First Encounter: How Pathogens Compromise Epithelial Transport

Pathogenic organisms trigger numerous signaling pathways that ultimately lead to drastic changes in physiological functions. Apart from altering structure and function of the epithelial tight junction barrier and activating inflammatory cascades, they induce changes in fluid and electrolyte transport. Pathogens do so by activating or by inhibiting ion channels and transporters, and the result might be to their benefit or to their disadvantage.

Pathogens alter epithelial structure and function

Our body is constantly challenged by pathogenic organisms, such as bacteria, viruses, and fungi (FIGURE 1). Most of these pathogens must overcome an epithelial cell barrier to establish an infection. The major portals of pathogen entry are the epithelial skin layer and the epithelial layers coating the gastrointestinal, respiratory, and urogenital tracts. Although both tissues clearly show transport function, the skin is complex with multiple keratinized cell layers, whereas internal surfaces are lined by only a single layer of highly polarized epithelial cells. These cells have distinct luminal and basolateral membranes composed of different phospholipids. They contain different sets of receptors and transport proteins and are electrically separated by tight junctions (TJ). Epithelia are not merely static barriers to the external environment. There is a rather complex and dynamic “cross-talk” between pathogens and the epithelium (2, 6, 24). Pathogen-host interactions are known to result in perturbations of the structure and function of TJ, induction of inflammatory responses, and other alterations in epithelial cell function (FIGURE 1). More recent evidence also indicates that fluid and electrolyte transport processes are affected, either specifically or as a consequence of altered cell function. Examples describing secretion-altering activities involve both pathogen-derived and pathogen-driven, host-derived molecules as well as direct pathogen binding to epithelial cells. In this short overview we will focus on the rapid effects that pathogens exert on epithelial electrolyte transport. This is a new and exciting field that may turn out to be of large pathophysiological relevance.

Pathogen-host cell contact: host recognition and pathogen attack

Pathogens may affect epithelial properties by means of secreted toxins, through directly pathogen-induced changes in enzyme function or protein expression, and through attachment to the cell membrane (FIGURE 2). In fact, attachment is regarded as the first contact of the intruder with the epithelium. Epithelial cells possess an array of cell surface molecules that monitor epithelial surfaces by binding various pathogen epitopes and eliciting cellular responses (FIGURE 2). The Toll-like receptor (TLR) family member TLR-4 and CD14, for example, cooperate in the recognition of the gram-negative bacterial cell wall component lipopolysaccharide (LPS) (48). Interestingly, the respiratory syncytial virus (RSV) also uses these receptors during transcytosis (48).

The cystic fibrosis (CF) transmembrane conductance regulator (CFTR) ion channel may also act as a pathogen receptor for Pseudomonas aeruginosa and Salmonella typhi uptake (36). However, this issue has been discussed controversially. Although some studies show attachment of P. aeruginosa to CFTR and its reduced clearance in CF airways, others do not find attachment to CFTR but rather to asialylated glycolipid (asialoGM1), which is upregulated in CF (9, 21, 25, 37). Thus underglycosylated proteins, abundant in membranes of CF cells, serve as binding sites for S. aureus and P. aeruginosa (9, 21).

Pseudomonas spp. translocate from the apical to basal cell surface via interactions with platelet-activating factor receptors and immunoglobulin receptors via choline-binding proteins (24). Some bacteria provide their own receptors, like enteropathogenic Escherichia coli, which are inserted into the host cell membrane. Others use basolaterally located host cell membrane proteins such as E-cadherin (Shigella, Listeria) or β-integrins (Yersinia) (24). These and other host membrane components (mannose receptor, proteoglycans, glycosaminoglycans, vitronectin, and CD66 proteins) may serve as bacterial attachment factors and initiate clathrin-mediated endocytosis of obligate intracellular parasites such as Chlamydia. Fungi and viruses are recognized by a number of protein-based receptors like TLRs but also bind to a variety of carbohydrate molecules that are very abundant at the epithelial cell apical surface. The common blood group antigen glycopospholipids are important in Candida albicans infection (4). Viruses bind to sialic acid-containing oligosaccharides and heparan sulfate proteoglycans. Fungal and viral pathogens also interact with common glycoconjugates on eukaryotic membranes such as lectins as well as receptors located on the extracellular matrix formed by fibronectin or laminin (unpublished observations).
**Pathogen-driven changes in cell signaling**

