Integrated Pathophysiology of Pyelonephritis

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ABSTRACT Pyelonephritis represents a subset of urinary tract infections that occur from bacteria ascending from the lower to the upper reaches of the genitourinary system, such as the kidney. The renal system contains a range of hydrodynamically and immunologically challenging, interconnected microenvironiments where the invading pathogen may populate during the course of the infection. The situation at the infection foci changes dynamically, vacillating between bacterial colonization and clearance, to which the outcome is a summation of all host-pathogen elements in play. A selection of important determinants includes factors of microbial origin, effects of eukaryotic cell signaling, physiological facets of the infected organ, and signals from distal organs. Improved understanding of the multifactorial aspects of molecular pathogenesis of infection requires intravital, cross-disciplinary approaches with high spatio-temporal resolution. The advancement of such approaches promises to eventually provide a comprehensive understanding of the integrated pathophysiology of pyelonephritis.

INTRODUCTION Urinary tract infections (UTIs) occur when bacteria, often originating from the fecal flora, migrate via the urethra to the bladder causing symptomatic cystitis or asymptomatic bacteriuria (1). Further ascension via the ureters leads to infection of the normally sterile kidneys, termed pyelonephritis, which is commonly defined as a tubulointerstitial disorder whose gross pathology includes abscess formation in the renal parenchyma and edema (1, 2). These conditions often lead to irreversible scar formation, and may contribute towards the development of renal insufficiency (3). The acute form of pyelonephritis is clinically defined as a syndrome of bacteriuria with accompanied uni- and bi-lateral flank pain and tenderness, chills, and sudden increase in temperature. It may encompass, or progress to, urosepsis, septic shock, and death (4). Chronic pyelonephritis, on the other hand, is a radiological diagnosis defined by histological changes to the renal tissue resulting from infection. Such changes may include renal scarring, fibrosis, tissue destruction, and interstitial inflammation (3).

Normally, the kidney is considered relatively resistant to infection owing to a number of challenges presented to any bacteria entering the urinary tract. To colonize in spite of the dynamically changing environment in these organs demands the adaptive flexibility of the invading pathogen. Moreover, the uroepithelium is far from uniform. Whereas the bladder is lined by a transitional stratified epithelium, the individual structure of the nephron contains different types of epithelia (5) (Fig. 1). The glomerular capillary tufts in the Bowman’s capsule are lined with thin squamous epithelial cells, whereas the tubular systems consist of single-layer epithelium with segment-specific expression of structure and function (5). The physiological process of urine production and

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discharge exposes microbes to mechanical stress originating from the continuous cycles of urine production, storage, and voiding. The chemical composition of the glomerular filtrate, which eventually forms the urine, is also changing throughout the different segments of the urinary tract due to reabsorption of filtered water, ions, and other solutes (6–8). Abnormalities in structure and function of the urinary tract can therefore increase susceptibility to infection (9). Voiding dysfunction and vesicoureteral reflux have been ascribed as risk factors in children, whereas behavioral factors are more relevant in adults (10). Also, genetic susceptibilities have been linked to pyelonephritis. Deficient CXCR1 expression in both adults and children has been linked to the typical symptoms of acute pyelonephritis and renal scarring because of suppressed innate defense mechanisms in these individuals (11).

Bacteria need to possess high metabolic versatility as well as a good degree of resilience to fluctuating physical conditions in order to enter the organs of the urinary tract. It is thus unsurprising that in the majority of diagnosed community-acquired UTIs, uropathogenic Escherichia coli (UPEC) is implicated in up to 80% of cases as the causative agent (12). Other Gram-negative bacterial species able to colonize the urinary tract include Klebsiella pneumoniae (6.2 %), Enterococcus (5.3 %), Proteus mirabilis (2 %), Klebsiella oxytoca (0.9 %), and Pseudomonas aeruginosa (0.8 %), whereas Gram-positive species include Streptococcus agalactiae (2.8 %), Staphylococcus saprophyticus (1.4 %), and the viridans streptococci group (0.9 %) (13). With UPEC being the unanimously major causative microbe for UTI, as well as the primary pathogen applied in numerous molecular studies of the UTI process, this chapter focuses on the current knowledge gained over the years from UPEC-induced UTIs. Specifically, this section will cover a selection of relevant facets of pyelonephritis, which have gained attention in intravital and animal models. For further details regarding specific bacterial and host factors, the reader is referred to relevant chapters in this book.

