptido-leukotrienes can profoundly increase the susceptibility of the rat stomach to injury induced by topical irritants (53). This effect appeared to be related to the vasoconstrictor properties of these substances and could be blocked by pretreating the animals with a leukotriene D_4 receptor antagonist.

The peptido-leukotrienes were recently implicated as mediators of the gastric damage associated with antigenic activation of mucosal mast cells. Administration of an antigen to which rats had previously been sensitized resulted in a significant increase in the extent of damage induced by an irritant applied topically to the mucosa (54). Pretreatment of the rats with a leukotriene D_4 receptor antagonist prevented this augmentation of gastric damage. As mentioned above, mucosal mast cells are likely the predominant source of peptido-leukotrienes in the stomach, and their release in response to antigenic activation of mast cells has been demonstrated (55). Peptido-leukotrienes have also been suggested to mediate the intestinal damage associated with mucosal mast cell activation (56).

There is little evidence supporting a role for peptido-leukotrienes in IBD. There is evidence of marked elevation of peptido-leukotriene synthesis in the inflamed colon (45, 57) and one small clinical trial suggested beneficial effects of a peptido-leukotriene receptor antagonist (58), but these results have not been confirmed or extended.

Thromboxane. Thromboxane is the major arachidonic acid metabolite produced by platelets (*via* cyclooxygenase). The platelet accounts for about 95% of serum thromboxane levels, although neutrophil can also synthesize this eicosanoid (59). Thromboxane is a very potent vasoconstrictor and stimulus for platelet aggregation. As any reduction in mucosal blood flow could potentially render the mucosa more susceptible to injury, it has been suggested that thromboxane may be an important contributor to the pathogenesis of ulceration in the gastrointestinal tract. As thromboxane also has the capacity to stimulate leukotriene B_4 release and the adherence of leukocytes to the vascular endothelium (60), thromboxane may also contribute to mucosal injury through modulation of inflammatory responses.

The first evidence to support this hypothesis came from Whittle and colleagues (61), who showed that close-arterial administration of arachidonic acid into the dog gastric microcirculation resulted in a profound reduction of gastric blood flow. They further demonstrated that this was attributable to generation of thromboxane from arachidonic acid. Subsequently, the same group (62) demonstrated similar effects with a thromboxane mimetic and further demonstrated that the susceptibility of the gastric mucosa to injury could be profoundly increased by administration of this mimetic.

With the development of inhibitors of thromboxane synthesis (blockers of thromboxane synthase), a number of studies were undertaken to determine the contribution of thromboxane to mucosal injury in experimental models of gastric injury. For example, thromboxane synthase inhibitors were shown to reduce the gastric damage induced by bile salts (63, 64), ethanol, and indomethacin (65). However, given that indomethacin itself will block thromboxane synthesis, it is difficult to implicate thromboxane in the pathogenesis of that particular type of injury, suggesting that the thromboxane synthase inhibitor used in that study may have reduced damage through a nonspecific effect. Moreover, Whittle (65) showed that greatly reducing the capacity for thromboxane synthesis, by rendering rats thrombocytopenic, did not alter their susceptibility to damage induced by ethanol or indomethacin. Very few clinical studies have been performed to evaluate the role of thromboxane in ulcer disease. Hawkey *et al.* (66) reported that there were no changes in thromboxane levels in gastric tissue taken from ulcer patients versus controls, irrespective of where the biopsy was taken from (at or removed from the ulcer site) or the presence or absence of inflammation at the biopsy site.

Thromboxane has also been implicated in the pathogenesis of damage in the small intestine. Boughton-Smith *et al.* (67) reported that endotoxin-induced jejunal damage was associated with marked increases in thromboxane and PAF production. Treatment with thromboxane synthase inhibitors markedly reduced both the production of thromboxane and the tissue injury, without affecting PAF synthesis. Using a model of indomethacin-induced small intestinal inflammation and injury, Bannerjee and Peters (68) reported that selective inhibitors of thromboxane synthetase reduced the severity of damage (epithelial permeability) and reduced the extent of granulocyte infiltration into the affected mucosa. However, given that the dose of indomethacin used to induce injury would itself cause a marked suppression of thromboxane synthesis, it is not clear how thromboxane can be implicated in this type of injury. Indeed, it is surprising that with the availability of a number of selective thromboxane receptor antagonists, some of which have been assessed in clinical trials (69), these agents have not been employed to further delineate the contribution of thromboxane to the pathogenesis of gastrointestinal mucosal injury.

