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MINIREVIEW

Waging War against Uropathogenic *Escherichia coli*: Winning Back the Urinary Tract[▽]

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Urinary tract infection (UTI) caused by uropathogenic *Escherichia coli* (UPEC) is a substantial economic and societal burden—a formidable public health issue. Symptomatic UTI causes significant discomfort in infected patients, results in lost productivity, predisposes individuals to more serious infections, and usually necessitates antibiotic therapy. There is no licensed vaccine available for prevention of UTI in humans in the United States, likely due to the challenge of targeting a relatively heterogeneous group of pathogenic strains in a unique physiological niche. Despite significant advances in the understanding of UPEC biology, mechanistic details regarding the host response to UTI and full comprehension of genetic loci that influence susceptibility require additional work. Currently, there is an appreciation for the role of classic innate immune responses—from pattern receptor recognition to recruitment of phagocytic cells—that occur during UPEC-mediated UTI. There is, however, a clear disconnect regarding how factors involved in the innate immune response to UPEC stimulate acquired immunity that facilitates enhanced clearance upon reinfection. Unraveling the molecular details of this process is vital in the development of a successful vaccine for prevention of human UTI. Here, we survey the current understanding of host responses to UPEC-mediated UTI with an eye on molecular and cellular factors whose activity may be harnessed by a vaccine that stimulates lasting and sterilizing immunity.

MISSION: ERADICATE UPEC-MEDIATED UTI

Urinary tract infection (UTI) is one of the most common infections in humans. Bacteria present in fecal matter inoculate the periurethral area, then the bladder (124, 176, 291), causing symptoms clinically termed cystitis. Left untreated, bacteria ascend the ureters to the kidney and establish a secondary infection, acute pyelonephritis. At this juncture, there is risk of permanent renal scarring, and bacteria can access the bloodstream (282). It is estimated that 40% of women and 12% of men will experience a symptomatic UTI, with incidences peaking in their early 20s or after age 85, respectively (75, 185). Approximately 25% of these women will experience recurrence within 6 to 12 months (75, 185). Uropathogenic *Escherichia coli* (UPEC) is the most common etiological agent responsible for uncomplicated UTI (93, 94, 282). Uropathogenic strains are classified as extraintestinal pathogenic *E. coli*, a broad grouping of *E. coli* that cause diseases other than gastroenteritis and typically lack a type III secretion system (171, 220, 221, 283, 284). Nonetheless, UPEC strains express an assortment of virulence and fitness factors that aid in successful colonization of the mammalian urinary tract (126, 136). In the United States alone, the estimated annual societal cost of UTI is more than 3 billion dollars (159).

Despite a relatively in-depth knowledge base for UPEC physiology and virulence mechanisms (reviewed in references 54, 126, 136, and 284), no licensed vaccine to prevent UTI exists in the United States. A more thorough understanding of the mechanisms involved in the natural immune response to UTI, however, may direct a new approach to harness these responses in a vaccination setting. In this review, current and potential treatments for UPEC-mediated UTI will be surveyed, as well as efforts to identify suitable vaccine candidates. Factors involved in host responses to UTI (summarized in Table 1) and the mechanisms by which UPEC stimulates and influences these responses will also be covered. Lastly, commentary on the steps needed to better understand infection and immunity in the urinary tract and to develop a vaccine that elicits heterologous protective immunity against UPEC is given.

TERRAIN: THE URINARY TRACT

The urinary tract is an impermeable barrier that undergoes continuous expansion and contraction (9). These fluctuations are achieved on a gross level by unfolding of the mucosal surface and on a molecular level by cellular membrane dynamics. Specialized fusiform vesicles are thought to be endo- and exocytosed at a rate that provides additional membrane surface area during expansion (9). This unique mucosa is a transitional epithelium with large, highly differentiated, multinuclear superficial facet (umbrella) cells lining the luminal surface (Fig. 1A and B) (9). The apical side of the umbrella cells consists of a detergent-insoluble membrane containing a family of integral membrane proteins termed uroplakins (9). Uroplakins are partly responsible for the barrier function of the uroepithelium and act as receptors for FimH, the tip adhesin of UPEC type 1 fimbriae (298). For the purposes of this

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TABLE 1. Summary of mammalian factors associated with the host response to UPEC-mediated UTI

Category or factor	Known or proposed role during UTI	Reference(s)
Adherence prevention		
Apoptosis/exfoliation	Removal of bound and intracellular UPEC	10, 60, 81, 166, 176, 177, 189
GAGs	Decreases uroepithelium affinity for and/or inhibits UPEC adherence factors	120, 158, 196, 197, 199, 237
THP	Binds FimH, innate-adaptive signaling	172, 190, 192, 193, 195, 225
Antimicrobial peptides		
α-Defensin	Disrupt UPEC membranes	234, 294
β-Defensin	Disrupt UPEC membranes, DC maturation	28, 174, 277
Cathelicidin	Disrupt UPEC membranes	46
Extracellular matrix		
Involucrin	Maintenance of uroepithelium	215
Suprabasin	Maintenance of uroepithelium	215
Signaling and cellular function		
Adaptor proteins		
ARH, Dab2, Numb	Alternative clathrin adaptors for UPEC internalization	64
AP-2	Primary clathrin adaptor for UPEC internalization	64
MyD88	Proinflammatory gene expression	73
TRIF	Proinflammatory gene expression	73
TRAM	Proinflammatory gene expression	73
Enzymes		
AC3	Proinflammatory gene expression, UPEC internalization suppression	243, 245
Histone deacetylase 6	UPEC internalization	53
Rho GTPases (Rac-1, Cdc42)	Mobilize actin for UPEC internalization	56, 161, 243
Lysozyme	Cleaves bacterial peptidoglycan	215
NOS enzymes	Production of bactericidal reactive oxygen radicals, modifies dynamin facilitating UPEC entry into host cells	130, 132, 181, 205, 281
Kinases		
Aurora A kinase	UPEC internalization	53
FAK	UPEC internalization	65
PI3-K	UPEC internalization	162
PKA	Proinflammatory gene expression, UPEC internalization suppression	243, 245
Src family kinases	UPEC internalization	65, 162
Tyrosine kinases	UPEC internalization	162
Membrane and cytoskeleton		
Actin	UPEC internalization	162
α-actinin	UPEC internalization	162
Bid	Apoptosis	141
Cholesterol	UPEC internalization	64
Clathrin	UPEC internalization	64
Caveolin-1	UPEC internalization	56, 64, 133
Dynamin	UPEC internalization	64, 281
Kinesin light chain-2 (kinesin-1 motor complex)	UPEC internalization	53
Microtubules	UPEC internalization	53
Vinculin	UPEC internalization	162
Receptors		
β1 and α3 integrin	UPEC internalization	65
CD46	Opsonized UPEC internalization	157
CD48	UPEC internalization	18, 139
CD55	Regulator of the complement system, UPEC internalization	133, 215
Crry	Opsonized UPEC internalization	247
GM-1 ganglioside	UPEC internalization	133
ICAM-1 and β2 integrin	Required for efficient neutrophil migration to the infected urinary tract	3, 227
TLR4	Recognition of UPEC by LPS and/or fimbriae in the bladder and kidney	12, 14, 72, 79, 104–106, 175, 228, 229
TLR5	Recognition of UPEC flagellin in the bladder	7
TLR11	Recognition of UPEC in the kidney	296
Uroplakin IIIa	Apoptosis/exfoliation	258, 259
Signaling molecules		
Calcium	Apoptosis/exfoliation, proinflammatory gene expression, UPEC internalization	64, 245, 259
cAMP	Intracellular UPEC vesicle dynamics, mobilize actin for UPEC internalization, proinflammatory gene expression	29, 243, 245
Transcription factors		
CREB	Proinflammatory gene activation	245