**PATHOPHYSIOLOGY OF PYELONEPHRITIS**

**The Cinematic View of Pyelonephritis**

The highly vascularized kidney consists of approximately 1 million nephrons that continuously filter blood from waste products. As filtrate flows through the nephron, tubulo-epithelial absorption and secretion turn the filtrate into urine. In pyelonephritis, invading bacteria cause deviation from this native-tissue physiology and histology, resulting in dramatic changes of the microenvironment at the infection site. In contrast to the widely used sacrificial animal model of pyelonephritis, which is limited to studies at defined time points or the end stage of infection, studies applying intravital imaging based on 2-photon microscopy allow for dynamic studies of organ physiology and disease pathophysiology at the cellular level in the live animal. Recently, this live-animal imaging technique was applied to perform a high-resolution study of a live-renal UPEC infection in real-time in the presence of all interplaying host factors, such as the immune, vascular, lymphatic, and nervous systems (Movie 1 <http://asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.UTI-0014-2012>) (14–16). The slow infusion of green fluorescent protein (GFP)<sup>*</sup>-expressing UPEC bacteria directly into the lumen of a superficial renal tubule allows for spatial and temporal control of the infection. Monitoring of this site revealed that very few bacteria initially adhered to the tubule epithelium in the face of the passing glomerular filtrate (Fig. 2A). These few bacteria, however, rapidly adapted to the microenvironment, and began colonizing the tubule (Figure 2B) (15).

As infection progresses, major alteration of the infected organ’s physiology occurs. Early tissue changes include vascular coagulation, epithelial breakdown, vascular leakage, immune cell recruitment, and general
tissue destruction (15, 17). Coagulation in local peritubular capillaries, and subsequent vascular shut-down, occur within 5–6 h of infection, and these events are accompanied by a dramatic loss of local tissue oxygen (17). At this very early stage on infection, the host response is highly focused to the infection site. One significant finding is related to the rapidity of renal responses to a local infection, leading to bacterial clearance within 22 h (Figure 2C) with no effect on neighboring tubules (Figure 2D). Nevertheless, clearance appears to have come at the cost of local tissue destruction and vascular shutdown. The resulting edema contains vast numbers of polymorphonuclear cells (PMNs) (Figure 2E, F), and this necrotic site showed great resemblance to abscesses seen on the superficial cortex in both the intravital model and the end-stage versions of the retrograde models of infection (16, 18). Whether the localized clearance of the infection is accompanied by bacterial colonization of distant nephrons is currently unknown.

**Bacterial Colonization in the Dynamic Renal Tubular Environment**

Whereas the reported shear stress for the renal tubule is low (19), bacteria must adhere to the tubulo-epithelium to withstand the associated hydrodynamic pressures. Shear stress in proximal tubules is estimated to be 0.17 dynes/cm²; however, fluctuations in tubular reabsorption and secretion as the body regulates renal function alter the viscosity of the filtrate, which imply

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**FIGURE 2** Real-time, 2-photon microscopy of UPEC strain CFT073: GFP⁺ (LT004) infecting the proximal tubule. Epithelium of the infected tubule (blue) and blood flow (red) are outlined by fluorophore-labeled dextran. Micro-infused bacteria are visualized by genetically encoded GFP⁺ (green). A: Foci of infection 1 h post-infusion of LT004. Arrow points at bacteria adhering to the epithelium. B: Foci of infection 5 h post infusion. Massive green fluorescence indicates bacterial multiplication. C: Foci of infection 22 h post infusion. Lack of green fluorescence indicates clearance of bacteria. D: Non-infected renal tissue adjacent to the infection site shown in C. Scale bar = 30 μm. E: Ex vivo histological analysis of the foci of infection shown in C by confocal microscopy. Nuclear stain Hoechst 33342 (blue) and leukocyte marker α-CD18-Cy3 (red) have been added. Fluorophore-labeled dextran outlining the infected tubule is pseudocolored (yellow). Scale bar = 50 μm. F: Magnification of image E. The arrow highlights a neutrophil phagocytosing bacteria. Scale bar = 10 μm (From Månsson LE, et al. 2007. Real-time studies of the progression of bacterial infections and immediate tissue responses in live animals. Reprinted from Cell Microbiol (16), with permission.) doi:10.1128/microbiolspec.UTI-0014-2012.f2
a degree of variability (19). Bacterial adhesion is thus an essential feature for successful colonization of the kidney. The wide and often redundant repertoire of UPEC attachment organelles includes P, type 1, F1C, S fimbriae, and Afa/Dr adhesins (20).