Epithelial receptors are a key feature in initiation of host innate immunity. As a paradigm, pathogen binding and/or internalization results in cell signaling changes, secretion of chemokines, and secretion of host defense molecules, in turn attracting immune cells that release an array of microbiocidal compounds and additional cell regulators (FIGURE 1). Both physical pathogen-cell interactions and the resulting changes in intracellular compounds have profound influences on epithelial cell fluid and electrolyte transport. In early observations, purified *Pseudomonas* rhamnolipids as well as LPS from *Klebsiella pneumoniae* were found to inhibit epithelial Na⁺ absorption through binding to TLR-2 or TLR-4, respectively (15, 49). Thus it will be interesting to determine if membrane attachment pathogens such as *P. aeruginosa* will effect Na⁺ absorption by the epithelial Na⁺ channel ENaC. *P. aeruginosa* has been shown recently in our experiments with isolated perfused mouse tracheal epithelium and previously in studies with bovine trachea and dog bronchial epithelia to induce changes in fluid transport, probably by inhibition of ENaC (11, 45). Pathogen attachment triggers a multitude of intracellular signaling pathways, most notably those involving MAPKs, phosphatidylinositol 3-kinase, protein kinase C (PKC), intracellular calcium, and nuclear factor-κB (NF-κB). It seems obvious that several of these messengers, besides other cellular effects, will also affect the activity of proteins mediating ion transport (FIGURE 2). *P. aeruginosa* activates Ca²⁺-dependent MAPKs in airway epithelial cells via binding to asialoGM1 receptors (41). Activation of this pathway also results in an inhibition of epithelial Na⁺ absorption (11, 45) and an increase in mucin production (11, 32, 33, 45). More recent studies indicate that binding by this CF pathogen elicits ATP release and autocrine activation of purinergic nucleotide receptors (33). Experiments in our laboratory indicate that asialoGM1-mediated...
EMERGING TOPICS

**TOXINS**
- Cholera toxin (CTX)
- E. coli LT and ST
- Clostridium Tox

**Pathogen-derived NO**
- E. coli ST
- V. parahaemolyticus TDH
- Salmonella SopB
- Rotavirus NSP4

**PATHOGEN INDUCED**
- Salmonella
- Shigella
- E. coli

**PATHOGEN ATTACHMENT**
- Bacterium
- Virus
- Fungus

**FIGURE 2.** Pathogens change epithelial ion transport by secreting toxins, activating enzymes, and attaching to the host cell membrane.

Pathogens are subdivided according to their effects on intracellular messengers cAMP, cGMP, or Ca²⁺ (PKC, phosphatidylinositol 3’-kinase, TDH, thermolabile hemosylin; NSP, nonstructural protein). Toll-like receptor; PAF, platelet-activating factor; PBP binding protein; PI3K, phosphatidylinositol 3-kinase; TDH, thermolabile hemosylin; NSP, nonstructural protein.

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The “electrically sealed” nature of epithelia may also be breached by disruption of the TJ. Pathogens target TJ integrity by direct binding or by activating cell PKC and proteases, which lead to redistribution and degradation of TJ proteins as found in ulcerative colitis (2, 28, 42) (FIGURE 1). Viral-epithelial interactions also have a number of effects on cell signalling (FIGURE 2), and recent studies raise the possibility that Na⁺ transport is also affected. Respiratory viruses such as rhinovirus have been reported to activate p38 MAPK (16), PKC is activated during RSV infection (16, 34), and the hemagglutinating influenza virus activates the Raf/MEK/ERK and PKC cascades (26, 38). This latter orthomyxovirus was shown to inhibit amiloride-sensitive Na⁺ absorption in mouse airways by a PKC-dependent mechanism (26). Similar observations were made in mouse airways exposed to the paramyxovirus member Sendai virus (27). Further exciting discoveries may be expected in future for other hemagglutinating viruses, because lectin binding has been demonstrated to cause changes in epithelial ion transport (13, 29). The reduced NaCl absorption is likely to contribute to accumulation of fluid in the respiratory tract during viral airway infection.

**Pathogen-driven cell-secreted molecules**

A major epithelial response to pathogen contact is the activation of an inflammatory cascade. Pathogen adherence triggers expression and secretion of a typical composition of cytokines, adhesion molecules, and major histocompatibility complex class II molecules (FIGURE 1). Neutrophils are attracted to the luminal side of the epithelium by cytokines like IL-8. Inflammatory cells release superoxide and induce cell damage and the release ofAMP, which is converted to adenosine and binds to A₃ receptors. Activation of these receptors increases intracellular cAMP and activates secretory ion channels (2, 28). Typically several MAPKs are activated during inflammation, including ERK, JNK, and p38 as well as PKC, leading to the activation of the transcription factors NF-κB and AP-1. As well as exerting proapoptotic activity on epithelial cells (23), NF-κB and AP-1 also induce the production and release of cytokines such as TNF-α, IL-6, and IL-8 and the inflammatory mediator nitric oxide (NO) through upregulation of inducible NO synthase expression. Released inflammatory mediators activate electrolyte secretion in intestinal and airway epithelial cells (3, 28, 31, 43, 46). For example, ENaC-mediated Na⁺ absorption is inhibited by TNF-α and NO (14, 18, 22). Other molecules are released by epithelia on exposure to various bacteria, fungi, and even protozoa with potent secretagogue activity, including the secretory neuropeptide galanin and prostaglandins.
Interestingly, Saccharomyces boulardii-conditioned medium has been demonstrated to modulate secretagogue-induced cAMP and Ca\(^{2+}\)-dependent Cl\(^{-}\) secretion in colonic epithelial cells (7, 8). This may have applications for the prevention and treatment of intestinal infections and diarrhea. Similarly, cytokines released during inflammatory cholangiopathies trigger NO release, inhibiting adenylate cyclase and thus CAMP-dependent HCO\(_3\)\(^{-}\) and Cl\(^{-}\) secretion. This process may largely contribute to bilary cholestasis (44). Alternatively, inflammatory mediators may stimulate submucosal nerve or immune cells to release proteases such as trypsin, which then stimulate type 2 protease-activated receptors located on epithelial cells and enteric nerves (28) (FIGURE 1). Several pathogens such as Salmonella and E. coli affect TJ integrity in parallel, causing release of inflammatory mediators and altering ion transport. Moreover, these events do not occur independently of each other. It is obvious that TJ barrier damage will distort the vectorial ion transport and that inflammatory mediators will affect a broad range of cellular second messengers (2).