In the case of inflammatory bowel disease, 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 and, in particular, in patients with Crohn's disease (70). Moreover, impaired mucosal perfusion, as could occur when a vasoconstrictor like thromboxane is overproduced, has been suggested to be a precipitating event in the pathogenesis of Crohn's disease (71). In a rat model of colitis, mucosal thromboxane symthesis was shown to be markedly elevated (72). Treatment with prednisone or 5-aminosalicylate, which reduced the severity of mucosal injury, also reduced thromboxane production. Moreover, treatment with either or two thromboxane synthetase inhibitors reduced the severity of mucosal injury. The results of trials of a compound, ridogrel, which is both a thromboxane synthetase inhibitor and a thromboxane receptor antagonist, in the treatment of ulcerative colitis have recently been reported in abstract form. Once-daily rectal treatment with ridogrel was found to produce a comparable reduction in the severity of colitis to that achieved with prednisolone (69). In a separate study, treatment with ridogrel for 8 weeks was found to be as effective in relieving the inflammation and symptoms of mild to moderate ulcerative colitis as a standard dose of 5-aminosalicylic acid (73).

Platelet-Activating Factor. Like the eicosanoids, PAF is derived from membrane phospholipids through the action of phospholipases (Fig. 1). PAF can be made

by most types of cells and can exert effects on a number of target cells and organs (74). Among its more potent actions are the ability to modulate smooth muscle tone and the ability to activate neutrophils and 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 (75, 76). At nanomolar doses, PAF caused hemorrhagic lesions in the stomach and intestine (75, 76) and at picomolar doses, predisposed the gastric mucosa to damage induced by topical irritants (77). PAF was also shown to be responsible for much of the tissue injury observed in the gastrointestinal tract following administration of endotoxin at doses sufficient to cause shock (78), or in experimental hemorrhagic shock and ischemia-reperfusion (79–81). In the cases of hemorrhagic shock and ischemia-reperfusion, there is good evidence that mucosal injury was mediated through the ability of PAF to stimulate leukocyte adherence to the vascular endothelium and to activate granulocytes to release reactive oxygen metabolites (79–82). In addition to upregulating expression of β_2 integrins on leukocytes (CD11/CD18), PAF itself can act as an adhesion molecule when it is expressed on the surface of endothelial cells (83). Pretreatment with PAF receptor antagonists has been shown to ameliorate gastrointestinal injury in several models of shock-associated damage (78, 79, 84). Some of the ulcerogenic effects of PAF may be mediated *via* release of other potent ulcerogens, such as the peptidoleukotrienes (85) and thromboxane (67).

PAF is produced in the intestine in large amounts during helminth infections (86). There is some evidence that PAF is derived primarily from mast cells in these circumstances (87), and good evidence that PAF contributes to the epithelial secretion that is aimed at clearing the infection (88). It is also likely that PAF contributes significantly to the marked recruitment of eosinophils into the lamina propria of the infected intestine (86).

PAF has also been implicated in the pathogenesis of disorders of the lower intestine, including neonatal necrotizing enterocolitis (76) and inflammatory bowel disease (89–93). In the case of the latter, PAF has been shown to be produced in increased amounts in the mucosa of humans (89) and animals (90–92) with colitis, as well as in the stool of IBD patients (93). 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 (89). 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 (90–92). It is likely that PAF contributes to mucosal injury in these circumstances through its chemotactic effects and through its ability to stimulate neutrophil release of free radicals and proteases. However, the ability of PAF to influence blood flow, epithelial secretion and smooth muscle tone could contribute to the promotion of tissue injury and the generation of symptoms (i.e., diarrhea) associated with intestinal inflammation (94, 95). With respect to effects of PAF on epithelial secretion, it is noteworthy that the epithelium has been suggested to be a major source of the production of this mediator in ulcerative colitis (96).

Nitric Oxide

As in other areas of physiology and pathophysiology, nitric oxide (NO) has been the subject of extensive studies with respect to its role in the physiology of gastrointestinal (GI) mucosal defense and the pathogenesis of mucosal injury. There remains considerable controversy regarding the predominant role of nitric oxide in the GI tract: protective or damaging. This has recently been reviewed in the form of a debate (97, 98). There can be little doubt that nitric oxide can influence gastrointestinal mucosal integrity. For example, nitric oxide has been shown to influence mucus secretion, mucosal blood flow, and enteric nerve function, all of which can have impact on resistance to injury. Suppression of nitric oxide synthesis renders the gastric mucosa more susceptible to injury (99), while administration of nitric oxide donors can protect the stomach from injury (100). Indeed, the latter finding led to the development of a series of nitric oxide–releasing NSAIDs which do not cause gastrointestinal damage (101–103). Interestingly, elevation of mucosal nitric oxide synthesis through administration of low doses of endotoxin has been shown to increase the resistance of the stomach to damage induced by irritants (104).