The P fimbriae, expressed in approximately 80% of the UPEC strains (21), are traditionally associated with pyelonephritis. A study undertaken comparing E. coli strains isolated from patients with acute pyelonephritis and those with asymptomatic bacteriuria showed that strains from the former group adhered in greater numbers to uroepithelium than the latter (22). Though renal colonization may also occur independently of PapG (18, 23–27), there is a strong indication that the presence of the P fimbriae increases the severity of infection, since PapG-positive clinical isolates induce more extensive renal damage as compared to the negative counterparts (21). When analyzing the role of P fimbriae for renal colonization using dynamic, intravital imaging, strains lacking PapG-mediated attachment showed compromised colonization kinetics with only a few bacteria visible before 8 h. This is in sharp contrast to the isogenic wild-type strain, which immediately established itself in the tubule. The important role of P fimbriae for early bacterial colonization in vivo was further demonstrated by the finding that successful, yet delayed, colonization only occurred in one-third of animals infected with the papG mutant strain compared to all animals infected with the P fimbriae-expressing counterpart. It is interesting to notice, however, that regardless of P-fimbriae-associated effects on the early colonization kinetics, the outcome 22 h post-infection was the same, with edema formation containing numerous neutrophils that had cleared the absolute majority of bacteria (14).

Synergistic Effects of P and Type 1 Fimbriae In Vivo

Traditionally, type 1 fimbriae have primarily been associated with bladder infections (18, 23–27). Gene expression studies on UPEC isolated from the kidney in the intravital model of infection showed, however, that bacteria also express type 1 during pyelonephritis. This suggests a novel role for type 1 fimbriae also in the upper compartments of the urinary tract. The ability to monitor bacterial behavior in real-time while exposed to physiological challenges of the nephron was critical to reveal a synergistic action between type 1 and P fimbriae. Intravital imaging showed that the combined action of the two fimbriae enabled UPEC as a population to resist the hydrodynamic pressure in the lumen of the proximal convoluted tubules. Whereas P fimbriae aid in the early stage of colonization by mediating binding to the epithelium, the type 1 fimbriae is essential for bacteria to colonize across the luminal center of the tubule while being exposed to the shear stress from the filtrate. Since bacteria are at risk to be washed away from the central part of the tubule lumen where no epithelium is available to hold on to, the population is forced to engage in inter-bacterial adhesion (Movie 2<http://asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.UTI-0014-2012>)(14). A role of type 1 fimbriae in inter-bacterial binding is corroborated by previous findings, showing that the FimH tip adhesin is essential for biofilm formation in vitro (28, 29). Inter-bacterial binding, as well as direct binding to biotic and abiotic surfaces, are key interactions for biofilm formation, making biofilms extraordinarily resistant to hydrodynamic-flow shear forces and allow bacteria to colonize perfused environments.

The binding capacity of the FimH tip adhesin is enhanced when exposed to shear stress of approximately the same magnitude as that which a bacterium experiences in the urinary tract (30, 31). This occurs through a force-enhanced allosteric catch-bond mechanism, operating via a finger-trap-like β sheet-twisting mechanism (30). The initial weak interaction of FimH shear-dependent binding is strengthened as the shear forces increase from 0.02 to 0.8 dynes/cm² (31). With the relaxation of shear strength, UPEC strains were observed to positively select for FimH variants that maintained attachment in comparison to fecal or vaginal E. coli isolates (32). The positive regulation of bacterial adhesion by hydrodynamic forces is particularly meaningful in the urinary tract where flow is pertinent albeit with fluctuations, allowing bacteria to bind and remain in the microenvironment rather than being washed out of the system.

Expression of P and type 1 fimbriae are controlled by ‘phase variation’, thereby allowing rapid adaptation and fine-tuning of the gene expression of the bacteria’s adhesive nature in response to the microenvironment (33). At the same time, this allows for development of heterogeneous bacteria populations (34). The genetic fim switch controlling the expression of type 1 or P fimbriae is an example of phase variation. This invertible genetic element carries the main promoter for the expression of fimbrial-structural subunits (34). PapB represses the FimB-promoted off-to-on inversion of the fim switch (35). Similarly, the PapX protein in UPEC strain CFT073 binds to the flbD promoter and represses
the transcription of the master regulator of flagella FlhD2C2, thereby negatively regulating motility (36). It can thus be envisaged that UPEC is able to tightly regulate the antagonistic forces of adhesion and motility in order to colonize the urinary tract.