Pathogen-derived effects on epithelial ion transport

Many pathogenic bacteria produce molecules with highly toxic activities against host cells. Many of these toxins not only directly affect the viability and integrity of the epithelium but also exert specific changes on cellular ion transport (FIGURE 3). Prominent examples are the toxins from Vibrio cholera (CTX) and the heat labile (LT) and heat stable (ST) enterotoxins from E. coli, which activate G\(_s\) proteins via mechanisms to induce Cl\(^{-}\) secretion. These toxins can act through different mechanisms to induce Cl\(^{-}\) secretion. CTX and LT stimulate G\(_\alpha\) proteins via ADP-ribosylation and activation of adenylate cyclase, whereas ST activates guanylate cyclase, resulting in activation of CFTR, basolateral cAMP/cGMP-dependent K\(_{CNQ2/KCNE}\)\(^{+}\) channels, and the basolateral NKCC1 cotransporter (2, 28) (FIGURE 3). Similar cellular mechanisms are exploited by Klebsiella pneumoniae and Yersinia enterocolitica toxins (17, 40). The SopB toxin from Salmonella not only acts on the cytoskeleton but also increases intracellular inositol 1,4,5,6-tetra phosphate, which activates Ca\(^{2+}\)\^-activated Cl\(^{-}\) channels during cell invasion (12). The thermostable direct hemolysin from Vibrio parahaemolyticus, a worldwide cause of gastroenteritis, is another example of a toxin that activates Ca\(^{2+}\)\^-dependent Cl\(^{-}\) secretion (39). A clinically relevant, non-bacterial example is the enterotoxin NSP4 from Rotavirus that activates phospholipase C and intracellular Ca\(^{2+}\) and thus induces Cl\(^{-}\) secretion (10, 47). Ca\(^{2+}\)-activated Cl\(^{-}\) secretion is due to luminal Ca\(^{2+}\)-activated Cl\(^{-}\) channels of unknown identity and parallel activation of basolateral Ca\(^{2+}\)-activated SK4 K\(^{+}\) channels (FIGURE 3). In addition to ion transport, toxins may also target the absorption of substrates such as glucose and amino acids, exemplified by the Rotavirus toxin NSP4 (19).

Summary

What are the consequences of pathogen-induced changes in epithelial fluid and ion transport, and who benefits? From the examples described to date, the vast majority of effects result in increased apical fluid secretion by epithelial cells and are rapid, occurring within minutes of pathogen interaction. This may be advantageous to both host and pathogen. Increased fluid secretion may enhance the “flushing” effect in the airway, intestine, and urogenital tracts, clearing noxious compounds and pathogens (20). The lung especialmente relies on apical fluid homeostasis for its defense, as illustrated by the high susceptibility to infection experienced in CF. Thus pathogen-stimulated apical electrolyte and fluid responses may represent an important but poorly understood host defense mechanism. On the other side of the coin, increased apical secretion may enhance the invading pathogen’s motility and host defense evasion and may increase potential for transmission to additional hosts. Future work should clarify who profi ts from the change in epithelial transport.

FIGURE 3. Regulation of epithelial electrolyte and substrate transport. Electroneutral absorption by the Na\(^{+}\)/H\(^{+}\) exchanger NHE3 is inhibited by CAMP, whereas anion exchange is activated. The Rotavirus toxin NSP4 inhibits the Na\(^{+}\)/glucose transporter SGLT1. Na\(^{+}\) absorption by the epithelial Na\(^{+}\) channel ENaC is activated by phosphorylase 4,5-bisphosphate (PIP\(_2\)) and cAMP but is inhibited by PKC, NO, and TNF-α. Cl\(^{-}\) secretion by the cystic fibrosis transmembrane conductance regulator (CFTR) is activated by cAMP, cGMP, and PKC, and NO is maintained by basolateral NKCC1 as well as K\(_{CNQ2/KCNE}\)\(^{+}\) channels. Ca\(^{2+}\)-activated Cl\(^{-}\) secretion (Ca\(^{2+}\), IP\(_3\)) occurs via a different class of luminal Cl\(^{-}\) channels along with parallel activation of basolateral SK4 K\(^{+}\) channels.
EMERGING TOPICS

1. Bachhuber T, Schreiber R, and Kunzelmann K. Increased in intracellular Cl\(^{-}\) concentration mediated by Cl\(^{-}\)-C and activation of purinergic receptors and 


