Mucosal Immunity and Inflammation
V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense*

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Podolsky, Daniel K. Mucosal Immunity and Inflammation. V. Innate mechanisms of mucosal defense and repair: the best offense is a good defense. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G495–G499, 1999.—Well-coordinated mechanisms have evolved that provide both innate protection against gastrointestinal mucosal injury and facilitation of rapid mucosal repair following mucosal damage. Generic protection from injury is provided by intrinsic structural features of the epithelium that form a highly competent barrier and a complex formed at the apical surface by trefoil peptides that comprise the interface between mucosa and lumen. When the epithelial barrier has been broken, regardless of the nature of the injury, epithelial surface continuity is rapidly reestablished through restitution as cells migrate and elongate. This process is promoted by trefoil peptides at the apical surface and a large array of cytokines and growth factors acting at the basolateral pole. Many of these regulatory peptides are products of the immune and other lamina propria cell populations, which are activated following disruption of the mucosal barrier. Thus efforts to repair the epithelium follow inherently from inflammatory effects after initial damage; the repair process in turn may allow abrogation of further inflammation. Ultimate repair of injury requires both proliferative replacement of damaged epithelial cells and remodeling of extracellular matrix and deeper cell populations to restore normal architecture and a fully functional mucosa.

growth factors; restitution; cytokines; epithelial barrier; trefoil proteins

ALTHOUGH AN IMMUNE SYSTEM capable of responding selectively and specifically is critical in defense against the myriad potential threats confronting the gastrointestinal tract, this system is largely activated after the integrity of the mucosal surface has been breached in some manner, via either direct destruction or invasion of bacteria through the epithelial surface. However, mechanisms that confer primary protection of the mucosal surface may be even more fundamental and evolutionarily ancient. Studies over the past several years have provided an insight into the key intrinsic processes that protect the mucosa and that appear distinct from conventional mechanisms of innate and specific immunity. At the same time, it has become clear that the same factors that protect the mucosa from injury also play a critical role in facilitating repair of the epithelium after injury has occurred.

The barrier formed by the intestinal epithelium as a result of tight junctions is the cornerstone of intrinsic mechanisms protecting the underlying elements of the intestinal mucosa from luminal agents. Significant progress has been made in recent years in defining key constituents of the complex junctional structures that result in a tight barrier (8). These include occludin, cingulin, ezrin, zonula occludens (ZO)-1, and ZO-2 as well as additional proteins that form the tight junctions and other subjunction components, including claudins. These junctions may be regulated in a dynamic fashion through modulation of the actin-myosin ring present in the apical region of the enterocyte. The latter may be especially important in facilitating controlled paracellular movement of solute (still the subject of controversy) without overall compromise of the epithelial barrier (17). However, understanding of the factors regulating the tight junction remains substantially incomplete, requiring further study. Given that most studies have utilized highly reductionist models, characterization of regulation of specific tight junction components in vivo as well as any differences between small intestinal and colonic epithelium is imperative.

In addition to the structural features of epithelial cells, which serve as a barrier, it is increasingly clear that a preepithelial compartment at the apical epithelial surface and composed of products secreted by the epithelium plays an essential role in protecting the mucosa from primary injury. The presence of large amounts of secreted mucin glycoproteins that form a viscoelastic gel has been long recognized, although evidence of functional contribution to the barrier has been limited. More recently, it has been recognized that trefoil peptides, a family comprised of three small proteins that share a distinctive and highly conserved structural motif, are also secreted by goblet cells and are present in a continuous fashion at the surface of the gastrointestinal tract throughout its length. The three

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members of the family, designated pS2, SP, and intestinal trefoil factor (ITF), are distributed in a region-specific fashion, with the first two secreted in the mouth, stomach, and hepatobiliary and pancreatic ducts (depending on the animal species) and with ITF present throughout the normal small and large intestine. The three peptides share a motif of six cysteine residues that result in the formation of three intrachain loops by disulfide bond formation. This secondary structure is likely responsible for the marked resistance to protease digestion that permits these peptides to remain structurally and functionally intact despite secretion onto the mucosal surface where they are exposed to a variety of proteases.

