Lessons from Enteropathogenic Escherichia coli

Some microorganisms interact with and respond to signaling pathways in host animal and plant species

John K. Crane

Both eukaryotic cells and microbes sense and respond to small molecules in their environments. Microbial cells communicate with one another via quorum-sensing molecules, antibiotics, and even hormones. In microbially rich soils, for example, bacteria and fungi communicate via antibiotics, while the Qse quorum-sensing system of pathogenic Escherichia coli cells responds to host hormones, including norepinephrine and epinephrine, in addition to its canonical signaling molecule, autoinducer-3 (AI-3). Many other kinds of bacteria, including staphylococci and Pseudomonas aeruginosa, respond to mammalian hormones and neurotransmitters—a phenomenon that Mark Lyte of Texas Tech University Health Sciences Center refers to as “microbial endocrinology” (Microbe, April 2009, p. 169). In effect, some microorganisms “listen” and respond to signaling pathways in host animal and plant species.

Within this broader field, my collaborators and I are studying how small molecules from intestinal cells of mammalian hosts that are infected with diarrheagenic E. coli affect the infectious process and host-pathogen interactions. We find, for example, that some diarrheagenic E. coli strains, especially enteropathogenic E. coli (EPEC), respond to the nucleoside adenosine, which is released from pathogen-damaged, host intestinal cells.

Once in the Public Health Spotlight, EPEC Is an Ancestor of Toxigenic Strains

Although enteropathogenic E. coli (EPEC) is no longer in the public health spotlight, several decades ago EPEC-induced disease was so widespread and well known throughout North America and Europe that it earned several descriptive slang terms, including “cholera nostra,” “cholera infantum,” “summer diarrhea,” and “summer complaint.” During the 1940s and 1950s, before the development of lifesaving oral rehydration therapy (ORT), EPEC caused large outbreaks of diarrhea among newborn infants, with fatality rates exceeding 50%. During that period, John Bray, a physician at Hillingdon Hospital in Middlesex, England, established the first link between a pathotype of E. coli with diarrheal disease. Erwin Neter, an infectious diseases physician in Buffalo, N.Y. (and cofounder of the ASM

Summary

- Small molecules from intestinal cells of mammalian hosts that are infected with diarrheagenic Escherichia coli affect the infectious process and host-pathogen interactions.
- In developing countries, both typical and atypical enteropathogenic E. coli (EPEC) are common causes of acute watery diarrhea in young children.
- ATP, ADP, AMP, and, eventually, adenosine have different but potent effects on intestinal cells, including stimulating cramping as well as secretion of fluid and electrolytes.
- Uric acid, the purine at the end of the catabolic pathway, signals danger, triggering inflammation and activating immune responses.
- In generating a series of nucleotide breakdown products, EPEC provides a vivid example of a “scorched-earth” infectious process.
journal *Infection and Immunity*) coined the term “enteropathogenic *E. coli*” shortly afterward.

EPEC decreased in incidence in developed countries during the 1960s and 1970s, dropping to a level where laboratories no longer found it worthwhile to test for EPEC by then-standard serotyping procedures. While classical EPEC remains rare in developed countries, newer variants of EPEC called atypical EPEC are now relatively common in both developed and developing countries. Further, in developing countries, typical and atypical EPEC coinmingle as common causes of acute watery diarrhea in young children.

Since EPEC was identified, several other diarrhea-producing *E. coli* expanded this group of pathotypes, including enterotoxigenic *E. coli* (ETEC) and enteraggregative *E. coli* (EAEC). During the past few decades, Shiga toxigenic *E. coli* (STEC, but also called EHEC), particularly the notorious *E. coli* O157:H7 strain, stole the spotlight from other diarrheagenic *E. coli* due to deadly outbreaks of disease in developed countries, including the United States. STEC is considered an evolutionary descendant of EPEC, and the two share many genetic and pathologic features, including the locus for enterocyte effacement (LEE), the intimate adherence phenotype, and Type III secretion. Unlike STEC, however, EPEC does not produce toxins.

### E. coli Pathotypes Interact with Host Cells and Effector Molecules

EPEC, STEC, *Salmonella enterica*, and several other pathogens release adenosine triphosphate (ATP) from intestinal cells (see box, p. 70). Once released from host cells, ATP may activate P2X receptors in nerves lining the gut mucosa, triggering pain sensations and cramping from the uncomfortable stretching and distention of hollow organs that typically accompanies intestinal infections that cause diarrhea. Once released into the extracellular milieu, microbial and host nucleotidases along the luminal surface of the intestinal tract convert much of this ATP into ADP, AMP, and, eventually, adenosine (Fig. 1). Adenosine has powerful effects on nearby intestinal cells, potently stimulating secretion of fluid and electrolytes into the gastrointestinal (GI) tract. These secretory effects are a major component of the watery diarrhea that occurs in response to EPEC, STEC, and similar pathogens.

In addition, adenosine has cytoprotective effects on intestinal cells, reducing their susceptibility to cell death. Third, adenosine can have strong anti-inflammatory effects, reducing the influx of neutrophils and the production of pro-inflammatory cytokines, including tumor necrosis factor-α.
**FIGURE 2**

Pro- vs. Anti-inflammatory properties of adenine nucleotides, nucleosides, and purines.

