MINIREVIEW

Gastrointestinal Inflammation: A Central Component of Mucosal Defense and Repair

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The mucosal layer of the gastrointestinal (GI) tract is able to resist digestion by the endogenous substances that we secrete to digest foodstuffs. So-called "mucosal defense" is multifactorial and can be modulated by a wide range of substances, many of which are classically regarded as inflammatory mediators. Damage to the GI mucosa, and its subsequent repair, are also modulated by various inflammatory mediators. In this article, we provide a review of some of the key inflammatory mediators that modulate GI mucosal defense, injury, and repair. Among the mediators discussed are nitric oxide, polyamines, the eicosanoids (prostaglandins and lipoxins), protease-activated receptors, and cytokines. Many of these endogenous factors, or the enzymes involved in their synthesis, are considered potential therapeutic targets for the treatment of diseases of the digestive tract that are characterized by inflammation and ulceration. Exp Biol Med 231:130–137, 2006

Key words: cyclooxygenase; lipoxins; prostaglandins; nitric oxide; ulcer disease; inflammation; hydrogen sulfide; annexin

Introduction

Inflammation is often considered as a harmful process that should be avoided. However, inflammation is a homeostatic response aimed at limiting entry of foreign materials to the body and of facilitating repair. In the gastrointestinal (GI) tract, the inflammatory process is a key component of mucosal defense against exogenous and endogenous factors. Impairment of this response can lead to mucosal injury and to impairment of repair processes. Of course, dysregulated inflammatory responses can greatly worsen injury in the GI tract and can contribute to the generation of symptoms. The inflammatory response is coordinated, to a large extent, by an array of mediators that are released from the epithelium and from cells within the lamina propria (e.g., mast cells, lymphocytes, neurons, and fibroblasts).

Inflammatory mediators can alter mucosal integrity by influencing the various components of "mucosal defense"; that is, the 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 microbes (1). The components of mucosal defense include the factors secreted into the lumen, such as acid (in the stomach), mucus, bicarbonate, and antibacterial substances (e.g., immunoglobulins and lactoferrin). The epithelium acts as a barrier to the passive diffusion of harmful substances. When damaged, epithelial repair can occur very quickly *via* migration of healthy epithelial cells from the gastric pits over the denuded region ("restitution"; Ref. 2).

The microcirculatory response of the mucosa is possibly the most important component of mucosal defense. It is modulated by the extrinsic and intrinsic nervous systems and by an array of inflammatory mediators. When toxins (including gastric acid) diffuse into the mucosa, there is a profound and rapid increase in mucosal blood flow. This is mediated *via* extrinsic primary afferent nerves, which release both calcitonin gene-related peptide (CGRP) and substance P in the vicinity of submucosal arterioles, resulting in vasodilation (3). The increase in blood flow acts to dilute and neutralize the toxin, as well as to prevent

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the toxin from accumulating within the mucosa to cytotoxic concentrations. The importance of this vascular response is evident from experiments in which ablation of capsaicin-sensitive primary afferent nerves or pretreatment with CGRP/substance P antagonists abolished the reactive hyperemic response, rendering the mucosa more susceptible to injury (4, 5). Thus, the release of CGRP and substance P from extrinsic afferents produces gastroprotective effects by increasing mucosal blood flow and inhibiting acid secretion within the GI tract. An inadequate reactive hyperemic response seems to underlie the increased susceptibility of the gastric mucosa to damage in experimental models of portal hypertension (6, 7).

In this review, we summarize the roles of several of the most important groups of inflammatory mediators that contribute to mucosal defense and repair, with a particular focus on those mediators that have been the subject of investigations in recent years.