**Bacterial Toxins Affect the Kinetics of Host Responses**

UPEC express several proteinaceous toxins. Though commonly referred to as virulence factors, their precise role in disease is still unclear. The virulence factor concept is further complicated by the notion that toxins are not ubiquitously expressed among all UPEC isolates. The lipoprotein α-hemolysin (Hly), considered to be an important UPEC virulence factor, is only expressed by circa 50% of E. coli isolates implicated in pyelonephritis. The traditional view of Hly as a pore-forming toxin had to be revised when it was found that Hly exerts an isogenic mutation on soluble CD14 in body fluids, the level of LPS detection of uroepithelial cells is as sensitive as that of macrophages (42–43). In uninfected kidneys, TLR4 is mainly found on the apical surface of the distal tubules (46). However, when the host is experiencing sepsis, TLR4 expression becomes ubiquitous across all segments of the kidney suggesting that TLR4 is upregulated during inflammation (46, 47). This variability may explain the discrepancies of inconsistent reports on TLR4 presence on renal cells (42, 43). It remains, however, that TLR4 plays a role in protecting the host from UTI. Studies of the genetic relationship of TLR4 with UTI have shown an association with different types of UTI. For example, children with reduced TLR4 expression on neutrophils have a higher prevalence of developing asymptomatic bacteriuria (48, 49). Individuals with the TLR4 A(896)G allele are also more susceptible to recurrent UTI (50).

While colonizing the kidney, UPEC have indeed been shown to express Hly (16). To analyze a role for Hly in vivo, intravital imaging using either a wild-type or an isogenic hlyA mutant strain showed that bacterial colonization of the proximal tubule was equally efficient, whether or not Hly was expressed (40). A striking difference was, however, observed when studying the host response. In the absence of Hly, delayed kinetics of the tissue responses, which involved the onset of ischemia, obstruction, and immune cell recruitment, were observed (16). Indirectly, these findings correspond to reduced pro-inflammatory signaling in the absence of Hly; however, the precise role for Hly-induced Ca2+ signaling in vivo remains to be analyzed. Important to note is, however, that the same end point of infection at 24 h is achieved regardless of Hly expression.

**Innate Immune Responses**

Toll-like receptors (TLR) play an important function to alert the host innate immune system to the presence of pathogens through detection of pathogen-associated molecular patterns. Binding of these leads to the dimerization of TLRs and the activation of TLR signaling by co-receptor recruitment or engagement, typically inducing a pro-inflammatory responses (41). Among the 10 and 12 members of TLRs that have been characterized in humans and mice, respectively, TLR4, TLR5, and TLR11 have been associated with the urinary tract.

Uroepithelial cells express TLR4, which confers sensitivity to Gram-negative bacteria via lipopolysaccharide (LPS) detection. While the system is uniquely dependent on soluble CD14 in body fluids, the level of LPS detection of uroepithelial cells is as sensitive as that of macrophages (42–43). In uninfected kidneys, TLR4 is mainly found on the apical surface of the distal tubules (46). However, when the host is experiencing sepsis, TLR4 expression becomes ubiquitous across all segments of the kidney suggesting that TLR4 is upregulated during inflammation (46, 47). This variability may explain the discrepancies of inconsistent reports on TLR4 presence on renal cells (42, 43). It remains, however, that TLR4 plays a role in protecting the host from UTI. Studies of the genetic relationship of TLR4 with UTI have shown an association with different types of UTI. For example, children with reduced TLR4 expression on neutrophils have a higher prevalence of developing asymptomatic bacteriuria (48, 49). Individuals with the TLR4 A(896)G allele are also more susceptible to recurrent UTI (50).

In mice models of UTI, TLR5, which binds bacterial flagellin (51), as well as TLR11 (ligand unknown) (52) are important pattern-recognition receptors (PRRs) involved in the innate immune system. TLR signaling induces the production of cytokines, which in turn coordinate the immune response. A well-known subset of this response is the extravasation of inflammatory cells such as (PMNs), or neutrophils, along a chemotactic gradient of cytokines to the site of infection in the tissue. Transepithelial PMN migration is promoted by the main human chemokine IL-8 and the corresponding receptor CXCR1 (53). The importance of this particular signaling pathway is highlighted by an increased susceptibility to pyelonephritis in individuals with polymorphisms and mutations in the CXCR1 gene (54).

The neutrophil is the primary cell type involved in clearing a bacterial infection. Intravital imaging suggested, however, the involvement of other cell types in both the early inflammatory response as well as in the...
cleansing of bacteria. PMN recruitment is observed as early as 4 h post-infection; however, other cell types of hitherto unidentified origin also appear at the site. At 8 h post-infection, PMNs constituted only 20% to 40% of recruited nucleated cells and several hours ensued before neutrophils became the predominant cell type at the site (16). PMNs play a significant role in bacterial clearance via phagocytosis, a process that may occur independently of prior opsonization.

