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Uropathogenic *Escherichia coli*-associated exotoxins

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Abstract

Escherichia coli are a common cause of infectious disease outside of the gastrointestinal tract. Several independently evolved *E. coli* clades are common causes of urinary tract and blood stream infections. There is ample epidemiological and in vitro evidence that several different protein toxins common to many but not all of these strains are likely to aid the colonization and immune evasion ability of these bacteria. This review discusses our current knowledge and areas of ignorance concerning the contribution of the hemolysin, cytotoxic necrotizing factor-1 and the autotransporters, Sat, Pic and Vat to extraintestinal human disease.

Introduction

From as long ago as 1929, a study by M. W. Lyon indicated that the *Escherichia coli* strains isolated from the urine of infected symptomatic individuals were different than the fecal isolates from healthy individuals (1). The *E. coli* urine isolates when grown on blood agar plate medium are commonly lytic to erythrocytes whereas the fecal isolates are infrequently hemolytic. This was the first suggestion that the *E. coli* involved in extraintestinal diseases (ExPEC) are phenotypically different than the commensal strains common to the gastrointestinal tract. We now appreciate that the presence of no single virulence gene including those encoding toxins is the sine qua non of ExPEC disease potential. This fact puts these *E. coli* in a much different context for disease potential than other classic human pathogens such as *Corynebacterium diphtheria*, *Vibrio cholerae* and *Bordetella pertussis* where specific toxin genes essentially define these bacteria as pathogens. I bring this issue up in the introduction to this chapter to make the point that among the variety of toxins that uropathogenic *E. coli* (UPEC) and more broadly, ExPEC possess, there are no examples of “must have” toxin genes. Why this is the case I will leave for the reader to ponder while reading this review. In my conclusion, I will provide some opinions about the matter. What will now follow are summaries of the research on several families of protein toxins associated with the pathogenesis of *E. coli* that cause urinary tract infections. This will include the repeats-in-toxin (RTX) family, cytotoxic necrotizing factor (CNF) and the auto-transporter (AT) family/type V-secretion family.

The *E. coli* hemolysin, the prototypical member of the RTX family

Before DNA-DNA hybridization technology made genotypic-based epidemiological studies of virulence genes in ExPEC possible, investigators were limited to a handful phenotypic

tests. As mentioned above, complete lysis of erythrocytes for bacterial colonies growing on red blood agar plates is a simple test. This phenotype ultimately proved to be a strong indicator of ExPEC strains that are more commonly associated with severe upper urinary tract infections, pyelonephritis and blood stream invasion (urosepsis) (2-5). Initially investigators separated these hemolytic *E. coli* into two, somewhat confusing categories. The alpha-hemolysin producing *E. coli* expressed a true extracellular, filterable hemolytic activity whereas the *E. coli* with a beta-hemolysin produced a hemolytic activity that was cell-bound and not found free in filtered culture supernatants (6). Until an example of each determinant was cloned and tested by DNA-DNA hybridization, it was unclear if the two hemolysins were different or similar. As it turns out they are encoded by a set of homologous genes organized in a four gene operon, *hlyCABD* (7, 8). The difference in extracellular activities reflects several factors. The *E. coli* hemolysin activity is relatively labile where aggregation leads to rapid loss of activity in even freshly prepared supernatants kept on ice. In addition, there is a curious variability in the promoter sequences among hemolysin determinants found in *E. coli* strains (8, 9). Among the different hemolysin determinants, there are non-homologous sequences immediately upstream of the start codon for the *hlyC* gene. In only a handful instances, have the 5' ends of the *hlyCABD* transcript been identified (8). What is clear is that this evolutionary event has led to significant differences in the levels of *hlyCABD* transcription and subsequent expression of hemolysin activity. The classic alpha-hemolysin producers possess stronger promoters whereas strains characterized as beta-hemolysin producers have weaker promoters. So when investigators tried to assess extracellular hemolytic activity in the beta-hemolysin isolates, the weak expression and lability of hemolytic activity lead them to the erroneous conclusion that the hemolytic activity was cell-bound. Therefore, I have been a proponent of dropping the antiquated alpha- versus beta-hemolysin nomenclature and simply referred to all *hlyCABD*-encoded activities as the *E. coli* hemolysin.