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TABLE 1—*Continued*

Category or factor	Known or proposed role during UTI	Reference(s)
NF-κB	Apoptosis, proinflammatory gene activation	73, 142, 245
Iron homeostasis		
Lactoferrin	Iron sequestration, disruption of Gram- negative bacterial membranes	2, 49, 61, 90, 279
Lcn2	Bind and sequester enterobactin and enterobactin-like siderophores	70, 74, 88, 110, 215
Transferrin	Bind and transport iron, disruption of Gram- negative bacterial membranes	61, 213, 279
Cytokines, chemokines, and complement		
CCL2, CCL4, CCL5, CXCL1	Migration of T cells, monocytes, macrophages, NK cells, DCs, neutrophils	121, 122
G-CSF	Neutrophil development and differentiation	121, 122
IL-6	Acute phase response, B and T cell activation	103, 104, 121, 122
IL-8	Neutrophil migration	3, 4, 99, 102, 169, 229, 287
IL-17	Induce cytokine production important for phagocytic cell migration	Sivick et al., submitted
IL-1β, IL-12p40, TNF-α	Fever; T cell, NK, macrophage, and endothelial activation; Th1 response, local inflammation	121, 122
C3, factor B	Complement factors upregulated in response to infection, C3 utilized for UPEC internalization	215, 247
Phagocytic cells		
Dendritic cells	Bacterial phagocytosis and killing, antigen presentation, IgA secretion	62, 109, 144, 227, 255
Macrophages	Bacterial phagocytosis and killing, antigen presentation	63, 109, 121
Neutrophils	Bacterial phagocytosis and killing	4, 100, 236
Innate-like lymphocytes		
B-1 cells	Secrete IgM	23, 115
NKT cells	Rapidly secrete cytokines, mediate UPEC clearance	168, 182
γδ T cells	Rapidly secrete cytokine, including IL-17	17, 40, 45, 115, 130, 163; Sivick, et al., submitted
Adaptive cells[a]		
B cells	Secrete antibody	109
Th1 cells	Provide B cell help for an appropriate humoral response	87, 130
Th2 cells	Unknown	NA
Th17 cells	May be dispensable for natural adaptive immune protective responses	Sivick, et al., submitted
Treg cells	Unknown	NA
Antibody[a]		
IgA	Neutralization, opsonization, complement activation	58, 116, 122, 217, 249, 263, 264
IgG	Neutralization, opsonization, complement activation	58, 116, 122, 217, 249, 263, 264
IgM	Mainly complement activation	58, 116, 122, 217, 249, 263, 264

[a] In addition to the references cited, Table 2 provides data on humoral and cellular responses to vaccine candidates.

review, "postinoculation" and "infection" refer to *E. coli* accessing the urinary tract by the transurethral route, either experimentally in animals and volunteers or naturally in patients.

WEAPONS SYSTEMS: CURRENT AND PROPOSED TREATMENTS AND VACCINE INITIATIVES FOR UTI

There are several practiced and proposed therapeutics for UTI management. Prophylactic treatments include estrogen in postmenopausal women (36, 41, 125, 140, 198, 208, 214) or cranberry juice (13, 77, 200), although the efficacy of the former remains controversial. Treatment of UPEC-infected mice with forskolin, a drug that increases intracellular cyclic AMP (cAMP) levels, expels UPEC from intracellular vesicles into the extracellular milieu, rendering the bacteria susceptible to immune responses and antibiotics (29). Similarly, exposing the bladder to protamine sulfate, a highly cationic protein, removes bound and intracellular UPEC by causing umbrella cells to exfoliate (179), unfortunately with a significant level of discomfort, as reported by study volunteers (158). In addition to a number of nonspecific chemical treatments (274), both small-molecule inhibitors (33) and specific antibody directed against FimH (256) demonstrated some utility in preventing bacterial adherence. While antibiotic therapy remains the standard treatment for UTI, overuse leads to deleterious alterations of the normal host microbiota (52) and selection for resistant strains (50, 76, 93, 94, 128, 178), prompting the need for vaccine-mediated prevention of UTI.

Early vaccine studies focused on the lipopolysaccharide (LPS) side chain (O) antigen (Table 2) (275). There are