Mediators Derived from Cyclooxygenase (Cox): Prostaglandins

Prostaglandins are 20-carbon fatty acids produced from arachidonic acid *via* the enzyme, Cox. Similar to the other eicosanoids (i.e., leukotrienes and thromboxanes), prostaglandins generally act in an autocrine or paracrine manner and have short half-lives (seconds to minutes) in the circulation. In 1972, John Vane *et al.* reported that suppression of prostaglandin synthesis was a major mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs; Ref. 8). He also proposed that this could be a mechanism underlying the ability of these drugs to cause ulcers and bleeding in the GI tract (8). The importance of these discoveries was recognized in 1982 by the award of the Nobel Prize to Vane. In the mid-1970s, Andre Robert *et al.* (9) were the first to describe the ability of exogenous prostaglandins to greatly reduce GI injury induced by topical irritants; a phenomenon he called "cytoprotection." This led to extensive research into the role of this family of lipid mediators in mucosal defense and repair. Prostaglandins modulate many of the components of mucosal defense: they stimulate mucus and bicarbonate secretion, elevate mucosal blood flow, increase the resistance of epithelial cells to injury induced by cytotoxins (10), and suppress the recruitment of leukocytes into the mucosa (11, 12). Prostaglandins can also downregulate the release of a number of other inflammatory mediators that have been suggested to contribute to the generation of mucosal injury in certain circumstances. For example, prostaglandin E_2 has been shown to be a potent inhibitor of tumor necrosis factor (TNF)-α and interleukin (IL)-1 release from macrophages (13–15) and of leukotriene B_4 and IL-8 release from neutrophils (16–18).

In the early 1990s, the existence of two isoforms of Cox (Cox-1 and Cox-2) was confirmed (19). The observation that Cox-2 expression is low in the GI tract of healthy humans and animals (20–22) contributed to the widely accepted, but likely incorrect, notion that Cox-2 played little, if any, role in mediating physiologic events in the GI tract. There is substantial evidence that Cox-2 plays an important role in mucosal defense (Fig. 1; see Ref. 23 for review). Moreover, it is established that NSAID-induced GI injury is a consequence of inhibition of both Cox-1 and Cox-2 (24). Selective inhibition of either of these isoforms, in the absence of other factors that predispose the mucosa to injury (e.g., ischemia) does not result in hemorrhagic erosion or ulcer formation in the stomach or small intestine (24–27).

Although basal expression of Cox-2 is low in the GI tract, it is not absent. Moreover, the expression of Cox-2 is rapidly increased in response to a number of stimuli. For example, a marked increase in Cox-2 expression in the rat stomach was detected as early as 1 hr after administration of aspirin or indomethacin (21). Pretreatment with prostaglandin E_2 prevented the induction of Cox-2 expression by aspirin. An increase in the expression of Cox-2 in the rat small intestine occurs after selective inhibition of Cox-1 (26). These findings suggest that the trigger for increased Cox-2 expression is the diminished mucosal prostaglandin levels (21). Perhaps the rapid upregulation of Cox-2 expression in response to aspirin or to a selective Cox-1 inhibitor represents a compensatory response to inhibition of gastric prostaglandin synthesis. Indeed, it is likely that rapid Cox-2 induction is a common response to luminal irritation aimed at enhancing mucosal resistance to injury.

Cox-2–derived prostaglandins also make an important contribution to the repair of ulcers. Such repair involves formation of granulation tissue at the ulcer base, formation of new blood vessels (angiogenesis), and reestablishment of the glandular architecture. Cox-2 is strongly expressed in cells at the ulcer margin (28), which is where epithelial proliferation primarily occurs, allowing for reestablishment of glands. Cox-2 is also strongly expressed in endothelial cells in the ulcer bed (28), which is the site of new vessel growth. Administration of selective Cox-2 inhibitors to rats or mice with gastric ulcers results in a significant delay in ulcer healing (28–32).