Experimental evidence that the *E. coli* hemolysin is a virulence factor for UPEC

One of the first applications of Molecular Koch's Postulates was with the demonstration that the *E. coli* hemolysin is a virulence factor in a rat model of intra-abdominal peritonitis (10, 11). A recombinant hemolysin plasmid, pSF4000 was transformed into an avirulent, non-hemolytic, normal human fecal *E. coli* strain, J198. When this construct was injected intraperitoneally into rats, the result was a 1,000-fold decrease in the lethal dose 50% (LD₅₀) compared to J198 alone. When a nonhemolytic transposon insertion mutant plasmid, pSF4000::Tn1 was transformed into J198 and used to challenge rats, there was an increase in the LD₅₀ back to the level of J198 alone. These results have essentially stood as the primary evidence that the *E. coli* hemolysin acts as a significant virulence factor for ExPEC. A subsequent publication that lent support to this showed that the differences in hemolysin expression that result from different promoters is directly related to the relative levels of virulence in the peritonitis model (9).

Despite the compelling epidemiological evidence that implicates the *E. coli* hemolysin is a significant virulence factor for pyelonephritis and urosepsis isolates and our in vivo

peritonitis model results, the application of Molecular Koch's postulates to demonstrate the hemolysin is a virulence factor for UPEC is not entirely persuasive. In vitro the *E. coli* hemolysin is clearly cytotoxic to primary cultured renal cells (12, 13). O'Hanley et al. showed that the hemolysin contributed to renal parenchymal damage in a murine model of urinary tract infection (UTI) but it did not influence colonization levels in the kidney (14). Interestingly, that group also showed using the mouse UTI model that immunization with denatured hemolysin provided significant protection against the hemolysin-mediated damage in the mouse kidney (14). There are some technical issues with this study that should be pointed out. The UTI model involved dehydration of the mice with the animals denied water for 24 hrs prior to bacterial inoculation. Second, the immunization protocol involved complete and incomplete Freund's adjuvant and repeated intramuscular injections. Lastly, there was elevated hemolysin expression in the *E. coli* challenge strains compared to natural hemolytic UPEC strains. The investigators took *E. coli* pyelonephritis isolates that were non-hemolytic and transformed them with a medium copy number, recombinant plasmid, pSF4000. As was the case with my earlier peritonitis studies, exaggerated expression of hemolysin clearly can lead to in vivo pathology and morbidity, but what role does hemolysin play in uropathogenesis when expressed at its native levels? A recent paper from Alison O'Brien's laboratory demonstrated that mice immunized with a hemolysin toxoid had 10-fold reduced bacterial numbers in the urine when compared to sham-immunized mice in the UTI model (75). The hemolysin immunized mice also had reduced evidence of bladder inflammation compared to the control mice (75). This report represents some of the best evidence to date that hemolysin plays a significant role in causing inflammation and a selective advantage to the UPEC strains that express this toxin.

A report by Nagy and coworkers described results with UPEC model strain 536 in several animal models where deletion mutations of the two chromosomally encoded *hlyA* genes result in 536 virulence attenuation (15). The authors concluded that their experiments demonstrate that the hemolysin is a virulence factor in an ascending model of urinary tract infection. The model they employed involved intravesical inoculations of 3–4 day-old suckling mice and the mice were not separated as to sex. Intravesical inoculation of male adult mice is difficult to do without causing urethral bleeding. Second, bacterial colonization levels were not the end-point of their analysis rather death of the pups was used. Thus this model did not measure bladder or kidney colonization or histopathology which are the end points commonly used in the adult murine UTI model.

In the late 1990s Shai Pellett, Rachel Quinn and I constructed a site-directed hemolysin knock-out mutant in the model UPEC strain J96. We tested *E. coli* J96 and J96*hlyA* in single infections in the broadly employed adult female mouse UTI model. The results went unpublished because we observed no statistical difference in the number of colony-forming units for the two strains in either the bladder or kidneys at 48 hrs post-inoculation. However, we did publish later that a hyper-colonization mutant of UPEC model strain CFT073, CFT073*dsdA*, lost its hyper-colonization ability with mutational loss of *hlyA* in bladders and kidneys at 48 hrs post-inoculation (16). Although this result suggests that the hemolysin can aid urinary tract colonization, the hyper-colonization mutant is certainly not a natural UPEC isolate. Alison O'Brien's laboratory tested an isogenic hemolysin mutant pair with UPEC model strain CP9 in the murine UTI model (17). These authors performed both single as