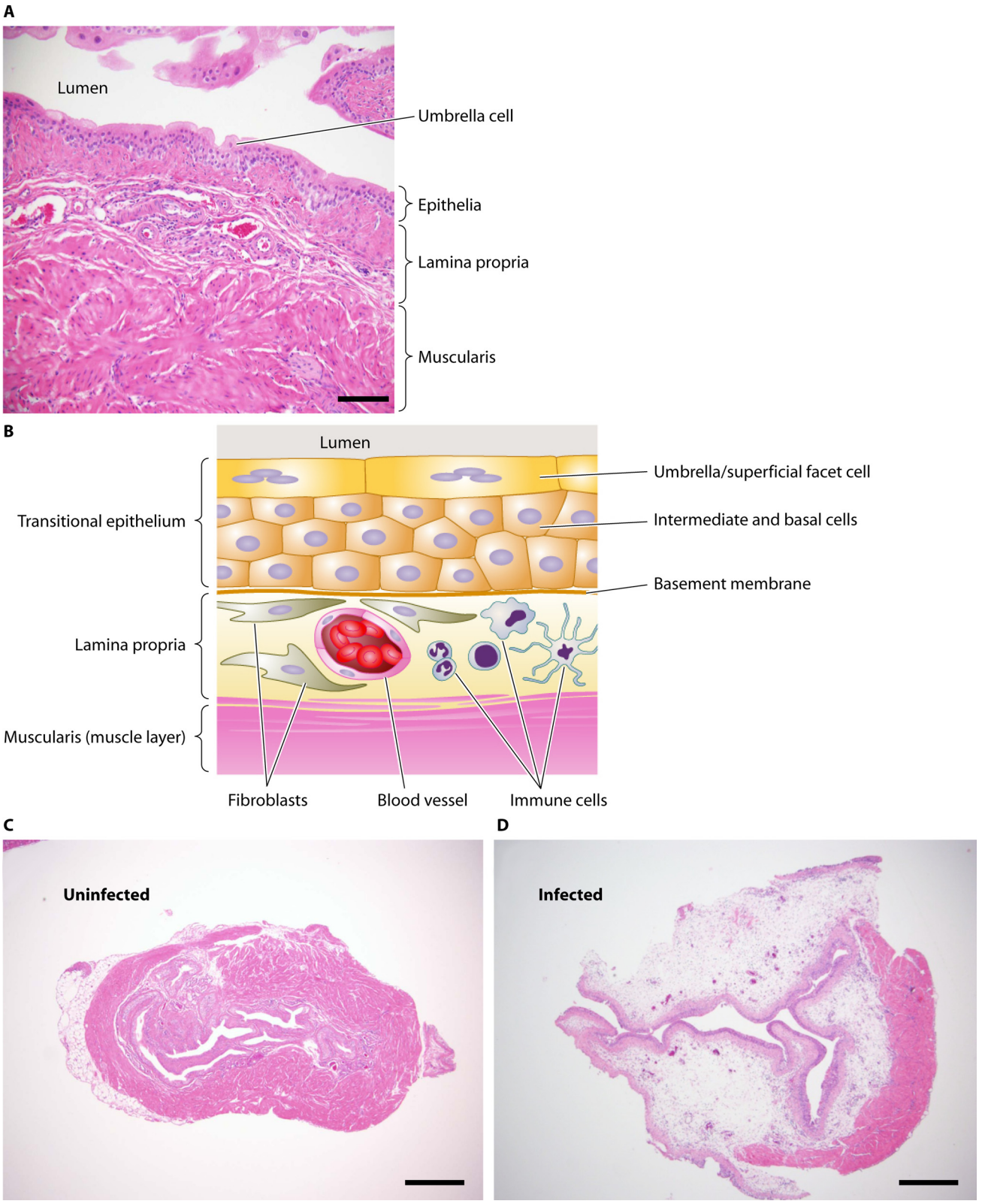

FIG. 1. Histological and schematic views of the murine bladder. (A) Hematoxylin and eosin (H&E)-stained section from a healthy wild-type C57BL/6 female mouse. Magnification, ×200. Scale bar, 100 μm. (B) Schematic representation of bladder physiology shown in panel A. Umbrella cells line the luminal surface of the transitional epithelium. The basal side of the umbrella cell layer consists of intermediate and basal cells, followed by the lamina propria, the primary site of edema and inflammation during UTI. (C and D) H&E-stained sections from wild-type C57BL/6 mice that were either left untreated (C) or infected for 48 h (D). Note the severe inflammation and edema in the lamina propria of the infected animal. Magnification, ×40. Scale bar, 500 μm.

TABLE 2. Previously tested vaccines for UPEC-mediated UTI.

Category/vaccine type	Tested in[b]	Route[c]	Adjuvant[d]	Immune response[e]	Protection[f]	Reference(s)
Surface structures						
O antigen	R	B, SC	ND[g]	H	B	275
OMP fractions	Swiss	IM, O	CFA	C, H	B	155
Adherence						
Dr fimbria	C3H/HeJ	ND	CFA, IFA	H	—	89
Type 1 fimbria	BALB/c	IM, SC	CFA	H	—	187
FimC-H or FimHt	C3H/HeJ	S	CFA, IFA	H	B, K	154
FimHt	BALB/c	IM, IN	CFA, IFA, CpG	H	B	204
FimC-H	P	IM	MF59	H	U	153
Fim peptides	Swiss	IM, SC	CFA, IFA	H	B	256
P fimbria	BALB/c	IM, SC	CFA, IFA	H	K, U	186, 187, 231
PapD-G	P	IP	AP	H	K	217
Pap peptides	BALB/c	IM, SC	CFA, IFA	H	U, K	231
Toxin						
Denatured HlyA	BALB/c	IM	CFA, IFA	H	K	186
Iron acquisition						
Denatured IroN	BALB/c	SC	ND	H	K	223
Native ChuA	CBA/J	IN	CT	C, H	—	5
Native Iha	CBA/J	IN	CT	C, H	—	5
Native IroN	CBA/J	IN	CT	C, H	—	5
Native Hma	CBA/J	IN	CT	C, H	B, K	5
Native IreA	CBA/J	IN	CT	C, H	B, K	5
Native IutA	CBA/J	IN	CT	C, H	B, K	5
Complex						
SolcoUrovac	BALB/c	IP, V	MO	C	B, K	272
SolcoUrovac	C57BL/6	IP, V	MO	C	B, K	272
SolcoUrovac	Swiss	IP	ND	H	ND	146
SolcoUrovac	R	IP	AP	ND	K	145
SolcoUrovac	P	IM, V	MO	H	B	270
SolcoUrovac	H	IM, V	ND	H	Y	91, 112, 114, 224, 266–269
Uro-Vaxom	BALB/c	IP, O	ND	C, H	ND	15, 117, 233
Uro-Vaxom	H	O	ND	H	U, Y	22, 51, 95, 156, 160, 232, 253
Live, live attenuated, or killed *E. coli*[a]						
L NU14	C57BL/6J	TU	ND	C, H	B	260
L and K CP9	C57BL/6J	IN	ND	H	ND	219
L and K CP923	C57BL/6J	IN	ND	H	ND	219
LA Δ*waaL*	C57BL/6J	B	ND	ND	B	27
K J96	BALB/c	IM, SC	CFA	H	—	187
K P678-54	BALB/c	IM, SC	CFA	H	—	187
K O6	R	V	IFA	N	B, K	273
K 1677	P	V	MO, MDP	H	U	265, 271

[a] L, live; LA, live attenuated; K, killed.
[b] If tested in mice, the strain is specified. R, rats; P, nonhuman primates; H, humans.
[c] B, bladder; IM, intramuscular; IN, intranasal; IP, intraperitoneal; O, oral; SC, subcutaneous; TU, transurethral; V, vaginal.
[d] AP, aluminum phosphate; CFA, complete Freund's adjuvant; CpG, CpG oligodeoxynucleotides; CT, cholera toxin; IFA, incomplete Freund's adjuvant; MDP, muramyl dipeptide; MO, mineral oil (for vaginal route only).
[e] C, cellular; H, humoral.
[f] K, reduction in kidney colonization/histopathology; B, reduction bladder colonization; —, no protection; Y, significant decrease in UTI incidence; U, reduction of UTI as determined by urinalysis.
[g] None disclosed.

trends regarding the frequencies of particular O antigens among UTI isolates (68, 249, 284), and O-antigen-specific antibodies demonstrate an antiadhesive effect (249). Nonetheless, significant structural heterogeneity may represent an insurmountable obstacle for development of an O-antigen-based vaccine. Furthermore, a study evaluating antibody responses in mice intranasally vaccinated with a killed *E. coli* lacking capsule and O antigen demonstrated that these surface features actually obstruct optimal humoral responses (219).

Later studies involved vaccines directed against particular virulence factors (Table 2). P fimbriae are adherence organelles that play a role in kidney colonization in mice and humans (187, 188, 288, 297); the pore-forming toxin alpha-hemolysin (HlyA) and P fimbriae are proposed minimal factors required for colonization of and dissemination from the kidney

(186). There are convincing data using both murine (186, 187, 231) and primate models (216, 217) that vaccination against P fimbriae or HlyA prevents renal colonization and damage. Additionally, to overcome P fimbrial allelic variability, linear peptide sequences that generated cross-reactive antibodies were evaluated as protective antigens (191, 231). Despite these successes, vaccines targeting P fimbriae may not be effective because of their limited role during bladder colonization. Type 1 fimbria is a *bona fide* virulence factor of UPEC and, in contrast to P fimbria, is critical for bladder colonization (11, 48, 92, 256). Animals vaccinated with various components of type 1 fimbriae had increased levels of antigen-specific antibodies and decreased levels of colonization upon challenge (153, 154, 194, 204, 256). Unfortunately, expression of type 1 fimbria is subject to phase variation, allowing UPEC to evade humoral responses targeting this organelle (59, 240). Additionally, since nonpathogenic isolates also express type 1 fimbriae (97, 126), targeting this population may result in detrimental disruption of the host microbiota. Also of note, both P and type 1 fimbriae were not necessary for colonization of the human neurogenic bladder, indicating the need for alternative targets in certain high-risk patient groups (118).