Mediators Derived from Cox: Lipoxins (LXs)

Concurrent administration of aspirin and a selective Cox-2 inhibitor results in significantly greater gastric damage than that produced by either drug alone. This synergistic interaction has been observed in rodents (33–35) and humans (36, 37). An initial interpretation of these findings was that combined inhibition of Cox-1 and Cox-2 would produce more gastric damage because prostaglandins from both isoforms of Cox contribute to mucosal defense (24). However, it now seems that a Cox-2–derived metabolite of arachidonic acid may explain this interaction. Aspirin acetylates a serine residue in Cox-2, rendering the enzyme inactive in terms of the conversion of arachidonic

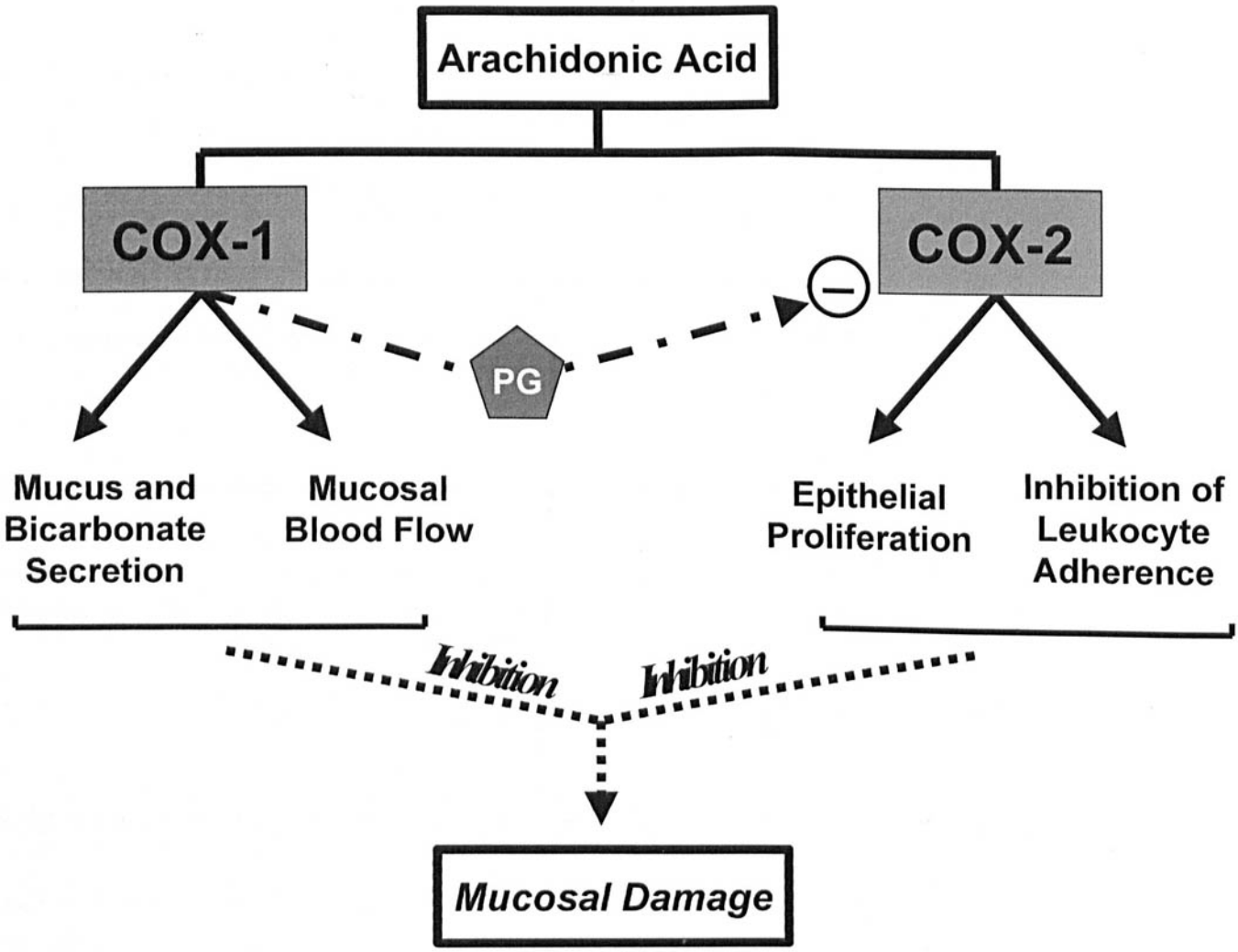

Figure 1. Schematic diagram illustrating some of the roles of Cox-1 and -2 in mucosal defense. Prostaglandins derived from Cox-1 contribute to mucosal defense in many ways, including stimulation of mucus and bicarbonate secretion and regulation of mucosal blood flow. Prostaglandins from Cox-2 also contribute to mucosal defense, such as by regulating epithelial proliferation and suppressing adherence of leukocytes to the vascular endothelium. Cox-1–derived prostaglandins (PG) seem to downregulate expression of Cox-2 in the GI mucosa. When both Cox-1 and Cox-2 are inhibited (but not when only one isoform is inhibited), mucosal defense is impaired to the extent that hemorrhagic lesions form.

acid to prostaglandins. However, acetylated Cox-2 is still capable of converting arachidonic acid to 15-*R*-hydroxyeicosatetraenoic acid (15-*R*-HETE). This product can then be converted *via* 5-lipoxygenase (mainly in neutrophils) to 15-*epi*-LXA_4 (or "aspirin-triggered LX" [ATL]; Ref. 38). ATL and LXA_4 have similar biological actions, including a range of anti-inflammatory effects that include suppression of many of the functions of neutrophils (38).

Like prostaglandins, LXA_4 has potent protective effects in the stomach. For example, our studies have shown that LXA_4 reduces the extent of aspirin-induced gastric damage in rats when administered parenterally in the low nanomolar range (33). These effects may be, in part, attributable to the ability of LXA_4 to suppress aspirin-induced leukocyte adherence within the gastric microcirculation (33). The importance of LXA_4 as an endogenous gastroprotective factor was further demonstrated by the observation that antagonism of the LXA_4 receptor significantly