well as competitive infections of mice with neither inoculation strategy revealing a impact on colonization levels in either the bladder or kidneys at three different time points, 1, 2 and 5 days post-inoculation. This makes it quite clear that hemolysin expression does not influence UPEC colonization numbers in the mouse urinary tract. However, they were able to make an important histopathological observation. They saw that the bladders taken from CP9 infected mice 24 hrs post-inoculation are more grossly inflamed and hemorrhagic than the bladders infected with the CP9*hlyA* mutant strain. This inflammation effect wanes at later time points in the infection. They also observed that the hemolysin was responsible for extensive sloughing of the urothelium in the bladder. The sloughing of the bladder urothelial cells during UTI is a common observation in this model and it is fundamentally similar to what is observed during human infections (18). A study that compares the hemolysin status of *E. coli* causing a clinical infection and the amount of urothelial cell sloughing and damage is needed to support this role in human infection.

***E. coli* hemolysin: structure, function and regulation**

Previously my colleagues and I have written reviews on the structure and mechanisms of cytotoxic activity of the *E. coli* hemolysin and I suggest consulting those publications for more specific details (19–21). For the purpose of this chapter I will provide a brief synopsis and a few new facts that have been published in the past several years. As mentioned above the hemolysin is produced through the direct action of four genes encoded on an operon, *hlyCABD*. The actual secreted protein product, HlyA is 110 kilodaltons in size and is encoded by *hlyA* (7). HlyA is post-transcriptionally modified heterogeneously at two lysine residues with 14-, 15- and 17-carbon fatty acids through the enzymatic activity of *hlyC*. The acylation is required for hemolytic and cytotoxic activities, but the modification is not required for extracellular secretion. The immature, unacylated and mature forms of the hemolysin are respectively referred to as proHlyA and HlyA. The *hlyB* and *hlyD* genes encode proteins that are involved in the secretion of the cytoplasmically modified HlyA polypeptide across the inner and outer membranes without amino-terminal cleavage of the polypeptide (22). These proteins form a type I secretion complex together with an unlinked gene product TolC (23). The *E. coli* hemolysin is generally considered to be the prototypical type I secretion protein in gram-negative bacteria. The family members share a motif of nine amino acid repeats near their C-terminal end that are involved in Ca²⁺-binding and it is this structural motif that led to the name of the repeats-in-toxin, RTX family (24). These proteins are secreted without aid of the general leader-peptide-dependent secretory pathway and the most C-terminal amino acids are involved in targeting these proteins for export by the HlyB-HlyD-TolC envelope complex (25, 26).

The RTX family of toxins produced by different gram-negative pathogens has members with narrow host and target cell specificities. For example, the non hemolytic *Mannheimia haemolytica* leukotoxin is cytotoxic to bovine and ovine leukocytes but not epithelial, and endothelial cells (27). Unlike the homologous RTX leukotoxins, the *E. coli* hemolysin appears to be cytotoxic to many different host cell types and towards many species of hosts, see review (21). The differences in target and host cell specificity appear to due to primary sequence and N-linked oligosaccharide modification differences in the primary receptors for the toxins, β 2 integrins on immune cells (27–29).

The mechanism for *E. coli* hemolysin cytotoxicity is commonly believed to involve the formation of membrane pores. When erythrocytes are used as a target cell, loss of intracellular K⁺ ions to the environment and subsequent influx of cations and water leads to osmotic lysis (30, 31). The use of extracellular osmotic protectants of defined size suggests a hemolysin-mediated membrane pore that is 2 nanometers in diameter (32). Mahtab Maoyeri and I published observations that suggested that although initial incubations of toxin with osmotic protectants and erythrocytes indicates a 2 nm pore, there is a hemolysin-concentration and time-dependent changes in the apparent size of the membrane pores (33). Helle Praetorius's laboratory recently published some elegant work that indicates there is a bi-phasic hemolytic process by the *E. coli* hemolysin (30, 34). She and her colleagues found that the initial pore formation leads to partial lysis of erythrocytes, but that the pore formation triggers purinergic receptor activation and creation of a host-mediated pannexin pore. These events then lead to full lysis of the hemolysin-treated cells. The initial pore does not lead to osmotic swelling of the erythrocyte, rather there is a delay until the secondary activation events occur when the erythrocytes then swell and lyse.