Iron is essential for nearly all organisms (83, 295), and UPEC strains encode a battery of genes involved in iron acquisition. Vaccination with UPEC outer membrane protein (OMP) fractions enriched for iron receptors protects against experimental sepsis (32, 57). Additionally, mice vaccinated subcutaneously with denatured IroN, an OMP siderophore receptor and urovirulence factor (222), had both increased levels of antigen-specific serum IgG and reduced kidney colonization upon challenge (Table 2) (223). Undetectable levels of IgA in the bladder mucosa after this vaccination may explain why these animals were not protected from cystitis (223). Recently, a broad functional vaccinology initiative was conducted using an "omics" approach to identify *PASivE* vaccine candidates: UPEC proteins that are pathogen-specific, antigenic, surface-exposed, and *in vivo* expressed (5, 238). Strikingly, the top targets identified by this approach were all OMPs functioning in iron uptake. Intranasal vaccination with three of six candidates afforded protection from cystitis and pyelonephritis (5), suggesting that combining antigenic motifs found in these proteins may be an effective multivalent vaccine for UTI.

Vaccines consisting of bacterial components or whole cells have also been assessed (Table 2). Transurethral immunization of mice with a live-attenuated UPEC strain lacking the ability to persist in the urinary tract engendered heterologous protection (27), a potential platform for further development. On the complex vaccine front, Uro-Vaxom is a daily oral capsule containing a lyophilized mix of membrane proteins from 18 *E. coli* strains (95, 253). The formulation elicits a number of immunological effects *in vitro* (230, 278, 289, 290), generates specific antibodies in mice and humans (15, 51, 117, 233), and generally reduces the incidence of UTI in patients (22, 51, 95, 156, 160, 232, 253). Unfortunately, complications can occur due to toxicity, and the necessity of daily administration presents supply and compliance issues. SolcoUrovac, a vaginal suppository containing 10 heat-killed uropathogenic strains, has been tested in mice (146, 272), in nonhuman primates (270), and in clinical trials (91, 112, 114, 224, 266–269). While safe, SolcoUrovac vaccination did not result in appreciable increases in local specific antibody (267, 269, 270), nor did it afford protection without periodic readministration (267, 269).

TACTICAL DEFENSIVE MANEUVERS: HOST FACTORS TO PREVENT UPEC COLONIZATION

Uroepithelial adherence is critical for establishment of UTI (284). UPEC strains possess an impressive repertoire of adhesins that enable them to aggregate and adhere to cellular surfaces (107, 137, 203, 235, 276). Consequently, the first line of host defense against UTI is concentrated on preventing UPEC adherence to the bladder mucosa. The luminal surface of the bladder is lined with highly sulfated and anionic glycosaminoglycans (GAGs) that contribute to bladder wall impermeability and afford an antimicrobial antiadherence property (120, 158, 196, 197, 199, 237). Intuitively, urine flow seems to be a convenient defense mechanism; however, FimH binds to mannose moieties using "catch-bonds," interactions that are actually strengthened by the sheer stress induced during urine flow (257). Thus, more active mechanisms, like umbrella cell exfoliation (10, 60, 81, 166, 176, 177, 189), remove adherent UPEC. Exfoliation occurs by an apoptosis-like mechanism that is promoted by FimH (142, 176). FimH induces cellular events consistent with activation of both extrinsic (death receptor) and intrinsic (mitochondrial) apoptotic pathways, with cross talk between the two signaling cascades mediated by the proapoptotic Bid protein (141). UPEC-induced urothelial cell death correlates with increased bladder cell differentiation and is also dependent on expression of the uroplakin IIIa receptor, a terminal differentiation marker (180, 258). Nuclear factor of kappa light chain polypeptide gene enhancer in B cells (NF-κB) is a transcription factor known for induction of proinflammatory genes concomitant with antiapoptotic genetic programs (20, 280). UPEC, independent of type 1 fimbriae, is able to suppress NF-κB and thereby promotes host cell apoptosis (142). Given the role for apoptotic cell exfoliation in UPEC host defense, promoting this sloughing activity may appear counter-productive for the bacteria. Nonetheless, cellular apoptosis may be an acceptable side effect of inhibiting the proinflammatory gene expression and ensuing cellular responses also initiated by NF-κB.

Tamm-Horsfall protein (THP) was first described in the early 1950s as a high-molecular-weight protein present in human urine (252); its ability to bind *E. coli* fimbriae was not recognized until some 30 years later (190, 192, 195). A detailed biochemical analysis revealed that soluble THP from both mouse and human urine was able to bind type 1 fimbriae by virtue of its mannose moieties, inhibiting fimbrial interaction with uroplakin receptors (172, 193). This phenotype translated *in vivo* as $thp^{-/-}$ mice were unable to control lower-UTI (21). THP also appears to act as an innate-adaptive immunoregulatory molecule that can activate dendritic cells (225).

BATTLEFIELD INTELLIGENCE: HOST SIGNALING IN RESPONSE TO UPEC RECOGNITION

Upon successful adherence to the uroepithelium, Toll-like receptor (TLR) recognition of pathogen-associated molecular patterns (123, 164) generates signaling cascades to control infection and direct adaptive responses (55, 242). It has been

known for over 2 decades that C3H/HeJ mice, harboring a mutation in the Toll/interleukin-1 receptor (TIR) domain of TLR4 (206), cannot resolve UTI as efficiently as LPS-responsive C3H/HeN counterparts (250). In accordance, *tlr4*$^{-/-}$ mice had significantly higher bacterial burdens in their bladders than similarly infected wild-type mice (12). This clearance defect is the result of insufficient downstream cytokine and chemokine production and neutrophil recruitment (96, 111, 201, 236). Data from mouse chimeras disclosed that TLR4 on both stromal and hematopoietic cells is critical for normal inflammatory responses and clearance of UPEC in the bladder (227) and kidney (201). Correspondingly, children with low TLR4 expression on their neutrophils display an asymptomatic bacteriuria (ABU) carrier state lacking both inflammation and bacterial clearance (211). A similar response is exhibited by C3N/HeJ mice following UPEC inoculation (210).

TLR4-mediated signaling in the urinary tract does not appear to be the result of the archetypal interaction with LPS. Both the role of LPS in and the molecular trigger of TLR4 signaling by UPEC are topics of debate (14, 106, 228). Studies using the A498 human kidney cell line indicate that TLR4 signaling in response to UPEC requires P fimbriae and can be mediated independently of LPS (79, 104, 106). Mechanistic details regarding this phenomenon include P fimbriae binding to surface glycosphingolipids (GSLs) and subsequent release of the GSL membrane-anchoring domain, ceramide (72). Ceramide appears to act as a TLR4 agonist and the putative intermediate for TLR4 signaling initiated by P fimbriae (72). In contrast to LPS-independent signaling by P fimbriae, there appears to be a cooperative stimulation of TLR4 by LPS and type 1 fimbriae (105, 229). This cooperative stimulation directly correlates with the level of cluster of differentiation 14 (CD14) expression on bladder cells (228). CD14 is an accessory molecule required for optimal TLR4 signaling in response to LPS (170). Immunohistochemical (IHC) analysis of human bladder biopsies revealed that CD14 expression is localized to the submucosa (106), suggesting that uroepithelial cells exposed to the lumen have little to no CD14 expression and therefore may not respond efficiently to LPS alone. These results support a role for both independent and cooperative TLR4 stimulation by UPEC fimbriae. Lastly, the FimH tip adhesin of type 1 fimbriae was recently shown to directly interact with TLR4, an additional means for LPS-independent stimulation by UPEC fimbriae (12, 175).

