Events at the Host-Microbial Interface of the Gastrointestinal Tract

I. Adaptation to a microbial world: role of epithelial bactericidal/permeability-increasing protein

Geraldine Canny and Sean P. Colgan
Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

Canny, Geraldine, and Sean P. Colgan. Events at the Host-Microbial Interface of the Gastrointestinal Tract. I. Adaptation to a microbial world: role of epithelial bactericidal/permeability-increasing protein. Am J Physiol Gastrointest Liver Physiol 288: G593–G597, 2005; doi:10.1152/ajpgi.00506.2004.—Epithelial cells of many mucosal organs have adapted to coexist with microbes and microbial products. In general, most studies suggest that epithelial cells benefit from interactions with commensal microorganisms present at the lumenal surface. However, potentially injurious molecules found in this microenvironment also have the capacity to elicit local inflammatory responses and even systemic disease. In this environment, the epithelium has evolved effective mechanisms to cope with microbial products and to provide appropriate responses to potential pathogens. Although our understanding of these mechanisms is clearly in its infancy, a number of recent findings provide insight into phenotypic characteristics that allow for this discrimination. Here, we briefly review some of these mechanisms, with particular attention to epithelial expression of the anti-infective molecule bactericidal/permeability-increasing protein.

mucosa; infection; inflammation; eicosanoid

MUCOSAL SURFACES such as the lung, intestine, and kidney are lined by a single layer of epithelial cells. Epithelial cells are uniquely positioned to serve as a direct line of communication between the immune system and the external environment. In their normal state, mucosal surfaces are exposed to high concentrations of foreign antigens, while at the same time they are intimately associated with the immune system via subepithelial lymphoid tissue. Consequently, the epithelium forms an important barrier, preventing the free mixing of luminal antigenic material with the lamina propria that houses the mucosal immune system. This latter capability is attributable to intercellular tight junctions, present as gaskets that circumferentially join epithelial cells at their apices. Tight junctions regulate the passive permeation of hydrophilic solutes through the “paracellular” space. Tight junctions are dynamic structures, the permeability of which is a highly regulated process. A second important function of the epithelium is the maintenance of mucosal hydration. A coordinated series of signaling events to epithelial stimuli (secretagogues) results in the activation of membrane ion channels and transporters. Depending on the net effect of such events (absorption or secretion of ions), an epithelial osmotic gradient is established and provides the driving force for paracellular water transport. Similar to many aspects of cell biology and immunology, this view has changed dramatically in the past decade. The epithelium is now viewed as an active player in normal homeostatic mechanisms of mucosal immunity. Here, we will briefly summarize current thinking regarding the many functional roles of epithelial antimicrobial peptides, with a particular emphasis on the recent discovery of epithelial-expressed bactericidal/permeability-increasing protein (BPI).

ANTIMICROBIAL PEPTIDES AND INTESTINAL EPITHELIA

The lumenal surface of many epithelia is exposed to an exceptionally large bacterial population. Surprisingly little is known about how epithelial cells adapt to such an environment. It is now well accepted that epithelial cells of broad origin express a variety of antimicrobial peptides, including defensins (7). Only recently have we come to appreciate the extent to which these peptides contribute to the health of mucosal tissues (16). For example, it is thought that defensin expression in Paneth cells of the small intestine maintains the crypt as an essentially sterile environment (16). Moreover, it was shown that animals genetically null of matriisin, the metalloproteinase that processes defensin precursors, and thus lacking mature defensins, are more susceptible to microbial invasion of the intestinal epithelium. These mice succumb more rapidly and to lower doses of Salmonella typhimurium than wild-type mice (25).