While neutrophils are necessary for the clearance of infection, their recruitment is also linked to severe tissue damage. Neutrophil granules released in response to the infection contain anti-microbial peptides, proteins, and proteolytic enzymes, which may exert acute and/or permanent detrimental effects on the extracellular matrix, cell structures, or functions (53). Evidently, PMNs isolated from acute pyelonephritic exudates cause lysis of a number of cell types in vitro within 24–48 h (55). Suppression of suppuration was found to reduce tubular epithelial cell damage and renal scarring albeit a higher bacterial burden (56, 57). In contrast to the traditional bacteriocentric view on tissue damage, the integrative view describes tissue damage as the result of several facets of an infection originating from both pathogen and host (14). This includes the inflammatory response as well as physiological injuries, such as ischemia and obstruction, during an infection.

Antimicrobial proteins and peptides produced by different parts of the nephrons’ urothelium constitute a major host defense mechanism against invading microbes. In contrast to TLRs, antimicrobial peptides can have a direct antimicrobial effect on pathogens, or indirectly function by modulating innate and adaptive immune responses. Antimicrobial peptides and proteins of importance in the kidney include the Tamm-Horsfall protein (THP), defensins, cathelicidins, lactoferrin, and lipocalin. THP is an evolutionarily conserved glycoprotein highly abundant in human urine. This protein is produced specifically by epithelial cells at the ascending loop of Henle (58, 59). THP stimulates both parts of the host’s innate and adaptive immune responses (60, 61). It is involved in cytokine production, cell-specific stimulation of granulocytes towards IL-8 production (60, 62), up-regulation of co-stimulatory molecules, major histocompatibility complex (MHC) expression on dendritic cells (DC), and DC maturation via TLR4 signaling. Correspondingly, the wide-reaching and strong effect of THP on immune responses can lead to interstitial nephritis when excessively stimulated (60, 61).

Defensins constitute another group of antimicrobial peptides of renal importance. Whereas β-defensins are produced by the local renal epithelium (61, 62), α-defensins are secreted from infiltrating neutrophils. These peptides exert dual effect, either showing direct antimicrobial activity on invading bacteria, or causing an indirect enhancement of the innate and acquired immune response. Via induction of secondary signaling from cells and tissues, defensins are indirectly affecting immune cell recruitment, the regulation of acute inflammation, angiogenesis, and wound healing (61).

Cathelicidins are produced and subsequently released into the tubular lumen of the urinary tract (63). Some peptides of this family, such as LL-37, are also produced in a wide variety of locations in the host, including epithelial cells of the skin, the gastrointestinal tract, the epididymis, lungs, neutrophils, and myeloid bone marrow cells (64, 65). Cathelicidin-based antimicrobial response to invading pathogens constitutes a two-stage process. Prior to leukocyte infiltration early during infection, cathelicidins are mainly produced by the epithelium. As the infection progress, cathelicidin production shifts to the recruited neutrophils (63). Intriguingly, the effect of cathelicidins is not impartial. The impact of the peptide on uropathogenic bacteria appears to be stronger than that with urogenital commensal bacteria (66). On the flipside, UPEC strains that cause severe UTI are likely to have a higher resistance to this peptide (66).

Infection-Associated Disruption of Mucosal Integrity

The intravital model of acute pyelonephritis reveals that local tissue destruction at infection site results from extremely rapid host responses (16). A cascade of signaling events is triggered, whose outcomes are observable as early as within 3 to 4 h from the entry of bacteria into the tissue. This includes massive rearrangement of the tubuloepithelial actin cytoskeleton, as well as sloughing of epithelial cells from the basement membrane (16, 17). The tight link between destruction of actin cytoskeleton and interference of the microfilament system, integrins, immunoglobulins, and cell-adhesion molecules are known to contribute to the disruption of epithelial-barrier function (67, 68). Additionally, intravital studies of non-infected kidneys have demonstrated that hypoxia-induced rearrangement of renal cortical-actin cytoskeleton interfere with cell–cell adhesion as well as cell–extracellular matrix adhesion, thereby disrupting tubular integrity (69–71).

A similar situation prevails in the UPEC-infected nephron, where epithelial signaling rapidly causes localized ischemia (see next section) (16). Dynamic imag-
ing shows ischemic injuries in the infected nephron, including the typical signs of local vascular leakage and loss of epithelial membrane-barrier functions, as soon as 4 h after onset of infection (Fig. 3 and Movie 3). A dynamic view on these events can be obtained using 2-photon microscopy to visualize the fate of systemically injected small molecular weight red-fluorescent dextran. Immediately upon injection, the red dextran is observed within the peritubular capillaries (Fig. 3, which represents selected images from Movie 3). In a non-infected nephron (upper part of Fig. 3 and Movie 3), dextran is filtered by the glomerulus and passes swiftly through the tubule lumens. The infected nephron showed a dramatically different situation (lower part of Fig. 3 and Movie 3). Loss of epithelial membrane-barrier function is obvious, as red dextran leaks into the epithelial cells. Careful inspection of the data reveals that leakage actually is initiated from the basolateral side, indicating a major contribution of vascular leakage in the interruption of epithelial membrane integrity. Inspection of the epithelial linings of the neighboring nephrons reveals that membrane disruption is strictly confined to the infected nephron.