(TNF-α) and interferon-γ (IFN-γ; Fig. 2). However, exactly how adenosine affects inflammation and immunity depends on which adenosine receptor subtypes are most abundant in a particular segment of the intestine, the species of host animal, and the pathogen that is causing the infection. Indeed, some adenosine receptor subtypes, such as adenosine receptors A1 and A2b, trigger pro-inflammatory signaling cascades.

Meanwhile, adenosine exerts strong effects on EPEC bacteria (Fig. 1). For example, adenosine stimulates EPEC bacterial growth, changes the pattern that EPEC displays when adhering to cultured cells, and changes expression of several virulence genes.

Further, when rabbits are infected with such pathogens, adenosine levels affect how each animal responds to its infection. Adding exogenous adenosine deaminase (ADA) enzyme to rabbit intestinal loops, for example, reduces the numbers of EPEC bacteria recovered 20 hours later by more than 10-fold. Notably, although ADA-treated intestinal loops contain fewer bacteria, the loop fluid becomes bloody and contains many leukocytes. In contrast, loops infected with EPEC but no ADA contain non-bloody fluid and few leukocytes (Fig. 3). In the opposite situation, when an inhibitor of ADA, called EHNA, is added to raise adenosine levels in vivo, both the numbers of recoverable EPEC bacteria and fluid secretion into the intestine increase, providing additional evidence that adenosine affects both the host and pathogen.

Because adenosine is readily soluble in water, it can act on nearby uninfected intestinal cells, triggering them to secrete fluid. This effect is important because EPEC infections are patchy, meaning that some areas of epithelium are actively infected while others are spared. However, adenosine released from EPEC-infected cells likely acts on nearby uninfected cells with intact secretory functions, thereby adding to the severity of the infectious process.

**Other Biochemical Activities Enhance or Quell Infectious Turmoil**

The breakdown pathway for nucleosides does not stop at adenosine, however (Fig. 1). We have evidence that adenosine catabolism continues during EPEC infection, producing inosine, hypoxanthine, xanthine, and uric acid within the gut lumen. Xanthine oxidase generates both uric acid and hydrogen peroxide, which exerts a range of effects on host tissues, the pathogen, and likely on bystander commensal microbiota within the GI tract.

It is noteworthy that human milk contains substantial concentrations of several nucleotides and nucleosides, in the 2- to 10-μM range, as well as very high concentrations of xanthine oxidase. In fact, the xanthine oxidase activity in human milk is about 10 times higher than in blood serum or any other body fluid. Thus, it seems likely that, for breast-fed infants, the nucleoside breakdown pathway provides yet one more way to protect against enteric infections—along with immunoglobulins, lactoferrin, and oligosaccharides.

While the effects of adenosine on the immune system are mainly anti-inflammatory, the nucleosides and enzymes after adenosine can have pro-inflammatory effects. Indeed, plotting the pro-inflammatory versus the anti-inflammatory effects of all the nucleotides, nucleosides, and purines generated from the breakdown of ATP yields a U-shaped or V-shaped curve (Fig. 2).

Consistent with that profile, the purine at the end of the catabolic pathway, uric acid, acts to signal danger, triggering inflammation and acti-
vating immune responses. The degree of inflammation in response to EPEC or STEC infection may depend, then, on the speed with which these products are generated during infection, the concentrations achieved, and how long the products persist once formed. Our preliminary data suggest that uric acid accumulates in high concentrations in the intestinal lumen (200–600 μM, or 60 to 100 mg/L) during 20-hour experimental infections.

If EPEC infections routinely produce such high amounts of uric acid, this key effect failed until recently to attract much attention. However, infectious disease experts caring for children noted that children who were infected with enteric pathogens such as rotavirus, STEC, or *Salmonella*, tend to have high levels of uric acid in their serum or uric acid crystals in their urine. Although I did not find reports of hyperuricemia during EPEC infection, this may be due to fact that the bulk of EPEC infections now occur in poor areas where extensive laboratory testing of such patients is rare.

**E. coli** Pathotypes Trigger a Range of Host-Immune Responses

EPEC and STEC infections can be fatal. However, among survivors the infections produce a strong, serotype-specific immune response that usually prevents reinfection. That strong immune response helps to account for why EPEC primarily infects young children in developing countries. EPEC follows a similar pattern in pets and farm animals, usually afflicting young animals shortly after weaning but seldom infecting or reinfecting older animals.