BPI

BPI is a 55- to 60-kDa protein originally found in neutrophil azurophilic granules, on the neutrophil cell surface, and, to a lesser extent, in specific granules of eosinophils (3). BPI selectively exerts multiple anti-inflammatory actions against gram-negative bacteria, including cytotoxicity through damage to bacterial inner/outer membranes, neutralization of bacterial LPS (endotoxin), as well as serving as an opsonin for phagocytosis of gram-negative bacteria by neutrophils (3). A growing number of proteins with primary structural homology to BPI and roles in lipid recognition and host defense is being identified. The plasma LPS-binding protein (LBP) is a product of the liver with an anionic net charge that serves to greatly amplify responses to LPS by delivering LPS monomers to a monocyte receptor-complex containing CD14, MD2, and Toll-like receptor 4 (11). Whereas LBP recruits LPS monomers for delivery to CD14, BPI stabilizes LPS aggregates thereby preventing LBP-mediated LPS monomer delivery (23). Thus BPI and LBP are functionally antagonistic. It is the COOH-terminal domains of LBP and BPI that determine the route and host responses to LPS complexes. Whereas LBP predominates in resting plasma, the higher affinity of BPI for LPS coupled with the greater concentration of BPI at neutrophil-rich inflammatory sites favors BPI-mediated endotoxin neutralization (4).
In addition to BPI and LBP, humans also express cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP), which apparently also maintain LPS-interactive properties (10). Homologues of BPI/LBP have recently been described in the head kidney and liver of a teleost fish (rainbow trout), suggesting that the BPI/LBP family is evolutionarily conserved. Of note, the BPI superfamily recently underwent a major expansion with the identification of homology to a murine epithelial expressed gene named plunc ( palate, lung, and nasal epithelium clone) (1). The PLUNC family includes proteins with extended structures corresponding to holo-BPI [long (L)] PLUNC and as well as smaller proteins with homology corresponding only to the NH2-terminal half of BPI [short (S) PLUNC]. The primary amino acid sequences of the PLUNC proteins reveal substantial divergence in the NH2-terminal region with greater homology (among the LUPNCs) in the region corresponding to the COOH-terminal half of BPI. Overall, the PLUNC family has modest amino acid sequence homology to BPI but very similar predicted fold and genomic organization to BPI. Rat and human PLUNC are secreted in nasal mucus, and rat PLUNC is upregulated in the nasal respiratory epithelium after bullectomy, suggesting roles in host defense subsequent to injury.

MICROBIAL ANTIGENS AND INFLAMMATORY BOWEL DISEASE

The inflammatory bowel diseases (IBD), including both Crohn’s disease and ulcerative colitis, are especially complex disorders. IBD often is a progressive and mutilating disease primarily localized to the gastrointestinal tract, although systemic complications are frequent. Despite extensive efforts, the basis of IBD has yet to be established. A number of etiologies for IBD have been proposed, including impaired epithelial barrier function, persistent infectious processes, abnormal regulation of intestinal immune responses, and multifocal vascularitis (20).

The hypothesis that human IBD might somehow be related to normal bacterial flora was first proposed more than 30 years ago. Accumulating evidence indicates that the luminal microflora is central to the development of IBD. First, studies in murine models of colitis have indicated that clinical manifestation of symptoms similar to those in human patients is less severe, if at all, in animals maintained in germ-free environment (5). If colonized with commensal bacteria, these same animals rapidly develop symptoms consistent with IBD (5). Colonization with commensal bacteria, these same animals rapidly develop symptoms consistent with IBD (5). Second, studies in humans and in a variety of animal models have indicated that broad-spectrum antibiotics alleviate some of the symptoms associated with IBD, suggesting that luminal flora (or their byproducts) contribute to ongoing inflammatory disease. Third, recent studies detailing the chromosome 16 map identified a gene responsible, at least in part, for genetic linkage of IBD to a large number of families. This gene, designated NOD2 (also referred to as caspase activation and recruitment domain 15, or CARD 15), encodes an intracellular protein that functions as a so-called pattern-recognition receptor for bacterial peptidoglycan. Although IBD patients appear to manifest increased activation, variants of NOD2 function result in a paradoxical reduced activation by bacterial products (5). This latter aspect remains to be explained. Nonetheless, significant recent evidence indicates that constituents of microbial antigens present within commensal flora may contribute to the IBD process. Studies directed at understanding endogenous protective strategies may aid in identification of future therapies for IBD.