A common assumption is that breakdown of epithelial integrity would open for further dissemination of bacteria, including systemic spread. It appears, however, that the tubular basement membrane confers an additional barrier function, attributed to the intact layer of connective tissue (17). Though bacteria can reach the basolateral side of the epithelium via paracellular migration, bacteria are confined within the tubule, lining up against the collagen IV-rich basement membrane. It can be hypothesized that the concerted actions of epithelial and basement-membrane barriers are important for the appropriate timing of the host response, keeping bacteria on-site to give sufficient time for an adequate, but not excessive, antimicrobial response to be mounted. Data shows that when UPEC eventually passes these barriers, neutrophils as well as mononucleated cells have arrived to the site to aid in solving the situation.

**FIGURE 3** Epithelial-barrier disruption and impaired renal filtration due to UPEC infection. Real-time 2-photon imaging of the foci of infection 4 h post-infusion of UPEC strain LT004. Images obtained 7, 20, and 80 s after intravenous bolus infusion of fluorophore-labeled 10 kilodalton (kDa) dextran are shown (red). In the non-infected nephron (upper part of figure), efficient filtration is observed as the tubular appearance of the bright-red fluorescence arising from the labeled dextran (20 s) is followed by an obvious drop in intensity (80 s). This indicates renal clearance. Renal obstruction of the infected nephron (lower part of figure) is observed as only limited fluorescent dextran enters the tubule. Epithelial-barrier function is destroyed in the infected tubule, as dextran is observed to enter the epithelial layer (arrowhead, 7 s), suggestive of epithelial and endothelial dysfunction. In contrast, the healthy epithelia in the non-infected nephron exclude the dextran (arrowhead, 20 s). Vascular clotting can also be observed in the vasculature next to the infected nephron (arrow, 20 s). (From Melican K, et al. 2011. Uropathogenic E. coli P and type 1 fimbriae act in synergy in a living host to facilitate renal colonization leading to nephron obstruction. Reprinted from PLoS Pathog (14), with permission.)

Loss of tubular integrity may therefore permit unbiased paracellular movements allowing both local pathogen migration as well as the extravasation of host neutrophils at the site of infection. From a clinical perspective, infection-associated breakdown of the epithelial integrity combined with cellular detachment and sloughing from the basement membrane strongly argue for a minor, if any, role for the tubule as a persistence reservoir for UPEC in the upper urinary tract. This is in contrast to the lower urinary tract where recurrent cystitis is considered to depend on UPEC persisting intracellularly in the underlying squamous epithelial layer of the bladder.

Clotting, Ischemia, and Hypoxia Prevents Dissemination

At the infected nephron, local hypoxia has been linked to the characteristic signs of ischemic injuries. Probing the local tissue oxygen tension using Clark-type micro-electrodes reveals that the infection site is completely devoid of oxygen within 4 h (oxygen tension = 0 mmHg). In vitro recording of oxygen consumption demonstrated that increased metabolic activity of UPEC-infected primary proximal tubule cells contributed to this effect. The high metabolic activity correlated to up-regulation of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, IL-1β, and IL-6 in vivo and in vitro, demonstrating the important role of the proximal tubule epithelium as an early responder to infection.

Onset of ischemia occurs rapidly, prior to neutrophil infiltration, suggesting the involvement of rapid infection-associated cell signaling. Intravital imaging shows that black silhouettes of the size of platelets are present in the peritubular capillaries, and eventually, clots are observed in the peritubular capillaries. Analysis of the local mRNA expression profile confirms that the vasculature shutdown is due to activation of the clotting cascade. Clot formation is usually associated with blood-borne infections; however, as local ischemia develops in the infected nephron, UPEC remain in the lumen of the proximal tubule without direct contact to the endothelium. Clot formation thus occurs as a consequence of infection-induced molecular epithelial–endothelial crosstalk of hitherto unknown nature.

Clot formation is shown to provide a novel innate immune-defense mechanism, as it protects the host from bacterial dissemination and systemic spread. By inhibiting clot formation via anticoagulant therapy, bacteria disseminate rapidly into the systemic circulation, causing death by septic shock within a few hours. Post-mortem examination of the succumbed animals shows bacterial spread to the blood, heart, liver, and spleen. Hence, clot formation adds to the list of host responses to infection associated with sepsis.