Like the prostaglandins, nitric oxide can also influence mucosal defense and injury through its ability to modulate the activity of mucosal immunocytes and by modulating acute inflammatory reactions (Fig. 2). For example, there is excellent experimental evidence supporting a critical role for nitric oxide in modulating mast cell reactivity, leukocyte-endothelial interactions, and intestinal epithelial permeability. Neutrophil adherence to the vascular endothelium following blockade of nitric oxide synthesis was first described by Kubes *et al.* (105), who studied this phenomenon in mesenteric post-capillary venules. The initial interpretation of this observation was that removal of a tonically produced mediator (nitric oxide) which was capable of inhibiting neutrophil function led to neutrophil adherence. However, subsequent studies which included careful study of the cells surrounding mesenteric venules led to the finding that mast cells became activated following NO synthesis blockade and contributed to the adherence of neutrophils to the neighbouring endothelium (106, 107). Interestingly, blockade of nitric oxide synthesis resulted in significant changes in intestinal epithelial barrier function (107, 108), which could be inhibited by pretreating the rats with mast cell stabilizers or receptor antagonists

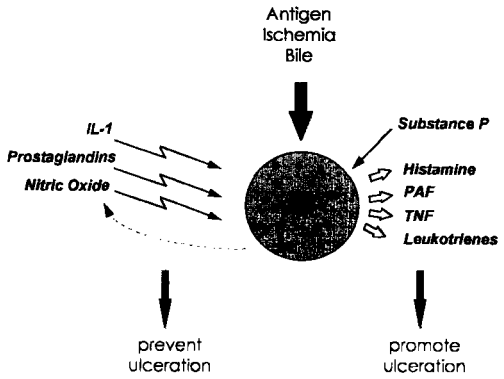

Figure 2. Schematic diagram showing some of the mediators that can be released by the mucosal mast cell which can influence mucosal defense and/or injury. Activation of the mast cell can occur *via* immunological (antigen), neural (substance P), or nonimmunological (ischemia, bile, others) means. Platelet-activating factor (PAF) and leukotrienes are newly synthesized upon activation, while tumor necrosis factor (TNF) and histamine are pre-formed (stored) within granules in the mast cell. Interleukin will inhibit the release of PAF through a nitric oxide–dependent pathway. Nitric oxide and prostaglandins are also capable of suppressing the release of PAF, TNF, and the leukotrienes. The mucosal mast cell spontaneously produces nitric oxide. Suppression of this basal production of nitric oxide leads to enhanced reactivity of the mast cell.

for histamine (H-1) and PAF, both of which can be released by mast cells.

The ability of nitric oxide to modulate mast cell reactivity was further demonstrated in *in vitro* studies performed by Hogaboam *et al.* (109). Mast cells spontaneously release nitric oxide. When exposed to interleukin-1, a profound and rapid increase in nitric oxide release was observed. Moreover, the release of nitric oxide appeared to exert feedback inhibition of PAF release from the mast cell, consistent with the findings of Kanwar *et al.* (107), which suggested that blockade of nitric oxide synthesis led to release of PAF. Others have demonstrated that exposure of mast cells to exogenous nitric oxide leads to a diminution of histamine release (110).

As alluded to above, the role of nitric oxide 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 nitric oxide as a contributor to mucosal injury has been most extensively characterized in experimental models of colitis. Nitric oxide 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 IBD (111). There is also evidence of peroxynitrite formation within the intestinal mucosa in other models of colitis (112). That NO contributes to tissue injury in colitis is supported by several studies demonstrating that administration of NO synthase inhibitors reduces the severity of colonic damage and inflammation (112–114). However, 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 (115).

Neuropeptides

The peptide mediators released from both extrinsic and intrinsic nerves within the gastrointestinal tract exert effects on virtually every component of the function of this organ. In this section, we will briefly review these roles of neuropeptides only as they pertain to inflammatory responses in the gut, and the impact this has on mucosal defense and injury.

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 from sensory afferent nerves of calcitonin gene-related peptide (CGRP), which through a nitric oxide–dependent pathway leads to dilation of submucosal arterioles. The resulting increase in mucosal blood flow helps to dilute and remove any back-diffusing toxin and neutralize back-diffusing acid. While best characterized in the stomach, it is important to note that the hyperemic response to irritants occurs throughout the gastrointestinal tract. In animals in which this pathway has been impaired, such as through ablation of sensory afferent nerves with capsaicin, the mucosa cannot mount a hyperemic response, and exposure to mild irritants results in the development of mucosal necrosis (4, 116). While conventionally regarded as a strictly 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 (Fig. 2). In addition to releasing CGRP, sensory afferent nerves in the gastrointestinal mucosa also release substance P. These nerves are found in close apposition to mucosal mast cells (117). Interestingly, substance P has been shown to cause histamine release from mucosal mast cells (118). In normal rats, a significant contribution of histamine to the gastric hyperemic response to topically applied acid or capsaicin cannot be detected (119). However, when rats with mastocytosis were studied, the hyperemic response to the irritants was significantly enhanced over that seen in normal rats, and this increase was abolished by pretreatment with histamine H_1 receptor antagonists or mucosal mast cell stabilizers (119). 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 then contributes to the protective mucosal hyperemic response. It is important to note that the mucosal hyperemic response also contributes significantly to the repair of ulcers. Blood flow to the ulcer margin, where healing

occurs, is markedly elevated above that in the surrounding tissue. While not yet studied in detail, it is conceivable that in a setting of mucosal inflammation surrounding an ulcer other inflammatory mediators and immunocytes may alter or contribute to the hyperemic response and may therefore influence ulcer healing.