Infection of knockout mice has revealed critical roles for myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adaptor inducing beta interferon (TRIF), and TRIF-related adaptor molecule (TRAM) in signaling for UPEC clearance (73). It is also apparent that different fimbrial types influence the corresponding downstream signaling pathways (73). Regardless of the fimbria involved in stimulation, all pathways involving these adaptor molecules result in activation of NF-κB and proinflammatory gene expression. Song and colleagues identified an accompanying proinflammatory bladder cell signaling pathway that is also dependent on TLR4 but results in a spike in intracellular calcium levels (245). This calcium spike leads to adenylyl cyclase 3 (AC3)-mediated increase in cAMP, protein kinase A (PKA) activation, phosphorylation of the cAMP response element-binding protein transcription factor (CREB), and proinflammatory gene expression (245). In response to UPEC inoculation, cytokine secretion by the CREB pathway occurs faster (1 h) than NF-κB translocation to the nucleus (2 h) and can also be activated by TLR2 and TLR3 ligands (245).

Other TLR pathways have been implicated in host defense during UTI. *tlr2*$^{-/-}$ mice appear to respond normally to acute UTI (210). Conversely, *tlr11*$^{-/-}$ mice are more susceptible than wild-type mice to UPEC kidney infection (296). The TLR11 ligand is a profilin-like molecule that was isolated from *Toxoplasma gondii* (292). While structurally related proteins are present in other apicomplexan protozoa (292), a UPEC-encoded homolog has yet to be identified. The fact that there is a stop codon in the open reading frame of human genomic and cell line *tlr11* sequences may help explain acute and recurrent UTI susceptibility in humans (296). In contrast to the kidney-specific role for TLR11 during UTI (296), TLR5 appears to play a UPEC recognition role in the bladder (7). TLR5 recognizes the structural subunit of flagella (101), which are essential for UPEC motility in the urinary tract (150, 152, 285). Flagellar expression peaks at 4 to 6 h postinoculation, coinciding with UPEC ascension of the ureters (150). At this time point, there is TLR5-dependent induction of inflammatory cytokines and chemokines (7). By day 5 postinoculation, *tlr5*$^{-/-}$ mice have increased inflammation and bacterial burdens compared to wild-type controls (7), highlighting the importance of early UPEC recognition to contain the infection.

Surface molecules other than TLRs are also involved in host-UPEC interactions. Upon UPEC exposure, the cytoplasmic tail of uroplakin IIIa undergoes phosphorylation, and intracellular calcium levels increase, presumably important events for uroepithelial cell apoptosis and exfoliation (259). Although uroplakin Ia is thought to be the main receptor for UPEC FimH *in vivo* (167, 256, 286), type 1 fimbriae may bind to a number of host molecules, including uroplakin complexes (259), extracellular matrix proteins (147, 207, 241), CD molecules (18, 85, 139), and integrins (65). The CD44 ligand, hyaluronic acid (HA), accumulates in the urinary tract during UTI; UPEC can bind HA, thereby facilitating interaction with CD44 and tissue invasion (218). In accordance with this, *cd44*$^{-/-}$ mice are more resistant to UPEC kidney colonization and successive dissemination (218). Also, there are still unidentified players in inflammation and clearance of UPEC. For example, LPS-responder C3H/OuJ mice were found to be equally susceptible to UTI as non-LPS-responder C3H/HeJ mice yet demonstrated elevated levels of inflammation (111), revealing a susceptibility locus to map.

UPEC has evolved mechanisms to counter host recognition and signaling. Clinical UPEC isolates encode the gene for TcpC, which has structural homology to the TIR domain of human TLR1 and binds to MyD88, thereby inhibiting cytokine responses (47). TcpC-mediated interference of MyD88 signaling is an immune evasion strategy particular to acute pathogens; targeting this major signaling "hub" deteriorates innate immune responses (38). In addition, Billips and colleagues noted that a type 1-fimbriated K12 strain elicited more robust cytokine secretion from cultured urothelial cells than UPEC strains (25). By screening UPEC transposon mutants, they identified a peptidoglycan permease (*ampG*) and an O-antigen ligase gene (*waaL*) responsible for the dulled cytokine secretion in response to uropathogenic strains (26). A similar screen

also identified the *rfa-rfb* operons and *surA*, encoding genes important for LPS biosynthesis and OMP biogenesis, respectively (119). These results suggest that UPEC utilizes gene products that modify bacterial membrane (especially LPS) to evade immune recognition and highlight the potential importance of TLR stimulation involving fimbriae and other organelles.

HAND-TO-HAND COMBAT: BATTLE FOR PRECIOUS RESOURCES

That *E. coli* strains causing UTI have several functionally redundant systems dedicated to iron uptake (39, 44, 283) suggests that the urinary tract, like other host niches, is an iron-limited environment (19). Siderophores are secreted iron-chelating molecules that allow bacteria to scavenge free and host protein-bound iron (37, 184). Enterobactin, for instance, can bind free ferric ions with a higher affinity than transferrin (70), a host iron transport protein responsible for regulating the free iron concentration in serum (213, 279). A transferrin family member, lactoferrin, evokes antimicrobial activity by sequestering iron over a range of pH (279). Lactoferrin is secreted by kidney cells (2) and is found in neutrophil granules (49) and thus could be involved in combating UTI. Both transferrin and lactoferrin have been shown to evoke direct antimicrobial activity by disrupting Gram-negative membranes (61, 90).

In addition to iron sequestration, there are host factors that directly counter the action of siderophores. Early studies indicated that serum albumin, alone or in concert with other serum proteins, can impede bacterial siderophore function (143). In addition, the mammalian protein lipocalin 2 (Lcn2) can bind and sequester enterobactin and similar catecholate siderophores (70, 74, 88, 110). Lcn2 inhibits enterobactin-dependent propagation of *E. coli in vitro*, and *lcn2*$^{-/-}$ mice are unable to control systemic *E. coli* burdens as well as wild-type mice (74). Production of Lcn2 is induced by TLR4, implicating iron regulation as a part of the immune response to infection (74). Murine GeneChip and quantitative PCR (qPCR) analyses confirmed that Lcn2 mRNA is upregulated by the uroepithelium of infected mice (215). Interestingly, these results were obtained in C3H/HeJ mice, indicating that a TLR4-independent signaling pathway can activate transcription of the *lcn2* gene in response to UTI. Not surprisingly, UPEC has evolved a mechanism to counter Lcn2 siderophore sequestration. Encoded within the *iroA* gene cluster are glycosyltransferases that modify enterobactin in such a way that it cannot be bound by Lcn2 (30, 71, 239). Thus, both the host and UPEC have systems in place to manage their own iron stores and to inhibit iron acquisition by the other—a molecular arms race for an essential nutrient.

The role for bacterial central metabolism during infection has only been recently appreciated (6, 69, 129). Genes important for glucose import were upregulated by the uroepithelium of C3H/HeJ mice experiencing UTI, possibly for either nutrient sequestration or energy to combat infection (215). This fact, coupled with the knowledge that UPEC does not chemotax toward glucose *in vitro* (151) or utilize glucose as a primary carbon source *in vivo* (6), implies that UPEC may have evolved to use alternative carbon sources in the urinary tract. These results imply that nutrient acquisition is also a crucial aspect of bacterial pathogenesis and the host response that may influence the outcome of UTI.