There are numerous studies on the in vitro effects of the *E. coli* hemolysin on host cells and it is difficult to assess which of these are relevant to the pathogenesis of UPEC. It seems unlikely that lysis of erythrocytes is significant although the canonical hypothesis is that release of iron-containing heme molecules is an important role for bacterial hemolysins in disease pathogenesis. As for the *E. coli* hemolysin activity against other host cell types, there are several variables that confound interpretations and conclusions about its role in uropathogenesis. The most significant variable is the presence of lipopolysaccharide (LPS) in *E. coli* hemolysin preparations. In general, at low, sub-lytic or apoptotic-inducing concentrations, the hemolysin triggers pro-inflammatory events (35–37). The critical issue to be resolved is to what degree are these proinflammatory events dependent on LPS present in hemolysin preparations. Mansson et al. showed that the classic Ca²⁺ signaling seen in many different cell types upon exposure to hemolysin is dependent on the presence of LPS co-purified with hemolysin protein, HlyA (38). In addition, the LPS present mediates the delivery of hemolysin via the classic LPS-CD14 pathway and not the pattern recognition molecule, TLR4 commonly stimulated by LPS (38). Although it may be entirely coincidental, it is intriguing that the hemolytic activity of the hemolysin is profoundly affected by the structure of LPS (39, 40). There is decreasing activity and loss of expression of the extracellular HlyA in strain backgrounds where there are successive truncations of the LPS to make progressively rougher strains. Associated with the loss of activity is an increase in the apparent size of the hemolysin. The conclusion drawn is that during or after secretion of HlyA, the polypeptide becomes associated with LPS. The rough forms of LPS forms become more hydrophobic with the loss of the O-antigen and outer core constituents. These species then more readily aggregate with HlyA into hemolytic or cytotoxic inactive forms. Shai Pellett and I find that in order to prepare endotoxin-free hemolysin as measured by the limulus assay, HlyA has to be denatured by boiling with 1% sodium dodecyl sulfate (SDS), and separated from LPS by SDS gel electrophoresis. HlyA is then eluted from excised gel fragments and dialyzed, before then being dissolved in 0.9% NaCl containing 1 mM CaCl₂ (38). In this purified form, HlyA hemolytic and cytotoxic activity is stable for months at 4°C in contrast with HlyA-containing material present in filtered bacterial culture supernatants.

Extracellular HlyA in its native, in vivo state is probably never free of LPS and experiments to assess HlyA events independent of LPS are unrealistic and likely do not reflect the in vivo situation. The caveat to this supposition is the possibility that in vivo, HlyA effects on the host are not the result of extracellular HlyA that comes in contact with host cells. Rather, it is easy to envision that HlyA is delivered upon close cell-to-cell contact between hemolytic *E. coli* and host cells. Aside from the flagellar apparatus common to nearly all UPEC strains, genomic sequence analysis indicates there are no type III secretion systems present in UPEC strains. However, common to the pathogenicity islands encoding the *hlyCABD* genes are the pyelonephritis-associated pili (pap) and this close linkage remains suspicious. The co-expression of hemolysin and pap fimbriae that mediate host-cell adhesion could provide a means to deliver the HlyA to host cells with little or no extracellular exposure.