exacerbated aspirin-induced gastric damage (33). Co-administration of a selective Cox-2 inhibitor with aspirin blocked the elevated formation of ATL, confirming that Cox-2 activity is required for its synthesis. This is associated with a significant increase in the severity of gastric damage (33–35). Thus, administration of aspirin results in the suppression of the synthesis of a family of mediators that are crucial to mucosal defense (prostaglandins), but at the same time, triggers the formation of a mediator (ATL) that can exert many of the same protective actions as prostaglandins. When Cox-2 is inhibited concurrently with these effects of aspirin, ATL synthesis is blocked, leading to more extensive mucosal injury.

Nitric Oxide (NO)

Oxidization of arginine by NO synthase (NOS) creates the volatile gas NO, which has numerous physiologic properties pertinent to the regulation of inflammation (39).

Three distinct isoforms of NOS have been characterized. Two are constitutively expressed, calcium-dependent isoforms: neuronal NOS (nNOS or NOS-1) and endothelial NOS (eNOS or NOS-3). One is an inducible, calcium-independent isoform: inducible NOS (iNOS or NOS-2).

The importance of NO in GI mucosal defense is well established (39). Studies with selective inhibitors of the constitutive forms of NOS, nNOS and eNOS, have helped to delineate the roles of NO in various processes in the GI tract. Interestingly, the actions of NO overlap considerably with those of prostaglandins: modulation of the activity of mucosal immunocytes (e.g., mast cells and macrophages), reduction of leukocyte-endothelial adhesive interactions, modulation of mucosal blood flow, reduction of epithelial permeability, stimulation of mucus, and bicarbonate secretion (40). NO has proven to be the primary nonadrenergic-noncholinergic neurotransmitter in the GI tract (41). Not surprisingly, therefore, inhibition of eNOS and/or nNOS results in disturbances of GI blood flow, motility, and secretion. NO also contributes to mucosal defense through its cytotoxic properties, a primary defense against ingested bacteria and parasites (42).

In the stomach, suppression of NO synthesis renders the mucosa more susceptible to injury (43), whereas administration of NO donors can protect the stomach from injury (44). Indeed, the latter finding led to the development of a series of NO-releasing anti-inflammatory drugs, with greatly reduced GI toxicity relative to the parent drugs (45–48).