FRIENDLY FORCES: HOST FACTORS INVOLVED WITH INTRACELLULAR UPEC

Over the past 12 years there has been a growing body of literature revealing that UPEC appears to have three distinct intracellular lifestyle components within the urinary tract (66). The first is uptake by apical endocytosis of Rab27b^{+}/CD63^{+} fusiform vesicles, which are subsequently recycled back to the cell surface and exocytosed (29). The other two pathways both begin with uptake into a membrane-bound compartment which can lead to either a quiescent nonreplicative existence (67, 179) or escape from compartmental life to undergo a highly replicative phase in the cell cytoplasm (131, 177). While internalization via the fusiform vesicle pathway may be a side effect of normal bladder epithelium function, cellular uptake by the other two pathways is perhaps intended by UPEC to establish a reservoir to persist in the urinary tract (67, 131, 138, 177, 179, 226). Indeed, UPEC has been shown to exist in the urinary tract for weeks, even after antibiotic treatment (138). Infection of 10 genetically distinct mouse strains also revealed that some strains were more susceptible to persistence than others, indicating that host hereditary components may also contribute to the ability of UPEC to persevere in the urinary tract (113).

Infected mouse bladder explants monitored by time lapse fluorescence videomicroscopy generated a model for the intracellular UPEC life cycle instigated after uptake in a membrane-bound compartment (nonfusiform vesicle route) (131). While the mechanism of compartmental escape remains undefined, once contained in cytoplasmic "intracellular bacterial communities" (IBCs), UPEC can undergo several changes in morphology, categorized as early, middle, and late IBC stages (8, 131). Late IBCs that escape exfoliation with umbrella cells contain filamentous UPEC that are not present in C3H/HeJ mice, indicating that this morphological change may be a bacterial stress response to TLR4-mediated immune activation (131, 177). This murine background also experienced increased incidence and severity of IBCs compared to immunocompetent mice (8, 84, 131). Urothelial cells proximal to IBCs in C3H/HeJ mice upregulate transferrin receptor, Lcn2, complement system components (C3, factor B, and CD55), and lysozyme (215). Involucrin and suprabasin transcripts were also increased, indicating that, in addition to gene products that function to eradicate bacteria, proteins important for epithelial integrity may be an imperative host response during UTI (215).

In vitro treatment of either 5637 cells with a small amount of the detergent saponin (67) or immortalized pediatric bladder cells with the cholesterol-sequestering drug filipin (24) recapitulates some *in vivo* features of intracellular UPEC. Additionally, much work has been done using the 5637 bladder epithelial cell line to further delineate molecular components and mechanisms surrounding UPEC intracellularity (29, 56, 64, 65, 67, 161, 162, 177, 229). UPEC internalization does not require bacterial viability (229) but is dependent on FimH (162). β1 and α3 integrins were shown to be receptors

for Fim-mediated UPEC internalization, mediated by signaling through focal adhesion kinase (FAK) and, in contrast to an earlier study, Src family kinases (65, 162). FimH-dependent uptake requires microtubules, histone deacetylase 6, the kinesin-1 light chain, and aurora A kinase (53). In addition to the involvement of cytoskeletal proteins, tyrosine kinases, and phosphoinositide 3-kinase (PI3-K) (162), UPEC engulfment has also been reported to be cholesterol and dynamin dependent and modulated by calcium levels, clathrin, and clathrin adaptors (64). Additional work on dynamin revealed that the nitric oxide synthase (NOS) enzyme is responsible for chemically modifying dynamin, redistributing it to the membrane for bacterial internalization (281). As hinted by the cholesterol dependence, UPEC internalization is often reported to be associated with lipid rafts (18, 133), dependent on caveolin-1 and Rho-family GTP binding proteins (56, 161). The association with lipid rafts was confirmed *in vivo*; UPEC inoculation in the presence of a lipid raft-disrupting chemical decreased the number of intracellular bacteria in the murine bladder (56).

Notably, TLR4 also plays a noninflammatory role in host defense against UPEC by modulating the activity of the observed secretory and vesicular internalization pathways. TLR4-mediated PKA activation suppresses the lipid raft endocytic pathway (243), a possible effort to prevent the establishment of persistence reservoirs. Also along these lines, UPEC exocytosis in fusiform vesicles was actually accelerated by TLR4-mediated recognition of LPS and dependent on the activities of cAMP, Rab27b, caveolin-1, and the scaffolding protein MyRIP (244).

CHEMICAL WARFARE: ANTIMICROBIAL PEPTIDES, CYTOKINES, AND CHEMOKINES

Antimicrobial peptides (AMPs) are short positively charged peptides secreted by both epithelial and hematopoietic cells that disrupt bacterial membranes and can be chemotactic for certain immune cells (246, 293, 294). Human β-defensin-1 mRNA and protein were found in kidney tissue, implicating this AMP in host defense against UPEC (277). More convincingly, mice deficient in *defb1*, a murine homolog of human β-defensin, have a significantly higher incidence of bacteriuria (174). Murine β-defensin is also a dendritic cell (DC) ligand that instigates upregulation of costimulatory molecules and maturation (28). The human cathelicidin, LL-37, and its murine homolog, cathelin-related antimicrobial peptide (CRAMP), are secreted in response to UPEC exposure (46). Studies using CRAMP-deficient mice revealed that epithelial-derived CRAMP is important during the early stages of UTI while leukocyte-derived CRAMP likely functions later when bacteria penetrate the kidney epithelium (46).

Human C-X-C ligand 8 (hCXCL8; interleukin-8 [IL-8]) is the main chemoattractant for neutrophils in humans, and murine CXCL1 (mCXCL1) and mCXCL2 (also known as KC and MIP-2, respectively) are the functional mouse homologs of IL-8 (122). Bladder and kidney cell lines secrete IL-8 in response to UPEC (102, 229, 287). Human and murine studies both demonstrate that neutrophil migration to the UPEC-infected urinary tract is dependent on IL-8 (3, 4, 99, 169). Additionally, mCXCL2 secretion is dependent on TLR4, as secretion was deficient in infected C3H/HeJ mice (100). hCXCR1 and hCXCR2 are receptors for a number of chemokines, including IL-8 (122). Both are expressed in bladder and kidney biopsies, and transmigration studies indicated that hCXCR1 plays a dominant role in IL-8-dependent neutrophil migration (86). Consistent with this, children prone to pyelonephritis tend to have low hCXCR1 expression and heterozygous hCXCR1 polymorphisms (78, 210). hCXCR1 deficiency results in impaired bacterial clearance but, unlike TLR4 deficiency, with intact inflammatory signaling that ultimately results in tissue damage (210). Similarly, mice lacking mCXCR2 (the functional homolog for hCXCL1) experience subepithelial accumulation of neutrophils, increased bacterial titers, and renal scarring after UPEC inoculation (78, 86, 98). These data indicate that normal function of neutrophils, their chemotactic ligands, and their chemokine receptors are required for bacterial clearance without postinflammatory sequelae.