In vitro and in vivo studies have demonstrated that these peptides can protect the epithelium from a variety of deleterious agents, including bacterial toxins, chemicals, and drugs (1, 13, 15). Thus trefoil peptides can protect intestinal epithelial monolayers in vitro from a number of agents, whereas topical administration, as well as transgenic overexpression, have protected mucosa in intact animals from standard agents of experimental mucosal injury, e.g., nonsteroidal anti-inflammatory drugs. As detailed below, these same peptides also appear to play a key role in promoting restitution after injury. The constitutive presence of high concentrations of trefoil peptides at the mucosal surface as well as the ability to rapidly stimulate further expression and secretion is consistent with a role in providing constant protection and in promoting repair after injury. Although the mechanisms through which trefoil peptides promote migration after injury are becoming better understood (see below), the mechanism of the protective effects of trefoil peptides is not well understood. Preliminary studies have demonstrated direct interaction with mucin glycoproteins resulting in a substantial increase in overall viscosity (D. K. Podolsky, unpublished observations). However, it remains unclear whether this interaction is necessary for either the protective or prohealing effects; in vitro, the trefoil peptides do confer significant protection against a variety of injurious agents when added to the apical surface in the absence of mucin glycoproteins. Nonetheless, coaddition of both trefoil peptides and mucin glycoproteins resulted in markedly greater protection.

Repair of intestinal mucosa after injury occurs through the aggregate effect of coordinated processes whose relative contributions are likely dependent on the depth rather than the extent of damage. Even the most superficial injury involves epithelial destruction. Thus restitution, the process through which epithelial continuity is reestablished, is central to healing after any form of injury, regardless of the underlying cause or severity (4, 9). Restitution is achieved through rapid migration of the residual epithelium from the wound edge. Reestablishing continuity is clearly of overriding importance to prevent bacterial invasion and penetration to the vascular space and the intense stimulation of mucosal immune response and subsequent inflammation due to dietary and bacterial antigens. Cells elongate and thin, enabling them to resurface surprisingly broad areas of denuded mucosal surface without actual proliferative replacement.

The rapidity of migration allows reconstitution of epithelial continuity much sooner than could be achieved through proliferation. Nonetheless, subsequent compensatory proliferation is needed to ultimately restore the normal mucosal surface. One as yet unexplained paradox is the means by which cell migration of restitution by a previously anchored epithelial population is accomplished without also precipitating programmed cell death. However, as noted below, recent studies on the functional properties of trefoil peptides suggest that they may be central to this coordination. It is also noteworthy that proliferation proceeds until sufficient to restore an appropriate cell population before returning to normal homeostatic turnover. Whether this results simply from the superseding effect of contact inhibition or other mechanisms is unclear. It has been suggested that a distinctive cell emerges at the site of injury that serves as a source of key regulatory peptides and possibly as a progenitor of the reparative epithelial cells.

When deeper injury occurs, which is commonly found in most clinically important injury, remodeling of the extracellular matrix, the submucosa, and deeper layers is also needed. Much less is known about the processes regulating these complex events. With repair of deeper injury, recapitulation of normal structure may be imperfect and, with excessive deposition of collagen and other extracellular matrix constituents, may result in fibrosis and stricture formation.

Many factors regulating the essential process of postinjury epithelial migration have been defined in the past several years through the use of in vitro models employing epithelial monolayers. These factors encompass a number of small nonpeptide molecules, including short-chain fatty acids, especially butyrate, polyamines, and prostaglandins, although it remains unclear whether they act predominantly to promote healing or whether they primarily contribute to constitutive epithelial metabolic maintenance (2, 27, 28).

The possible roles of a number of metabolic products notwithstanding, it has become increasingly clear over the past several years that restitution is fundamentally regulated by several regulatory peptides that act in a highly coordinated manner to ensure rapid restoration of epithelial continuity. These include members of several distinct growth factor and cytokine families that act through cognate cell surface receptors present on the basolateral surfaces of the epithelium (3, 6, 7, 19, 21, 22). It is noteworthy that this variety of promigratory peptides includes ligands that are produced by the epithelium itself and products of various cell populations present in the submucosa as well as some factors that are produced by both, e.g., transforming growth factor-β (TGF-β). Thus it is likely that autocrine and paracrine actions are important.

Among the mucosal cells that contribute to the milieu of peptides promoting restitution are the myofibroblasts that are immediately subjacent to the epithe-
lum and that secrete hepatocyte growth factor (HGF) (and prostaglandins), which appear to be targeted to receptors present exclusively on the epithelial basolateral surface (11, 24). KGF is also produced by mesenchymal cells and targets receptors on the epithelium to both stimulate migration and proliferation. Perhaps even more important is the capability of immune and inflamatory cell populations to produce cytokines and growth factors that can stimulate this critical process of repair. Because inflammatory responses to bacterial infection and other challenges can result in significant injurious and clinically important effects, their critical role in healing may be overlooked. The complex cascades of host response have evolved to help achieve the ultimate goal of reestablishing normal homeostasis, and these recent studies have provided insight into the mechanistic pathways through which inflammatory cells can contribute to mucosal healing by secretion of cytokines and growth factors.