EPEC belongs to a group of human-specific pathogens in which each strain has the opportunity to infect a particular individual only once. This group of microbes includes the viruses that cause measles, chickenpox, and dengue fever, as well as bacterial pathogens such as *Bordetella pertussis* and *Corynebacterium diphtheriae*. If General William Tecumseh Sherman had planned to

---

**FIGURE 3**

Effect of exogenous adenosine deaminase (ADA) on the tissue response to infection with rabbit enteropathogenic *E. coli* strain E22 (O103:H2), H&E stain, x200 original magnification. (A) Rabbit ileal loop infected with 10⁸ CFU EPEC E22, showing villus blunting and crypt hyperplasia. EPEC bacteria are stained blue on H&E stain and are visibly adhering in thick mats or biofilms on the intestinal epithelium (black arrows). Lumenal debris is scant and consists mostly of bacteria and sloughed epithelial cells. (B) Rabbit ileal loop infected with the same inoculum of EPEC bacteria, but with 35 units/ml ADA added at the time of infection. In the presence of ADA, there is bleeding into villi and into the intestinal lumen (turquoise arrows). In addition, there is now a massive lumenal cellular exudate, with many heterophiles (a type of polymorphonuclear leukocyte found in rabbits) in the lumen. Villus blunting is still observed. EPEC bacteria are present but are obscured by the inflammatory reaction. Despite the increased tissue damage, the number of EPEC bacteria recovered was significantly reduced in panel B (CFU/loop and CFU/ml). Size bars in both panels are 30 μm.
build a retirement home for himself in the Georgia Piedmont, he probably would not have burned crops, killed livestock, and destroyed farmhouses, towns, and villages from Atlanta to Savannah during the U.S. Civil War. Such behavior typically is seen when someone does not plan a long stay.

Some pathogens cause infections that simulate Sherman’s “scorched-earth” strategy. These pathogens inflict severe disease on their hosts, replicate to high levels, and trigger dramatic symptoms, but then must find new hosts, lest the immune responses that trigger EPEC replication lead, which provides opportunities for EPEC to disseminate to nearby hosts, including siblings, playmates, and attendees at daycare centers. However, some substances that EPEC infections release, such as hypoxanthine, xanthine, and uric acid, eventually help to eliminate those infections, both by activating innate immune responses of the host and later acquired immune responses.

Uric acid crystals are one of the best examples of a damage-associated molecular pattern (DAMP), also known as a “danger signal,” that is a natural counterpart to pathogen-associated molecular patterns (PAMPs). EPEC infection alerts the immune system by generating not only PAMPs like flagellin and bundle-forming pilus, but also the uric acid DAMP. Thus, EPEC is recognized not only as a stranger but also as a danger, to borrow phrases from immunologists Charles Janeway, Jr., late of Yale University, and Polly Matzinger of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health.

Uric acid activates acquired immunity in several ways. Monosodium urate crystals signal epithelial cells, neutrophils, and other host cells by receptor-independent pathways. In addition, uric acid crystals signal host cells via receptors, such as Toll-like receptor 2 (TLR2), TLR4, CD14 receptors, and the NALP3 receptor/inflammasome pathway on various cell types. Urate crystals can then activate cytotoxic T-cells, stimulate dendritic cells, trigger interleukin-1β secretion, or increase the antibody response to antigens.

In better understanding the pro- and anti-inflammatory pathways that EPEC infections trigger, we may learn how to better control overactive immune responses and to develop safer, more effective vaccine adjuvants. Thus, we hope eventually to reduce the numbers of deaths due to EPEC, STEC, and related pathogens.

EPEC generates a series of nucleotide breakdown products, providing a vivid example of a “scorched-earth” infectious process. Adenosine stimulates EPEC growth and replication, while the pro-secretory activity of this nucleoside in the GI tract of the host helps to generate profuse watery diarrhea, which provides opportunities for EPEC to disseminate to nearby hosts, including siblings, playmates, and attendees at daycare centers. However, some substances that EPEC infections release, such as hypoxanthine, xanthine, and uric acid, eventually help to eliminate those infections, both by activating innate immune responses of the host and later acquired immune responses.

Uric acid crystals are one of the best examples of a damage-associated molecular pattern (DAMP), also known as a “danger signal,” that is a natural counterpart to pathogen-associated molecular patterns (PAMPs). EPEC infection alerts the immune system by generating not only PAMPs like flagellin and bundle-forming pilus, but also the uric acid DAMP. Thus, EPEC is recognized not only as a stranger but also as a danger, to borrow phrases from immunologists Charles Janeway, Jr., late of Yale University, and Polly Matzinger of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health.

Uric acid activates acquired immunity in several ways. Monosodium urate crystals signal epithelial cells, neutrophils, and other host cells by receptor-independent pathways. In addition, uric acid crystals signal host cells via receptors, such as Toll-like receptor 2 (TLR2), TLR4, CD14 receptors, and the NALP3 receptor/inflammasome pathway on various cell types. Urate crystals can then activate cytotoxic T-cells, stimulate dendritic cells, trigger interleukin-1β secretion, or increase the antibody response to antigens.

In better understanding the pro- and anti-inflammatory pathways that EPEC infections trigger, we may learn how to better control overactive immune responses and to develop safer, more effective vaccine adjuvants. Thus, we hope eventually to reduce the numbers of deaths due to EPEC, STEC, and related pathogens.
ACKNOWLEDGMENTS
This work was supported in the past, but not currently, by the National Institutes of Health, via grants R21 AI 066055 and RO1 AI 50652. I thank Irina Shulgina, Tonniele M. Naeher, Swasti Majumdar, and Shilpa Choudhari for excellent technical assistance.

SUGGESTED READING