Despite ample information on IL-8 *in vitro* and *in vivo*, a complete picture of the cytokine and chemokine dynamics during UTI was lacking. In response, a longitudinal assessment using a Bio-Plex format was conducted (121). Chemokine (C-C motif) ligand 2 (CCL2 or MCP-1), CCL4 (or MIP-1b), CCL5 (or RANTES), CXCL1, IL-1β, IL-6, IL-12p40, IL-17, tumor necrosis factor alpha (TNF-α), and granulocyte-colony stimulating factor (G-CSF) were all upregulated in bladder homogenates from UPEC-infected C57BL/6 mice (121). These results agreed with patient and cell line data regarding upregulation of IL-6 in response to UPEC (103, 104). In mice, TNF-α expression was elevated at 1 h postinoculation for rapid mobilization of acute responses (121); this waned at later time points, likely to prevent the deleterious effects of uncontrolled TNF-α signaling (16). Expression of most cytokines and chemokines peaked around 24 h postinoculation, returning to near baseline at 2 weeks (121). These dynamics correlate well with the peak and resolution of bacterial burdens in C57BL/6 mice (121). One notable exception was IL-17, which was highly upregulated from 6 h to 1 week postinoculation, remaining above baseline through the 2-week experimental duration (121). Importantly, IL-17A (the Th17 signature cytokine) contributes to innate clearance of UPEC through a mechanism involving cytokine and chemokine secretion and macrophage and neutrophil influx (K. E. Sivick, M. A. Schaller, S. N. Smith, and H. L. T. Mobley, submitted for publication).

Similar to TLR adaptor molecule usage (73), the type of fimbriae expressed also seems to influence the repertoire of chemokines secreted. Specifically, kidney cells exposed to type 1-fimbriated UPEC secrete neutrophil-associated chemokines, while P fimbriae-stimulated cells secrete chemokines targeting antigen-presenting cell (APC)- and Th1-specific cytokines, exemplified by CCL2 and CCL5 expression (87). In addition, IFN-γ and IL-4 (signature cytokines of the Th1 and Th2 lineages, respectively) and IL-10 (a T-regulatory [Treg] effector cytokine) knockout mice were tested for susceptibility to both acute cystitis and pyelonephritis (130). While *il4*$^{-/-}$ and *il10*$^{-/-}$ mice appear to experience infection dynamics similar to the wild type, *ifn*γ$^{-/-}$ mice had increased incidence and severity of UTI (130), implying a role for IFN-γ and Th1-mediated inflammatory responses during UTI.

THE INFANTRY: NEUTROPHIL AND APC RESPONSES TO UPEC-MEDIATED UTI

Infected mouse bladders examined histologically display thickening of epithelium accompanied by robust infiltration of inflammatory cells and edema in the lamina propria (Fig. 1C and D) (124). Neutrophils are the most rapid and abundant responders to the infected urinary tract (4, 100, 124, 236). Efficient migration of neutrophils requires intracellular adhesion molecule 1 (ICAM-1) expression by epithelial cells and β2 integrin (CD11b/CD18) expression by neutrophils (3, 227). G-CSF is also required for the neutrophil response, and unexpectedly, mice with neutralized G-CSF are more resistant to UTI (121). Although monocyte/macrophage numbers were similar in anti-G-CSF-treated mice, cytokines important for macrophage activation were upregulated, potentially leading to accelerated clearance by enhanced phagocytic killing (121). Despite counterintuitive phenotypes with respect to cytokine knock-down, antibody-mediated knockdown of the neutrophil population confirmed their crucial role in bacterial clearance, especially within the kidney (100). Lastly, the electrostatic properties of the UPEC P fimbrial tip adhesin may interfere with neutrophil binding, a potential host response evasion tactic specific to the kidney (31, 254).

Compared to the neutrophil response, relatively little is known about APCs in the context of UTI. In mice, resident $CD11c^+$ cells that express low to intermediate levels of F4/80 and CD11b macrophage markers were found in the kidney (144), while $CD11c^+$ cells expressing the major histocompatibility class II activation marker were found in the bladder (109, 227). In spite of macrophage marker expression, $CD11c^+$ kidney cells had physical and functional characteristics of DCs (144). At 24 h postinoculation, $CD11c^+$ cells that migrate to the bladder did not express CD8α, Gr-1, or B220 and thus were not plasmacytoid or lymphoid but appeared to be TNF-α- and inducible NOS (iNOS)-producing (Tip)-DCs (62) that express intermediate levels of CD11b. Infection studies in mice lacking Tip-DCs suggested that they are not necessary for the host response to acute UTI (62). Since Tip-DCs are necessary for the generation of mucosal IgA (255), their role may lie in mediating the humoral response to UPEC. Similar to what was observed for DCs, there appears to be a resident population of macrophages in bladder tissue that increases by several orders of magnitude in response to UTI (63, 109, 121). Monocytes expressing high levels of Gr-1, which can give rise to macrophages or DCs, are also recruited to the bladder in response to UPEC infection. Release of these cells from the bone marrow was dependent on CCR2 (63), and, correspondingly, CCL2 is upregulated in the bladder response to UTI (121).

Some of the factors utilized by neutrophils, macrophages, and DCs for pathogen uptake and destruction have been described during UTI. iNOS generates the antimicrobial compound nitric oxide (NO) from L-arginine and was originally reported to be secreted by macrophages (108, 183, 248). Although iNOS is rapidly upregulated in the inoculated bladder (181), two independent groups reported that *inos*$^{-/-}$ mice are equally as susceptible to UTI as wild-type mice, suggesting that neuronal NOS, endothelial NOS, or myeloperoxidase may act as compensatory factors (132, 205). Alternatively or in addition, *inos*$^{-/-}$ animals may lack a colonization phenotype because there are several factors (Hfq and Nsr-regulated genes, polyamines, and flavohemoglobin) expressed by UPEC that enhance tolerance to reactive nitrogen species *in vitro* (34, 35, 148, 251), suggesting that NO production may be an ineffective host defense against UPEC. With respect to the complement system, it appears that UPEC is able to bind C3 to enter host uroepithelial cells via the surface receptors Crry or CD46 (157, 247). Correspondingly, *c3*$^{-/-}$ mice are more resistant to renal damage and infection (247). As C3 levels are significantly higher in the urine of UTI patients (157), UPEC may stimulate C3 production for pathogenic means or has evolved to exploit this host defense factor.

SPECIAL OPERATIONS: ILLs IN THE INNATE IMMUNE RESPONSE TO UPEC-MEDIATED UTI

Infection studies using severe combined immunodeficient (SCID) mice that lack functional B and T cells and nude mice that lack thymically derived T cells provide preliminary evidence of a role for innate-like lymphocytes (ILLs) in acute UTI host defense (115). Epithelial γδ T cells, B-1 cells, and natural killer T (NKT) cells are ILLs: cellular subsets that have relatively invariant receptors and reside in specific locations of the body (122). After a 2-day primary infection, SCID mice had significantly higher bacterial counts in their bladder and kidneys, while nude mice were colonized similarly to wild-type animals (115). The lack of a colonization phenotype in nude mice suggests that either antibody responses independent of thymus-derived T-cell help or extrathymically produced T cells may play a role in innate clearance of UPEC. The latter suggestion has some experimental support. γδ T cells can be produced extrathymically and rapidly secrete cytokines in response to stimulation (1, 17, 40, 45). Resident γδ T cells found in the bladder increase in response to UTI (163; also K. E. Sivick, M. A. Schaller, S. N. Smith, and H. L. T. Mobley, submitted for publication), and TCR $\delta^{-/-}$ mice are more susceptible to UTI than isogenic controls (130). As γδ TCR^+ cells express IL-17A during UPEC-mediated UTI (Sivick et al. submitted), this rapid-response cell population may function in concert with other innate factors to mediate neutrophil influx for clearance of UPEC. B-1 cells spontaneously secrete large quantities of polyspecific IgM against bacterial and self-antigens, and in contrast to conventional (B-2) B cells, do not require T-cell help (23). While IgM secreted by B-1 cells might play a role in innate clearance of UPEC, current evidence suggests otherwise. J_HD mice, lacking both B-1 and B-2 cells (43, 212), infected and monitored over a 14-day time period exhibited no significant increase in incidence or severity of cystitis (130). On a final note regarding ILLs, administration of α-galactosylceramide (α-GalCer), a ligand for CD1d-restricted NKT cells, alleviates renal UPEC infection (168). Consistent with this, we have observed a resident population of $NK1.1^+$ cells (potentially NK or NKT cells) in the bladder of C57BL/6 mice that increases in response to UTI (K. E. Sivick and H. L. T. Mobley, unpublished data). Studies using a systemic *E. coli* infection model suggested that, similar to γδ T cells, NKT cells may act as early amplifiers of the innate immune response to UTI by rapid cytokine secretion (182).