Studies from this laboratory have suggested that there may be a hierarchy in the action of these growth factors and cytokines in stimulating restitution. In vitro studies using monolayers of intestinal epithelial cell lines indicate that TGF-β plays a central role (6). Immunoneutralizing anti-TGF-β was able to abrogate stimulation by many peptides (though conversely, anti-sera against other peptides had no effect on TGF-β stimulation), suggesting that enhanced production of active TGF-β is either a necessary common final “pathway” or a necessary cofactor for many, if not all, of these ligands acting at the basolateral pole. However, it appears that the relative hierarchy of peptides, if present, may differ at other sites and perhaps by species. Thus studies using primary cultures of rabbit gastric and esophageal epithelium have suggested that HGF and fibroblast growth factors (FGFs) may be especially important and not dependent on TGF-β (24, 25). However, all these studies suffer from the intrinsic limitation of in vitro approaches, and the validity of these concepts of relative hierarchy remains to be explored in vivo.

Although the spectrum of regulatory peptides that promote restitution has been increasingly well defined, the cellular and subcellular mechanisms that result in cell migration remain to be more completely characterized. Nonetheless a number of important insights have been gained in the past few years. Not surprisingly, migration seems to be dependent on activation of small GTPases, specifically of the Rho subfamily, given the central role these have been found to play in modulation of cell cytoskeletal changes associated with cell movement in other biological processes (23). However, details of the other coordinated intracellular responses that must necessarily occur to facilitate cell movement in response to growth factors and cytokines remain to be defined. These include modulation of cell-cell and cell-extracellular matrix interactions as well as avoidance of precipitating programmed cell death and subsequent cell proliferation. It is axiomatic that each of these properties must be altered in a coordinated manner to permit cell migration in restitution. Although the detailed intracellular pathways that effect these changes have not been determined (cf. trefoil peptides discussed below), it is clear that rapid activation of mitogen-activated protein (MAP) kinase may play a central role in at least some of these responses to injury (5, 10, 18).

Evidence has already been obtained that specific cell-extracellular matrix interactions are essential for migration. It is clear that some regulatory peptides that promote migration (most importantly TGF-β) modulate production of extracellular matrix constituents as well as the expression of the cell surface receptors that bind to these constituents (especially integrins) (12, 14, 16, 20). In vitro studies have suggested that the extracellular matrix of the epithelial cell is composed of products of both the epithelial cell itself and mesenchymal cells. Epithelial cells contribute component subunits of laminin (α and β), fibronectin, and entactin among other more minor constituents, whereas the mesenchyme contributes laminin subunit (γ) and collagen. Production of several of these matrix components is rapidly upregulated after injury, TGF-β appears to play an important role in this enhanced expression. Competitive inhibitor studies indicate that integrin interactions with fibronectin (Arg-Gly-Asp dependent) and collagen are absolutely necessary for migration to occur. Other studies suggest that these may be mediated by the integrin heterodimers. Although further studies are needed to delineate the factors modulating integrin expression during mucosal healing, as noted, TGF-β and perhaps other factors such as the FGFs may regulate composition of the extracellular matrix. This may be accomplished both directly through regulation of expression and indirectly through regulated expression of metalloproteinases and other proteases. The latter may be especially important in the processes of tissue remodeling following deeper injury. Excessive production of collagen and other components of the extracellular matrix and possibly a relative lack of appropriate proteases could result in the eventual development of problematic fibrosis.