A second experimental variable when considering the significance of the hemolysin in uropathogenesis is the hemolysin concentrations employed in the different studies. As mentioned above, at sub-lytic or sub-necrotic concentrations, the hemolysin can influence different host signal pathways and in vitro appears to be pro-inflammatory in nature. It is also clear based on the in vivo studies mentioned above that the hemolysin can cause cell destruction and inflammation in the bladder or kidney. In contrast to those reports, Wiles et al. recently showed that the hemolysin inhibits activation of host cell Akt (protein kinase B) and may inhibit inflammation (41). Hemolysin causes uncontrolled activation of host protein phosphatases and proteases (41-42). The negative affect on Akt activation was proposed to lead to a reduction in NFκB. These investigators compared Akt activation states in 5637 human bladder epithelial cells that were challenged with either model UPEC strain UTI89 or an isogenic UTI89*hlyA* mutant. The inhibition of Akt activation by the *E. coli* hemolysin was not dependent on fluxes in intracellular Ca²⁺ nor extracellular K⁺ leakage, events commonly attributed as the very initial events mediated by HlyA upon membrane insertion. In this study two other pore-former toxins, the *Staphylococcus aureus* α-toxin and the *Aeromonas hydrophilia* aerolysin also caused inhibition of Akt activation. The authors speculate that the osmotic-stress created by three different pore former toxins leads to the identical event (41). Dhakal and Mulvey recently showed with cultured bladder epithelial cells or mouse peritoneal macrophages that the *E. coli* hemolysin activates host cell serine proteases such as mesotrypsin. This results in degradation of the cytoskeletal scaffolding protein paxillin, components of the pro-inflammatory NFκB signaling cascade and caspase 3/7 (42). There is a significant reduction in the production of the inflammatory cytokine, IL-6 attributed to hemolysin intoxication. The induction of the proteolytic cascade does not occur with treatment of the cells by two other pore-forming toxins, the *S. aureus* α-toxin or the *A. hydrophilia* aerolysin. Dhakal and Mulvey propose that the hemolysin-inhibition of cellular inflammation helps explain that UPEC cause UTIs because they suppress inflammation when in the urinary tract. This is an interesting hypothesis supported with results in several laboratories (43-45).

Lastly, the significance of the hemolysin to uropathogenesis is indirectly supported by the observation that expression of critical virulence determinants for UPEC, O-antigens, type II capsules and the ChuA hemin-utilization factor are positively co-regulated with the hemolysin operon by the specialized virulence NusG-like transcriptional anti-terminator, RfaH (46-50). As expected, a mutation in *rfaH* renders UPEC highly attenuated in rodent

models of disease (51). Interestingly, it has been recently shown that RfaH represses biofilm formation (52), a trait often touted to be important for uropathogenesis (53, 54)

A second chromosomal RTX family member, UpxA in UPEC

A second RTX-like gene, *upxA* and linked type I transport genes are apparent in the chromosome of classic UPEC model strain CFT073 (55). This gene was originally annotated as *upxA* with this designation standing for u^{ro}pathogen RTX A gene which is consistent with the general pattern of including X in the names of RTX genes. This nomenclature was disregarded by Parham et al in 2005 when they renamed *upxA*, *tosA* which they designated for t^{ype} o^{ne} secretion gene A (56). Recently, Vigil et al. have published interesting results that indicate *upxA* (*tosA*) provides a critical adherence function for CFT073 in the kidney in the murine model of urinary tract infection (57, 58). In addition UpxA (TosA) promoted CFT073 survival during experimental murine sepsis and could serve as a protective antigen during murine urosepsis (79). Unfortunately, these authors utilized the *tosA* nomenclature (58).

Cytotoxic necrotizing factor type 1 (CNF1)

CNF1 was originally described as a toxin that caused dermonecrosis when CNF-producing *E. coli* strains were injected intradermally in rabbits. Its first described cytotoxic activity was the formation of multi-nucleated cells in culture (59). Caparioli et al. found that 40% of UTI *E. coli* isolates produce CNF1 whereas only 1% of the normal fecal isolates produced the toxin (60). Epidemiologically like the *E. coli* hemolysin, CNF1 production is more often associated with UPEC strains responsible for more severe UTIs, Andreu et al. showed that 48% of the pyelonephritis isolate were *cnf1* positive (61). The curious and enigmatic observation is that when *cnf1* occurs in UPEC strains, it is always linked to the *hlyCABD* operon (62). It occurs 3' to that operon and is co-transcribed with the *hlyCABD* genes and positively regulated by RfaH (62). The converse is not true however, where UPEC strains can be commonly found with chromosomally encoded *hlyCABD* operons without *cnf1* being present (55).

CNF1 is a 115-kDa protein that catalyzes the deamidation of a conserved glutamine residue in three members of the Rho family of GTP-binding proteins. This leads to the activation of three specific GTPases, RhoA, Cdc42 and Rac (63, 64). The CNF1 protein is divided into three functional domains (65). The enzymatic C-terminal half of the CNF1 polypeptide is present in the host cytosol after cleavage and release from late endosomes (66). The very N-terminal CNF1 domain appears to be responsible for binding to a laminin receptor precursor protein (67, 68). An internal CNF1 domain is responsible for translocation across the endosomal membrane (69). The activation of these regulators leads to cytoskeleton rearrangements, cell cycle disruption and interruption of host cell signaling pathways (70). Hofman and coworkers found that CNF1 treatment of neutrophils led to increased production of reactive oxygen molecules but a decrease in the ability of those cells to phagocytize bacteria (71). Later, Davis et al. showed that CNF1 synthesis leads to increased survival of UPEC in association with isolated human neutrophils (72). CNF1 treatment of cultured epithelial cells leads to uptake of noninvasive bacteria and latex particles (73).