INTESTINAL EPITHELIAL CELLS EXPRESS BPI

Our recent studies identified, for the first time, expression of BPI in human intestinal epithelia (2). This observation was based on transcriptional profiling studies aimed at identifying differentially expressed genes elicited by epithelial exposure to analogs of endogenously occurring anti-inflammatory eicosanoids [aspirin-triggered lipoxins (ATLa)]. Lipoxins are bioactive eicosanoids derived from membrane arachidonic acid by the combined action of 5-lipoxygenase (LO) and 12-LO or 15-LO involving two different cell types (i.e., transcellular metabolism). A number of recent in vitro and in vivo studies have revealed that lipoxins, and specifically lipoxin A4 (LXA4), serve as an innate “stop signal,” functioning to control local inflammatory processes. For example, LXA4 has been demonstrated to inhibit polymorphonuclear neutrophil transmigration across both endothelia and epithelia, in vitro and in vivo (22). Synthetic lipoxin analogs exhibit greater potency for these actions than the native compound, likely due to decreased metabolism to inactive compounds. In particular, the synthetic LXA4 analog 15 (R/S)-methyl-LXA4, the structure of which resembles that of epimeric derivatives of lipoxin (15-epi-LXA4), a native lipoxin generated in vivo in the presence of aspirin, may partially contribute to the anti-inflammatory actions of aspirin. Similarly, the epi-lipoxin analogs have proven effective in vivo.

Interestingly, the original microarray suggested that a prominent gene cluster elicited by ATLa included a number of antimicrobial peptides. In addition to BPI, this cluster identified ATLa-dependent induction (threshold >3-fold increase) of several known epithelial antimicrobials, including lysozyme, defensin-5, and cathespin E (unpublished data). In parallel, recent transcriptional profiling studies suggest that at least two antimicrobial peptide genes (phospholipase A2 and lysozyme) are induced in human IBD tissues (12). Nonetheless, we pursued the finding that BPI might be expressed in epithelia, particularly because BPI had previously been associated only with cells of the myeloid lineage. Initial studies directed at verifying microarray analysis revealed that a broad range of intestinal epithelial cells express BPI and each was similarly regulated by anti-inflammatory lipid mediators. Studies aimed at localization of BPI revealed that such expression occurs on the cell surface of cultured epithelial cell lines and dominantly localizes to epithelia in healthy human mucosal tissue. Functional studies employing a BPI-neutralizing antiserum revealed that surface BPI blocks endotoxin-mediated signaling in epithelia and kills S. typhimurium. Our studies identified a previously unappreciated “molecular shield” for protection of mucosal surfaces against gram-negative bacteria and their endotoxin. These studies will be described in detail later. Of note, BPI expression had also been reported in human nasocriclal duct epithelium (17).

Ongoing studies within the laboratory have revealed that intestinal epithelial cell lines as well as native epithelia prominently express BPI. We have concentrated our efforts in the colon, but recent work (2) indicates that such BPI expression was not confined to the colon and was more widely distributed within the alimentary tract (e.g., squamous epithelia of the

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BPI in epithelial-microbial interactions

Bacterial infection of the intestinal epithelium represents a critical interface between the host and the microbial flora and is crucial to the development of innate immunity. In most cases, intestinal epithelial cells respond to endotoxin, and endotoxin-mediated induction of ICAM-1 surface protein. To this end, epithelial cells preexposed to anti-BPI and subsequently activated with a combination of endotoxin and human serum (source of soluble CD14) revealed that anti-BPI significantly increased endotoxin-induced ICAM-1 surface protein. Thus, a tenuous balance may exist between LPS recognition and activity, and endogenous mechanism(s) likely exist to diminish aberrant activation of epithelial cells. Our present work identified the expression of BPI mRNA in epithelial cells, and extensions of these findings revealed broad expression on epithelia of diverse origin. Before its discovery in epithelium, BPI expression had been described only in cells of myeloid lineage (7). Two conceptual points exemplify the potential importance of BPI expression on mucosal epithelia. First, epithelia provide the initiation point for host-microbial interactions. Although microbial flora are necessary and beneficial to the host, some degree of selectivity is also requisite for homeostasis. Epithelial-expressed BPI could provide such a role. BPI is remarkable for its potent (nanomolar) and selective bioactivity against gram-negative bacterial species (14). Moreover, the finding that functional BPI is expressed on the epithelial surface, and not in the soluble milieu, could provide an additional degree of selectivity for invasive/host-interactive pathogens. Second, a basic feature of many mucosal surfaces is the presence of high concentrations of endotoxin. Previous work (18) has indicated that under appropriate conditions, certain epithelial cells can respond to endotoxin, and recent studies have clearly defined the existence of LPS receptors (e.g., CD14 and Toll-like receptors) on epithelial surfaces (11), the latter of which may be differentially regulated in selective mucosal diseases. In this context, an elegant recent study demonstrated that commensal bacteria and bacterial components such as LPS, via activation of Toll-like receptor, are necessary for homeostatic processes in the intestine (19). Thus a tenuous balance may exist between LPS recognition and activity, and endogenous mechanism(s) likely exist to diminish aberrant activation of epithelial cells. Our present findings indicate that surface-expressed BPI could provide an innate dampening mechanism against endotoxin by effectively competing for the binding of endotoxin, as such, preventing endotoxin binding to such proinflammatory receptors. Indeed, endotoxin activation of epithelia (i.e., ICAM-1 induction) was significantly enhanced by the addition of functionally inhibitory anti-BPI sera, suggesting a protective role for BPI in mucosal endotoxin homeostasis. In summary, epithelial BPI may contribute to the innate biochemical barrier characteristic of mucosal surfaces but may also provide a degree of selectivity necessary for effective host responses.