Although there is clear evidence for an important role of NO in mediating mucosal defense under normal conditions, the situation is much more complex in circumstances in which the mucosa is inflamed or damaged. Some studies suggest that NO contributes to tissue injury, whereas others suggest that it acts in a protective manner. 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 colitis (49). The observation of tyrosine nitration being colocalized with iNOS immunoreactivity in the inflamed colon adds substance (although not proof) to the suggestion that iNOS may be responsible for tissue injury *via* the formation of peroxynitrite (50). The hypothesis that NO contributes to tissue injury in colitis is further supported by several studies demonstrating that NOS inhibitors can reduce the severity of colonic damage and inflammation (50–52). On the other hand, agents that release NO in small amounts over a prolonged period have been shown to greatly reduce inflammation and to accelerate healing in experimental colitis (53). At least some of the discrepancy in results from one study to another is related to differences in selectivity of NOS inhibitors for one or more of the various isoforms of NOS. Although the role that NO plays during inflammation is contentious, most studies would suggest that there is a net protective effect of this molecule in the GI tract (39).

Polyamines

The initiation of the repair phase of the inflammatory response is accompanied by an increase in polyamine synthesis. Polyamines are initially produced after the conversion of arginine to ornithine by the enzyme arginase. Ornithine can then be converted to the pro-proliferative polyamines *via* ornithine decarboxylase (ODC), the first rate-limiting enzyme in the strictly controlled polyamine biosynthetic pathway (54). The initiation of biosynthesis of cationic higher polyamines (putrescine, spermidine, and spermine) by ODC has proven to be an important stimulus for cell proliferation. Under homeostatic conditions, cells contain putrescine, spermidine, and spermine in millimolar concentrations. Increases in intracellular polyamine levels and in ODC activity are associated with rapid growth rates. They are also considered essential for life, because inhibitors of polyamine biosynthesis block cell growth (55, 56).

There is considerable evidence for a key role of polyamines in mucosal repair. In rats, ODC activity in the mucosa is increased after small-bowel resection (54, 57), parasitic or enteropathogenic bacteria–induced small intestinal inflammation or colitis (58, 59), ischemia reperfusion (60), and after partial obstruction of the lumen. Mucosal ODC activity in the colon and rectum has been reported to be significantly elevated in ulcerative colitis and Crohn's disease patients (61).

Polyamines have also been shown to be protective of DNA during the S-phase of the growth cycle (56, 62) and, in part, may explain the protective effects of trophic peptides (epidermal growth factor and glucagon-like peptide-2) after the administration of chemotherapeutic agents (63, 64). Considering that the trophic effects of many growth-promoting GI hormones are blocked after ODC or polyamine synthesis inhibition, it would not be surprising if polyamines are involved as a downstream effector of induced intestinal growth.

Proteinase-Activated Receptors (PAR)

PAR represent a distinct subclass of G-protein–coupled receptors that are activated by proteinase cleavage at a specific site in the extracellular NH_2-terminus. This results in exposure of a new N-terminal domain of the receptor, which acts as a tethered ligand, binding and activating the receptor (65). Four PARs have been cloned to date. PAR-1, PAR-3, and PAR-4 can be activated by thrombin, whereas PAR-2 can be activated by trypsin or human mast cell tryptase. Neutrophil-derived elastase (66) and matrix metalloproteases (67), derived from many types of cells, are also capable of activating PARs.

PARs are expressed on the surface of many cells, but those on the platelet have been the most thoroughly characterized. PAR-2 is expressed throughout the GI tract, including on epithelial cells and sensory afferent neurons (65). Vergnolle *et al.* (68) described a PAR-2–like receptor