Neuropeptides and the effects they exert on mucosal immunocytes may also influence acute inflammatory responses within the gastrointestinal tract. 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 (120). Substance P can also promote neutrophil recruitment into the lamina propria, both by directly upregulating endothelial adhesion molecule expression (121) and through the ability of this neuropeptide to activate mast cells, as discussed above (122).

Cytokines

Other than *in vitro* studies showing regulation by numerous cytokines of the production of other inflammatory mediators (e.g., prostaglandins, nitric oxide), there is very little information available on the roles of most cytokines in regulating mucosal integrity. However, two cytokines have been extensively characterized in this regard: interleukin-1β (IL-1β) and TNF-α. 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 (123).

While IL-1 has the capacity to increase expression of adhesion molecules on the vascular endothelium and to stimulate the release of other chemotaxins, most evidence to date, at least with respect to the upper gastrointestinal tract, suggests that IL-1 increases resistance to injury. In addition to being able to inhibit gastric acid secretion (124–128), at least partly through centrally mediated actions (128), IL-1 also can markedly reduce the severity of gastroduodenal damage in several models (124, 127, 129, 130). While the mechanisms responsible for the protective actions of IL-1 are not fully understood, this cytokine may, in the case of NSAID-induced gastric damage, reduce injury through a paradoxical inhibitory action on leukocyte adherence (127). IL-1 may also reduce gastroduodenal injury through its ability to stimulate prostaglandin and nitric oxide release (it can induce the enzymes responsible for synthesis of these mediators) and to inhibit the release of other ulcer-promoting mediators (e.g., PAF, histamine) from mast cells (109, 110) (Fig. 2). Endogenous IL-1, induced through administration of low doses of endotoxin, has also been suggested to exert protective effects in the stomach through a nitric oxide–dependent mechanism (131).

TNF-α has been implicated as 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 NSAIDs, there is convincing data from animal models demonstrating that TNF-α release into plasma is markedly elevated by NSAIDs and that blockade of TNF-α synthesis results in attenuation of the damaging effects of NSAIDs in the stomach (18, 21). Prostaglandins are potent inhibitors of TNF-α release from both the macrophage (16, 17) and the mast cell (25), and this may account in part for the ability of prostaglandins to reduce the severity of NSAID-induced gastric injury.

TNF-α has also been suggested to be a key mediator of the intestinal damage induced by endotoxin (132, 133), and this may be in part mediated by generation of PAF (134). One of the mechanisms through which TNF-α can amplify inflammatory responses and tissue injury is through its ability to regulate expression of receptors for other inflammatory mediators, including LTB_4 and PAF (135).

In recent years there has been considerable interest in the potential role of TNF-α as a mediator of tissue injury in inflammatory bowel disease (IBD), as well as in the potential for TNF-α as a therapeutic target. For example, TNF-α levels in plasma (136) and stool (137) 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 (138). Moreover, impressive data suggesting beneficial effects of treatment of Crohn's disease with an anti-TNF antibody have recently been reported (139). This clinical trial needs to be confirmed with a larger sample of patients, and further studies are necessary to elucidate the mechanism through which TNF-α contributes to inflammation and tissue injury in IBD.

Conclusions

The discovery of an association between infection with *H. pylori* and peptic ulcer disease, along with the discovery of the remarkable ability of prostaglandins to protect the gastrointestinal tract against injury, had led to a re-evaluation of the factors that contribute to "mucosal defense." In light of the close association between mucosal inflammation and mucosal ulceration, particular emphasis has been placed on the impact that a number of inflammatory mediators have on mucosal defense. Several of these mediators, such as PAF, the peptidoleukotrienes, and TNF-α, are considered as ulcerogens, since they either directly cause mucosal injury, or increase the susceptibility of the mucosa to injury induced by luminal irritants. Other inflammatory mediators, such as nitric oxide and IL-1β, can prevent mucosal damage induced by irritants and/or counteract the ac-

tions or production of ulcerogenic mediators. Identifying the key mediators responsible for reducing mucosal defense during inflammatory reactions may help in the development of novel therapies for the treatment of ulcerative diseases of the gastrointestinal tract.

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