Although it is clear that several peptides promote the process of migration, many of these same factors exert additional functional activities that may be integrated into an overall response to injury. Some of the ancillary properties of two growth factors may be especially noteworthy. TGF-β, which appears to play such a pivotal role in promoting restitution through enhanced migration and modulation of extracellular matrix, is paradoxically a potent inhibitor of intestinal epithelial proliferation. Indeed, in studies of its effects on intestinal epithelial cells in vitro, its inhibitory effects override the pro-proliferative actions of other growth factors. Given the essential need for eventual replacement of cells lost through injury, it is evident that TGF-β must be quickly downregulated or inactivated or mechanisms must supervene that render the reparative epithelium resistant to its growth suppressive effects. Conversely, TGF-α and/or other members of the epidermal growth factor family may play an even more important role in promoting the proliferative phase of...
repair than cell migration. Indeed, immunoneutralization studies suggest that TGF-α is the dominant autocrine factor responsible for extracellular-regulated protein kinase activation that leads to subsequent proliferation. Despite these broad inferences, the mechanisms that coordinate proliferation with the more immediate need to reestablish epithelial continuity by restitution remain to be defined through further research. Similarly, more work is needed to understand the processes that remodel tissue to restore normal architecture, although it appears that HGF may be especially important to this dimension of the healing mucosa.

As progress has been made in defining the variety of growth factors and cytokines that promote restitution, it has become clear that their actions mediated through basolateral receptors are complemented by the actions of trefoil peptides at the apical surface. Indeed trefoil peptides are the most potent peptides in promoting restitution in wounded monolayers in vitro. The actions of trefoil peptides appear to promote migration through pathways distinct from those of cytokines and growth factors; their effects are entirely independent of TGF-β. Trefoil peptide production is markedly enhanced in immediate proximity to sites of ulceration regardless of the nature of the underlying diathesis, e.g., peptic ulceration and Crohn's disease. Of interest, upregulation of trefoil peptide at sites of ulceration is not confined to the specific trefoil peptide normally present in that region of the gastrointestinal tract. Thus all three trefoil peptides have been found in the reparative epithelium adjacent to ulcers of both the proximal and distal intestines. Recent studies have demonstrated coordinate cross-regulation of the trefoil peptides in vitro and in vivo (26). ITF could induce expression of itself as well as the other two trefoil peptides, providing a potential basis for the concurrent presence of all trefoil peptides in proximity to sites of ulceration detected by immunohistochemistry.

In vivo studies support the inference that the role that trefoil peptides play in facilitating restitution may be even more important than their primary role as a component of innate mucosal protection. Mice made ITF deficient by targeted gene deletion develop normally and sustain normal mucosal integrity in the absence of a superimposed insult. However, these ITF-deficient mice are exquisitely sensitive to mucosal injury and succumb to modest forms of injury from which wild-type mice rapidly recover. This vulnerability is associated with a virtual absence of any histopathological signs of epithelial repair. The latter can be essentially entirely reconstituted by topical application of exogenous recombinant ITF.

These findings not only indicate that trefoil peptides may be more important in repair than in sustaining normal homeostasis but suggest that they are the most important factor in achieving effective restitution or at the very least are a necessary cofactor to ensure repair. It is noteworthy that mice rendered deficient in growth factors and cytokines, which appear to contribute to restitution in in vitro studies, may have some increased injury to the same standard agents, the apparent defect is not profound, and these mutant mice are ultimately able to recover. These observations lend support to the notion that trefoil peptides may have overriding importance in mucosal response to injury.

Recent studies in this laboratory have provided insight into the intracellular mechanisms through which trefoil peptides affect cell migration without precipitating apoptosis as a consequence of movement of an otherwise fixed epithelium. ITF induces movement of E-cadherin away from sites of cell-cell adherence to the cytoplasm. Redistribution of E-cadherin may facilitate cell movement by dissociating cells from attachment to adjacent cells. In addition, ITF leads to activation of MAP kinase and, most importantly, blocks induction of p53-dependent and p53-independent apoptosis. Although considerable more study is needed to define the mechanisms of action of trefoil peptides, it appears that they play a pivotal role in the coordinated response to injury that results in epithelial restitution. This overview has considered key components of the intrinsic mechanisms that act to prevent injury of the mucosa. It is clear that many of the same properties that enable a key group of peptides to confer protection also promote the rapid restoration of epithelial continuity after injury disrupting mucosal barrier integrity has occurred. In this context, this "nonspecific" innate host defense is closely complementary to rather than dissociated from the inflammatory responses mediated by the specific immune system. These inflammatory responses ultimately aim to promote tissue healing, even though they may occasionally themselves have deleterious effects. In this context, innate mechanisms promoting epithelial healing may be central to the eventual downregulation of immune and inflammatory responses, which if sustained themselves lead to disease by abrogating the further sustained stimulation of those responses due to ongoing exposure to bacterial proinflammatory products and antigens through the disrupted epithelial surface.

The author regrets that, due to space constraints, it was not possible to cite all of the many contributions by investigators who have advanced understanding in this area.

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REFERENCES