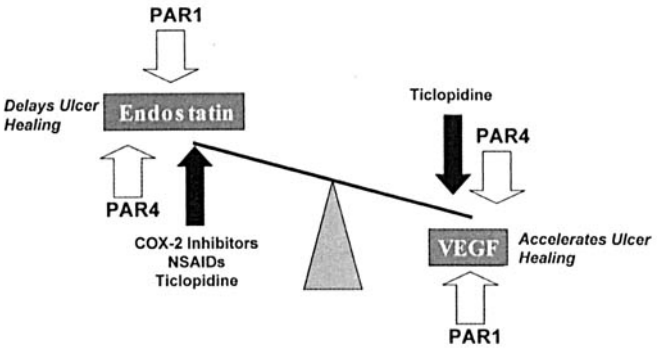

Figure 2. The angiogenic balance and its modulation by PARs and certain drugs. New blood vessel growth (angiogenesis) is a key element of healing, and is regulated by both proangiogenic and antiangiogenic factors (e.g., VEGF and endostatin, respectively). Both of these factors are contained within platelets and can influence healing of gastric ulcers. Drugs such as selective Cox-2 inhibitors, NSAIDs, and ticlopidine can shift the ration of VEGF to endostatin in platelets and in serum, such that inhibition of angiogenesis (thus, healing) is favored. PARs play an important role in regulating release of these factors from platelets and can thereby affect ulcer healing. Activation of PAR-1 results in stimulation of VEGF release from platelets, and inhibition of endostatin release (pro-healing). In contrast, activation of PAR-4 results in stimulation of endostatin release and inhibition of VEGF release (anti-healing).

in the intestine, the activation of which leads to epithelial chloride secretion. In the stomach, activation of PAR-2 (using selective PAR-2–activating peptides) triggered secretion of mucus (69). This effect was mediated *via* sensory afferent nerves, because ablation of those nerves (through previous treatment with capsaicin) abolished the effect. Administration of a PAR-2–activating peptide also greatly reduced the extent of injury in the stomach induced by ethanol or indomethacin (69). However, the mechanism of action for this effect is not yet clear, although the role of sensory afferent nerves is likely important, given the importance of those nerves in regulating mucosal blood flow.

Protection of the gastric mucosa from injury induced by ethanol was also observed after intravenous administration of a PAR-1 agonist. As in the case of PAR-2–mediated gastroprotection, this effect was abolished by previous ablation of sensory afferent nerves (with capsaicin; Ref. 70). An effect of the PAR-1 agonist on mucosal blood flow is further suggested by the observation that this agent produced an increase in gastric mucosal blood flow.

In the past 5 years, several studies from our laboratory have suggested an important role of platelets in gastric ulcer healing, and a potential contribution of PARs to this process. We demonstrated that rats that had been immunodepleted of their circulating platelets exhibited delayed gastric ulcer healing relative to normal rats (71). Transfusion of platelets from normal rats into the thrombocytopenic rats restored normal rates of gastric ulcer healing (71). The ability of platelets to contribute to ulcer healing is largely attributable to the many growth factors that they can release at sites of injury. For example, platelets can promote new blood vessel growth (angiogenesis) through the release vascular endothelial growth factor (VEGF). On the other hand, platelets also contain a potent antiangiogenic factor, endostatin. Whether the platelet acts to promote or inhibit angiogenesis depends on the relative content of these proangiogenic and antiangiogenic factors, and on the rates of release of one compared with the other (Fig. 2). PARs may play an important role in the latter. PARs act as the key receptors mediating the proaggregatory and prosecretory effects of thrombin on platelets. Recent studies suggest that PAR-1 and PAR-4 act in a counter-regulatory manner in terms of the release from platelets of VEGF and endostatin. Thus, activation of PAR-1 leads to release of VEGF and inhibition of release of endostatin (72). Activation of PAR-4 leads to release of endostatin and inhibition of release of VEGF (59). The relative activation of PAR-1 versus PAR-4 can, therefore, regulate angiogenesis and healing. Consistent with this notion, we observed that oral administration of a PAR-1 antagonist significantly impaired ulcer healing in rats (72).