From this vantage point, we proposed that one function of epithelial BPI is to modulate responses to bacterial endotoxin. The addition of endotoxin to epithelial cells in the presence of 5% heat-inactivated normal human serum induced a concentration-dependent induction of ICAM-1 mRNA. Having shown that epithelial cells respond to endotoxin, we determined whether inhibition of epithelial BPI might function to enhance endotoxin-mediated induction of ICAM-1 surface protein. To this end, epithelial cells preexposed to anti-BPI and subsequently activated with a combination of endotoxin and human serum reveal that anti-BPI significantly increased endotoxin-induced ICAM-1, suggesting that BPI provides an endotoxin-neutralizing function for epithelial cells and that surface-expressed BPI might normally function to dampen epithelial endotoxin responses. Taken together, these findings implicate epithelial BPI as a direct line of bacterial-epithelial communication to submucosal tissues in this setting.

**EPITHELIAL BPI IS BACTERICIDAL**

One function of the epithelium is to provide a barrier to prevent the free flow of luminal contents to submucosal tissues. Included within the barrier property of the epithelium is the modulation of bacterial-epithelial interactions and the modification of bacterial localization along the intestinal wall. For example, antimicrobial peptides produced and released by Paneth cells at the base of the crypt determine bacterial populations within the crypt lumen, and for all intents and purposes, this is considered a sterile space (16). Although not

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**Fig. 1.** Potential roles of epithelial bactericidal/permeability-increasing protein (BPI). In both physiological and pathophysiological states, epithelial cells are central to the coordinated coexistence with microbes and microbial products. During infectious episodes, the rapid induction and release of chemokines results in accumulation of neutrophils at epithelial surfaces. As part of the adaptive response, lipoxins are generated by transcellular biosynthesis. Ensuing induction of epithelial BPI provides a protective, antimicrobial shield for the resolution of acute inflammation (see text). LXA, lipoxin A₄; LXA₄R, lipoxin A₄ receptor.
well understood, the epithelium is thought to possess a surveillance mechanism(s), which is likely to comprise an important part of the innate immune response.

As part of the original characterization of epithelial BPI, we determined whether intact, adherent epithelial cells kill BPI-sensitive bacteria. To do this, confluent Caco-2 epithelial cells were exposed to S. typhimurium and examined for bacterial killing in standard colony-forming unit (CFU) analysis. Such analysis revealed a nearly 1-log order reduction in CFU over a 90-min period and that anti-BPI significantly inhibits bacterial killing compared with control normal goat serum. Parallel experiments assessing epithelial killing of a gram-positive bacterium (e.g., Enterococcus faecalis) revealed no influence of anti-BPI.