There is also a significant increase in vitro Rac1-dependent invasion of epithelial cells by UPEC strains expressing CNF1 (74- 75).

As is the case for hemolysin, the role of CNF1 in uropathogenesis is not entirely clear despite the evidence that it induces neutrophil dysfunction and increased epithelial cell invasion. Johnson et al. reported in 2000 that an *E. coli* cystitis isolate, F11 was not attenuated in the mouse UTI model in terms of bladder and kidney colonization at either 2 or 7 days post-inoculation upon the mutational loss of *cnf1* (76). There also were no changes in the relative amount of inflammation of the infected bladders or kidneys between single challenges of F11 and F11 *cnf1*. These results are in contrast to a report a year later where in a competitive murine bladder colonization model, the model UPEC pyelonephritis strain CP9 outcompetes a CP *cnf1* mutant (77). However, Alison O'Brien's laboratory did show with the same strains in single strain, murine bladder infections that there is no statistical difference in the mean number of colony-forming units (CFUs) of the wild type strain and the *cnf1* mutant at one day post-inoculation (17). A CP9 *hlyA cnf1* mutant was also tested in the single challenge murine UTI model and again there were no statistically significant differences in the mean of CFUs from bladders or kidneys for this mutant and the wild type. In this same study, it was demonstrated that CNF1 does cause bladder inflammation and edema at 3 and 5 days post-inoculation (17). However, the decrease in bladder inflammation attributable to CNF1 does not result in greater colonization ability of a CP9 *cnf1* mutant. Real et al. described a study where they monitored the levels of inflammatory mediators, IL-8, MCP-1 and MIP3a in the urine of patients with UTIs (78). They found that the patients with the strongest inflammatory responses and with high red blood cell counts in their urine were disproportionately infected with UPEC strains possessing the genes for *hlyA* and *cnf1*. A recent publication by Boyer et al. that suggests that in some contexts, CNF1 acts as an avirulence factor (79). They show using a *Drosophila melanogaster* traumatic wound model that the presence of CNF1 in UPEC strain J96 induces a protective immune response and that there is an increased infectious burden in the flies when challenged with a J96 *cnf1* mutant. They demonstrate that CNF1 activates Rac2, this activates innate immune adaptors, IMD and Rip1-Rip2 and ultimately leads to increased production of protective antimicrobial peptides.

A recent more detailed review of the enzymatic mechanism and in vitro and in vivo, effects of CNF1 is available (80). Interestingly, in this review there is discussion of how CNF1 is being extensively studied as a pharmacologic tool to control pain. CNF1 apparently holds some promise as another bacterial toxin that may have practical clinical benefit in some settings.

Toxins in Type V Secretion Family

Based on genomic DNA sequence analysis of model UPEC strains there are 10 or more different genes of the Type V Secretion family (T5S) present in the chromosome (81). This family is also often referred to as the autotransporter (AT) family. In UPEC model strain CFT073, there are 10 such genes (82). The function of many of these remain to be elucidated, but those that have been characterized fall into two broad functional categories, serine proteases (the SPATE AT subfamily) and adhesins such as Antigen 43 (Ag43) (83)

and UpaB (82). The SPATE family members are further divided into two classes, 1 and 2 which respectively represent toxic vs. nontoxic proteases (84). These extracellular serine proteases are produced by a variety of *E. coli* pathovars besides UPEC.