COVERT OPERATIONS: CELLULAR AND HUMORAL ADAPTIVE IMMUNE RESPONSES TO UPEC-MEDIATED UTI

Existing data regarding adaptive immune responses to UPEC are relatively limited. In a seminal study, Thumbikat and colleagues engineered a strain of UPEC to express ovalbumin to examine mechanisms behind antigen-specific adaptive immune responses in experimental UTI (260). In response to reinfection, $CD4^+$ and $CD8^+$ cells infiltrated the bladder and expressed the CD69 activation marker in the spleen (260), extending the findings of early IHC studies probing T- and B-cell populations in infected bladders (109). Furthermore, splenocytes, enriched splenic T cells, or serum antibodies from previously infected donor mice each protected wild-type naïve recipient mice against UPEC challenge (260). This result suggests that protection derived from natural infection is antibody mediated, as UPEC-specific antibody-secreting plasma cells could be present in both splenocyte and enriched T-cell preparations. As expected, transfers from naïve donor mice did not facilitate enhanced protection to recipients (260). This result is in contrast to a previous murine adoptive transfer study where SCID recipients receiving splenocytes from either naïve or vaccinated wild-type donors exhibited equal levels of enhanced clearance, despite the presence of antigen-specific plasma cells in the vaccinated donor cells (115). This result suggests that simply reconstituting immunosuppressed mice with lymphoid cells provides the means (likely stimulatory cytokines for phagocytic cells) for enhanced clearance. Conversely, wild-type recipient mice used in the former study only exhibited enhanced clearance when given cells or serum from antigen-educated, vaccinated donors (260), indicating that enhanced protection in individuals with intact immune systems will be provided only by stimulation of an effective adaptive immune response.

T-cell subsets are characterized by transcription factors and cytokines involved in their differentiation and the particular effector cytokines they secrete. To date, studies have not implicated a skew toward Th1- or Th2-mediated UTI immunity (5, 260). DC phagocytosis of infected apoptotic cells is the key event required for DCs to secrete the cytokine milieu necessary for Th17 development (262), and both DCs and infected apoptotic cells are present in the bladder during UTI. Despite this connection, IL-17A is dispensable for the generation of a protective response in a murine reinfection model, suggesting that Th17 cells may not play a role in adaptive responses to UPEC infection (Sivick et al., submitted). Similar to APCs and other lymphocytes, there are resident $CD8^+$ cells in the bladder that increase in response to infection (260; also Sivick and Mobley, unpublished). We speculate that the observed $CD8^+$ cells are either classical cytotoxic T cells or intraepithelial lymphocytes that exert cytotoxic effects on UPEC- or virus-infected cells or rapidly secrete cytokines to mobilize innate immune responses. Lastly, the role of Treg subsets in UTI host defense has not been formally examined.

Despite the lack of detail regarding T-cell responses to UTI, there is ample evidence for antibody-mediated clearance of UPEC. Since the 1970s, the genitourinary tract has been recognized as part of the secretory immune system (82, 261). UPEC-specific antibodies are detected in the urine of infected patients (202) and in the urine or serum of animals exposed to UPEC antigens (116, 217, 260, 264). Urinary IgG and IgA from UTI patients are capable of inhibiting UPEC adherence (58, 249, 263). Patient studies have also suggested that antibody responses to pyelonephritis are, in general, stronger and last longer than humoral responses to cystitis (80, 134, 135). Analysis of murine urine and serum samples collected before and after vaccination with OMP iron receptors allowed identification of immunological correlates of vaccine-induced protection against UTI (5). Specifically, levels of either urinary IgA or serum IgG (relative to serum IgM; denoted the class switch index) inversely correlated with bladder colonization in vaccinated mice (5). Presumably, urinary IgA plays a direct role in UPEC clearance from the bladder mucosa, while IgG may be a marker for class switching by B cells or also play a direct role in mucosal bacterial clearance. As mentioned earlier, infected J_HD mice had wild-type levels of colonization in response to primary infection, suggesting that B cells have no role in innate clearance of UPEC (130). However, this result is not unexpected since both antigen presentation and antibody-mediated protection provided by B cells would likely play a role in adaptive responses, indicating a need for reevaluation of these mice in UPEC reinfection and vaccination challenge models.

RECONSTRUCTION AND RECOVERY: UROTHELIAL REGENERATION IN RESPONSE TO UPEC INFECTION

One of the consequences of UPEC infection is exfoliation of the superficial facet cell layer that lines the surface of the bladder lumen (10, 60, 81, 166, 176, 177, 189). Microarray analyses probing regenerative signals revealed that, in addition to genes involved in cell biological processes, inflammatory cytokines, chemokines, signaling molecules, and transcription factors are also upregulated in response to inoculation (180, 181). While regeneration itself appears to be a function of basal stem/progenitor cells in the transitional epithelium (180), studies of the gut epithelium unveiled macrophages as "cellular transceivers" that relay MyD88-dependent inputs from the epithelium to colonic epithelial progenitors via direct contact (209). Whether or not macrophages play a similar role in the urinary tract remains unknown.

THE WAY AHEAD: VACCINE AND IMMUNIZATION STRATEGIES

While treatments have been proposed to expel intracellular UPEC from the bladder (29, 179), an *E. coli* reservoir harboring potential UPEC strains will always be present in the intestine (127, 173, 291). The involvement of TLRs in the immune response to UTI and current knowledge of their ability to incite innate and direct adaptive responses make them attractive adjuvant candidates for UTI vaccines (149, 242). These and other mucosal adjuvants and variations in vaccination routes and schedules must be tested in an effort to generate UPEC-specific local and systemic antibodies (155, 204) and optimize production of immunological memory, not tolerance (42, 165). There is considerable work to be done to better understand the mechanisms of protective immunity against UPEC in the bladder. Specifically, available knockout mouse

strains could be used to systematically evaluate the role of various receptors, signaling molecules, cytokines and chemokines, and cell types in controlling UPEC-mediated UTI and eliciting potent adaptive and memory immune responses. Ideally, the field can acquire insights on UTI immunity at a level suitable to rationally develop a much-needed vaccine that elicits sterilizing immunity against UPEC in the human urinary tract.

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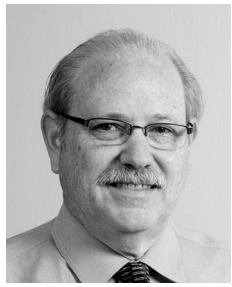

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