**FIGURE 4** Clot formation in mucosal infections. Real-time 2-photon imaging of a UPEC strain LT004 (green)-infected nephron (blue) shows platelets (arrow) in the form of black silhouettes surrounded by blood (red) within the peri-tubular vasculature. 2.5 h post-infection. Black masses adhered to the vessel wall (arrow head) suggest the presence of platelet aggregates. The high-intensity-red fluorescence indicates stagnant blood flow and a lack of red blood cell movement. Scale bar = 30 μm. (From Melican K, et al. 2008. Bacterial infection-mediated mucosal signaling induces local renal ischemia as a defense against sepsis. Reprinted from Cell Microbiol, with permission.) doi:10.1128/microbiolspec.UTI-0014-2012.f4
mucosal infection as cessation of vascular flow helps to contain bacteria locally at the infection site, while neutrophil infiltration and other defense mechanisms become activated.

**Infection-Associated Nephron Obstruction**

Glomerular filtration is tightly associated with renal UPEC infection. Featured both as tubulopathic or free aggregates, the increasing bacterial population has severe impact on renal filtration, leading to obstruction of the nephron (14). Sloughing of the tissue, cast formation, and cellular blebbing following ischemic injury further adds to the tubular obstruction (75). This is a severe condition, since physical obstruction of the nephron can rapidly lead to acute renal failure (6).

Obstruction of the filtrate flow leads to major changes in hydrostatic pressure and dilation of the tubules (75). The increased intra-renal pressure can also effect renal filtration, leading to critical reduction of the glomerular-filtration rates (GFR) (75). In severe conditions, associated injuries may extend to arteriole vasoconstriction and a drop in renal blood flow (75, 76). Actually, minute injuries, such as single-nephron obstruction, are severely affecting the pathophysiology of renal injuries, as local induction of an inflammatory response, tubular-cell injury, changes in glomerular-capillary pressure and eventual disuse atrophy have been demonstrated (76–80). A strong link between infection and renal hydromechanics is further illustrated as LPS is shown to impair ion-transport functions of renal tubules in a TRL4-dependent manner (81, 82).

Feedback between the tubules and renal vasculature, so-called tubuloglomerular feedback (TGF), is regulated by the juxtaglomerular apparatus (JGA) (83). The JGA is composed of specialized distal tubule epithelial cells called the macula densa, afferent arteriole, and juxtaglomerular cells. Found between the vascular pole of the renal corpuscle and the returning distal convoluted tubule, changes in glomerular filtrate volume and osmolarity is sensed by macula densa and juxtaglomerular cells, which in turn modulate the renal system through renin and vasopressin release, respectively (84). Whether the renal injuries associated with UPEC infection induces a JGA-directed response is currently unknown.

**Inter-Organ Communication During Infection**

Renal injury, such as ischemia, can initiate molecular crosstalk of inflammatory nature between the kidney and distant organs (85). Studies of such complex events call for the use of an all-inclusive intravital model of infection. A transcriptomic analysis of biopsies obtained from the spatio-temporally well-defined intravital model of pyelonephritis showed that the host response involves active crosstalk at the site of infection. Microarray data from biopsies isolated within the first 8 h of infection revealed circa 60 genes to be significantly differentially expressed as compared to non-infected controls (86). A Gene Ontology (GO) analysis showed these genes to cluster into functionally defined categories, many of which are associated to inflammation. The tissue response is, however, very versatile, and it embraces other GO categories, such as wound healing, response to hypoxia, cell death, and apoptosis. Collectively, this confirms the multifaceted events, such as ischemia and tissue disruption, observed in the real-time model of infection.

A comparative tissue transcriptomics approach applied to study early (8 h post-infection) tissue responses in pyelonephritis revealed a common core of 80 significantly upregulated genes in Gram-negative infection and inflammation (86). Interestingly, 25% of identified genes are interferon (IFN-IFN-γ regulated. A marked increase of IFN-γ serum levels is accompanied by upregulation of splenic ifng gene expression involving the IL-17/IL-23 pathway (86). This suggests a rapid engagement of the local infection site in communicating with distant sites. The mechanisms governing this communication remain to be identified.

**ACUTE KIDNEY INJURY**

Acute kidney injury (AKI) of septic or non-septic origin is a common clinical complication that can occur from a range of kidney pathologies, to which renal failure is the end result. The pathophysiology includes renal hypoperfusion and ischemic injury, cardiogenic or distributive shock, followed by tubular necrosis (87, 88). More recently, septic AKI has been linked to maintained global renal (medullar and cortical) blood flow (89), highlighting that symptoms and treatments of infection-associated kidney injuries differ amongst septic cases. In the U.S.A., AKI shows a varying incidence of 1% (community-acquired) up to 7.1% (hospital-acquired) of all hospital admissions (90, 91).