The UPEC toxin SPATE family member that has received the greatest attention is the secreted autotransporter toxin (Sat). The *sat* gene is present along with the hemolysin and pap pili determinants within the large *pheV*-associated pathogenicity island of UPEC model strain CFT073. The *sat* gene encodes a 142 kDa protein that possesses the three characteristic domains of SPATE proteins (85). ATs possess a long N-terminal signal sequence, a secreted passenger domain and canonical C-terminal autotransporter domain that provides the cell envelope export channel. In the case of Sat, the mature extracellular polypeptide is a 107 kDa in size (86). The *sat* gene is present by 68% of *E. coli* strains isolated from pyelonephritis but occurs in only 14% of normal fecal *E. coli* isolates (86). Guyer et al. demonstrated Sat possessed potent cytopathic effects to cultured cells and in the murine model of UTI, histopathological lesions in the kidney can be attributed to Sat (87). However, in single strain, challenge experiments, a CFT073*sat* mutant does not have a statistically significant reduction in bacterial load in either the bladder or kidney compared to the parent strain CFT073. Mononcle et al. demonstrated through construction of active mutants that Sat proteolytic activity was responsible for cytotoxic activity, cytoskeleton rearrangements and proteolysis of host proteins (85). Recently Moal et al. showed in cultured HeLa cells that the Sat proteolytic activity initiates disorganization of F-actin that results in detachment of the cell monolayers and loosening of cell-to-cell junctions (88). These events then induce host cell autophagy. These authors speculate that Sat may contribute to the exfoliation of the urothelial cells, an event commonly seen during model UTIs.

There are two other SPATE family members commonly found in UPEC, Pic and Vat which were first described respectively in *Shigella flexneri* and avian pathogenic *E. coli* (89-90). In UPEC model strain CFT073, the genes for these two ATs are located respectively at the *aspV*- and *thrW*-associated pathogenicity islands. Pic possesses mucinase activity towards O-linked glycoproteins, such as CD43, CD45 and fractalkine commonly found on neutrophil surfaces (91). Pic treatment of neutrophils in vitro results in chemotaxis and transmigration defects. The Pic treated neutrophils are also stimulated to produce an oxidative burst (91). Lloyd et al. demonstrated that a CFT073*pic* mutant colonizes the mouse bladder ~ 50-fold less than wild type CFT073 (92). Vat is vacuolating AT toxin that occurs in more than half of *E. coli* cystitis and pyelonephritis isolates (81). There are no reports of the significance of Vat in UPEC in vitro or in vivo model systems. It has been demonstrated to be a significant virulence factor in avian pathogenic *E. coli* using respiratory and cellulitis infection models of disease in broiler chickens (90).

Summary

A wealth of epidemiological evidence supports that the UPEC toxins reviewed in this chapter are virulence factors. It is clear that UPEC exotoxins cause dramatic in vitro effects to primary and cultured host cells relevant to human UTIs. It does remain a conundrum, why in the murine UTI model, do we see that UPEC *hlyA*, *cnf1*, or *sat* mutants cause less

inflammation without a subsequent increase in the number of mutant bacteria recovered in the infected tissues? The pervasive rationale for this observation is that UPEC strains evolved redundant in exotoxin activities that cumulatively impede host immune responses. There is certainly precedence for redundancy of virulence factors in UPEC as seen adhesins and systems for iron acquisition. I contend that another rationale should be considered, the murine model of UTI simply lacks sufficient functional similarity to human urinary tract infection. The mouse model has aided in our understanding of UPEC colonization and growth in the urinary tract, but in terms of the interplay of toxins, host cells and inflammation, the mouse model fails us in its present form.

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Table 1Prominent Protein Toxins of Uropathogenic *Escherichia coli*

Toxin	Exoprotein Family	Action	General Epidemiology
Hemolysin (HlyA)	Type I	Membrane pore-formation	Common to UPEC strains that cause pyelonephritis and sepsis, less common to cystitis isolates, infrequent in fecal isolates
Cytotoxic Necrotizing Factor-1 (CNF-1)	Genetically linked to Type I hemolysin operon	Deamidation of GTPases, RhoA, Cdc42 and Rac	~30–40% of pyelonephritis UPEC isolates, rare in fecal isolates
Secreted Autotransporter Toxin (Sat)	Type V	Serine protease that targets cytoskeletal proteins	Common to UPEC strains that cause pyelonephritis and sepsis, less common to cystitis isolates, infrequent in fecal isolates
Protein involved in intestinal colonization (PicU)	Type V	Serine protease that targets O-linked glycoproteins	~50% in cystitis and pyelonephritis UPEC isolates
Vacuolating autotransporter toxin (Vat)	Type V	Serine protease that causes vacuole formation in cultured bladder cells	~50% in cystitis and pyelonephritis UPEC isolates