Although PARs can regulate the release of growth factors from platelets, other drugs seem to be able to shift the content of certain growth factors within platelets, and, in doing so, can also influence ulcer healing. As mentioned previously in the section entitled "Mediators Derived from Cyclooxygenase (Cox): Prostaglandins," NSAIDs and selective Cox-2 inhibitors can delay ulcer healing. We observed that NSAIDs and selective Cox-2 inhibitors result in a decrease in the ratio of VEGF to endostatin (31), which is consistent with delayed ulcer healing (Fig. 2). We also observed that treatment of rats with ticlopidine resulted in a relative increase in endostatin to VEGF within platelets and serum, as well as an impairment of ulcer healing (31).

PARs have recently been implicated in the regulation of antimicrobial peptide expression in epithelial cells. Chung *et al.* (73) demonstrate that expression of peptides of the β-defensin family, which are produced by crypt epithelial cells in the intestine and play an important role in mucosal defense against luminal microbes, was dependent on the presence of bacterially derived proteases. Moreover, these proteases induced expression of the β-defensin through activation of PAR-2.

Cytokines

Cytokines play a central role in the regulation of the mucosal immune system, and are, therefore, 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 inflammatory bowel disease. The role of cytokines in GI inflammation and repair of injury has recently been reviewed in detail (74).

IL-1β and TNF-α are released early in an inflammatory reaction. They contribute to the systemic response to inflammation or infection, such as the acute phase response.

reduce appetite, and participate in the generation of fever (75). Various types of cells produce IL-1β, including monocytes, macrophages, neutrophils, endothelial cells, and fibroblasts (76). An endogenous IL-1 receptor antagonist (IL-1ra) is produced by many of the same cells that produce IL-1β, and a recombinant form of this antagonist has been shown to inhibit many of the biological activities of IL-1β *in vitro* and *in vivo* (76).

Regarding the upper GI tract, the administration of IL-1β has been shown to both increase the resistance to injury and to reduce the severity of gastroduodenal damage in several experimental models (77–79). The mechanisms 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 (77). Substantial evidence reveals that IL-1β is a potent inhibitor of gastric acid secretion (77, 78, 80–82), at least partly through centrally mediated actions (82), and is capable of inducing Cox-2 (83) and iNOS expression (84). Thus, IL-1β might 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., platelet-activating factor) from mast cells (85).

TNF-α seems to be a key contributor to many forms of gastric mucosal injury, including that associated with *Helicobacter pylori* infection and the use of NSAIDs. Regarding the latter, plasma TNF-α levels are markedly increased after the administration of NSAIDs, and that inhibition of TNF-α synthesis results in an attenuation of the damaging effects of these drugs in the stomach (86, 87). Interestingly, prostaglandins, which can greatly reduce the severity of NSAID-induced gastric damage, are potent inhibitors of TNF-α release from both the macrophage (13) and the mast cell (88). NO may also exert its protective effects in the stomach, in part, through modulation of cytokine production. One of the ways in which TNF-α contributes to mucosal injury induced by NSAIDs is through activation of caspases, which can trigger apoptosis. NO can inhibit activation of these caspases through S-nitrosylation. This has been suggested as a major reason that NO-releasing aspirin does not produce significant damage in the stomach of animals or humans (47, 89–91).

Conclusions

During the past 30 years, studies aimed at better understanding the pathogenesis of ulcer disease have shifted their focus from the factors that can damage the mucosa to the endogenous factors that make the mucosa resistant to injury. It has become increasingly apparent that the acute inflammatory response is an essential element of mucosal defense. Exposure to an irritant results in an almost immediate increase in blood flow, and to changes in vascular permeability and leukocyte-endothelial adhesive interactions. Immunocytes within the mucosa rapidly release chemicals that mediate these effects. The identification of some of the key chemical mediators involved in mucosal defense (e.g., prostaglandins and NO) has allowed for the development of novel agents that can protect the GI tract.

Inflammation is also an important element of the process of ulcer healing in the GI tract. As a better understanding is gained as to how specific inflammatory mediators contribute to this process, novel therapies that can accelerate healing will undoubtedly be developed. Moreover, it may be possible in the future to improve the quality of ulcer healing, such that recurrences or bleeding of ulcers can be greatly reduced or prevented altogether.

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