**BPI AND IBD**

The high affinity of BPI for the lipid A region of LPS targets its cytotoxic activity to gram-negative bacteria. Binding of BPI to the gram-negative bacterial outer membrane is followed by a time-dependent penetration of the molecule to the bacterial inner membrane where damage results in loss of membrane integrity, dissipation of electrochemical gradients, and bacterial death (14). The actions of BPI are amplified by extracellular factors including the complement system and secretory phospholipase A2 (4). Members of the defensin and cathelicidin antimicrobial peptide families synergistically enhance the antibacterial activity of BPI (14). Whether this is of relevance to intestinal immunophysiology remains to be determined. Although the anti-infective properties of BPI are best defined with respect to gram-negative bacteria, BPI congeners and BPI-derived peptides have also shown activity against cell wall-deficient gram-positive bacteria and fungal organisms.

BPI binds the lipid A region of LPS with high affinity (14) and thereby prevents its interaction with other (proinflammatory) LPS-binding molecules, including LBP and CD14 (8). Because BPI binds the lipid A region common to all LPS, it is able to neutralize endotoxin from a broad array of gram-negative pathogens (14). Thus BPI is capable of inhibiting all of the many proinflammatory activities of LPS, including induction of cytokine release, activation of the neutrophil oxidase enzyme, and nitric oxide formation (14). The opsonic activity of BPI occurs via binding of the NH2-terminal domain to gram-negative bacteria and promotion, via the COOH-terminal portion of BPI, of bacterial attachment to neutrophils and monocytes (4). Whether such opsonic effects occur via ligand receptor interactions between the COOH-terminal half of BPI and membrane receptors or other mechanisms is unclear.

The selective and potent action of BPI against gram-negative bacteria and their LPS is fully manifest in biological fluids including plasma, serum, and whole blood (14). In multiple animal models of gram-negative sepsis and/or endotoxemia, administration of BPI congeners is associated with improved outcome, and these properties have rendered BPI an attractive target of biopharmaceutical development (15). BPI has proven safe and nonimmunogenic in phase I trials and has demonstrated potent anti-endotoxic activity in phase II trials (9). In a large, multinational phase III double-blinded, placebo-controlled trial, adjunctive treatment with rBPI21 was associated with reduced complications, including limb amputation, due to meninogocccal sepsis (13). Additional indications for the use of BPI congeners are being actively explored, as are activators of epithelial BPI. For instance, stable lipoxin analogs have recently proven protective in murine models of colitis (6). Given their size (<400 Da), stability, and composition, the lipoxins look especially promising as future therapeutic modalities for mucosal inflammation, for which some of this protection may be afforded by induction of epithelial BPI.

Of interest, BPI has been suggested as a marker molecule for IBD. For example, high levels of neutrophil-associated BPI are found in the colonic mucosa of patients with ulcerative colitis, and autoantibodies directed against BPI are proposed seromarkers for the IBDs (21). Indeed such autoantibodies have recently been shown to inhibit BPI bioactivity, indicating that the blocking of BPI could be involved in the disease process (21). Moreover, BPI congeners are currently being evaluated as novel therapies for diseases in which endotoxin is thought to play a role. Considering that IBD may be caused by a persistent reaction to antigen(s) secondary to a failure of normal immunoregulatory mechanisms and the observations that the ability of the epithelium to “hold back” flora in IBD may be abrogated, it is desireable to elucidate the contribution of proteins such as BPI to intestinal immunity.

In conclusion, the initial encounter of microbes with human tissue frequently takes place at mucosal surfaces. If the microbes survive, proliferate, and reach sites not normally colonized by microbial flora, the influx of neutrophils and other inflammatory cells into the affected area ensues. Normal host responses include the initiation of counterregulatory measures, including the local production of anti-inflammatory lipids (Fig. 1). The effectiveness of host defenses as innate mechanisms of adaptation to a microbial world is now an area of intense investigation. Studies in recent years have focused much attention on microbicidal peptides and proteins as common components of both epithelial and phagocytic innate host defenses. Intestinal epithelial cells form a single layer of cells that isolate the host from the potentially hostile gut lumen and are recognized as important immune effector cells, capable of responding to a wide array of biologically active agents commonly found in the intestinal environment. With its antibacterial and endotoxin-neutralizing properties, epithelial BPI may play a significant role in innate defense at the mucosal interface.

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