A number of mechanisms are proposed by which UPEC infections induce renal injury. Damaging toxins expressed by infecting bacteria are considered major factors for tissue destruction (55, 57, 92, 93). This is acting in parallel to the damaging effects from the strong inflammatory response. Upon immune suppression, renal scaring is reduced despite a consequential...
increased bacterial load (94, 95). Neutrophils isolated from acute pyelonephritic exudates are known to kill syngeneic renal cells in vitro, which is corroborated by in vivo studies, showing neutrophil-induced oxidative injury of renal cells (17). The intravital model of pyelonephritis identified local ischemia occurring only hours after the first bacterium entered the tubule, which together with bacterial multiplication in the tubule lumen led to severe obstruction (17). Both are complex syndromes to which severe episodes of either condition can result in renal scarring (96) and lead to end-stage renal failure.

Cell death associated to ischemic renal injury occurs either via necrosis or via induction of apoptotic-signaling pathways. Adenosine triphosphate (ATP) and guanosine-5′-triphosphate (GTP) act as small-molecule inducers of either one of the pathways (97). In the latter situation, hypoxia, resulting from a combination of reduced local blood flow and increased respiratory demand, depletes the pool of the small nucleotide GTP, thus altering the ratio of GTP/guanosine diphosphate (GDP) that is known to regulate the GTPase activity (97). It is postulated that GTP depletion induces apoptosis through Ras and Rho family GTPases (97).

Despite the original cause, AKI is not an isolated event. Inflammatory mechanisms causing increased cytokine production, oxidative stress, edema, and leukocyte trafficking may act in concert to instigate dysfunction of distal organs. For a comprehensive review of extrarenal organ dysfunction, the reader is referred to Yap et al. (87). Uncontrolled activation of inflammation in response to AKI leads to increased epithelial and endothelial apoptosis, increased membrane/vascular permeability, and effects on water channels (aquaporin) and sodium-potassium pumps (87, 98). In the lung, histological and physiological changes in response to septic AKI are associated with the activity IL-1, IL-6, TNF-α, and macrophages, as intervention therapies conferred attenuation of the pathways leading to dysfunction (99, 100). Within the gastrointestinal tract, AKI leads to increase in IL-17A, which brings about histological alterations through increased necrosis and apoptosis (101). Additionally, a major increase of proinflammatory cytokines such as IL-6 and TNF-α is observed (87). As cytokines are being drained into the liver through the portal circulation, this organ will also succumb to inflammation-related cell death and tissue damage (102–104). Pertaining to AKI and cardiac function, increased cytokines TNF-α, IL-6, and IL-1 and neutrophil infiltration are clinically associated with congestive heart failure (105).

**TREATMENT**

As the primary cause of pyelonephritis is a bacterial infection, the treatment of the condition is similar to that of UTI, which is mainly by the application of antibiotics. For in-depth reading into UTI treatment, the reader is referred to Wagenlehner et al. (106), which provides extensive statistics and efficacy analysis of clinically prescribed antibiotics. In Wagenlehner’s analysis, the options for UTI treatment is based on the assignment of the patient into categories of uncomplicated and complicated UTI. (106, 107). However, treatment of both UTI categories follows the fundamental aim of providing fast and efficient intervention against recurrent infections and prevention of resistance generation, as well as exacerbation (106).

Wagenlehner et al. (106) defines the two categories as follows:

“Uncomplicated UTI denotes UTI without relevant structural and functional abnormalities arising from the urinary tract (uropathies), without relevant kidney diseases (nephropathies) and without relevant comorbidities. Conversely, complicated UTI is a complex condition of the following conditions: (1) Anatomical, structural or functional alterations of the urinary tract. (2) Impaired renal function by parenchymal and renal nephropathies. (3) Accompanying diseases or conditions that impair the patients’ immune status.”

Acute pyelonephritis is a complex syndrome, which may not consistently exhibit the same symptoms at the time of diagnosis. In addition, this stage of infection can open to a wide variety of complications. Treatment of acute pyelonephritis therefore follows under the category of complex UTI. Complex UTI follows a bidirectional approach in which treatment is directed to remove the disease-causing pathogen, as well as the complicating factors arising from the infection (107).

Calibration of the antibiotic treatment follows several criteria (106). An individual’s risk when undergoing antibiotic treatment, the pathogen’s sensitivity spectrum and concentration to antibiotics, clinical efficacy of an antibiotic, possible effects on the commensal microbiota, side effects, resistance development, and the possibility that the causative agent in complex UTI may be polymicrobial in nature with wide-spectrum antibiotic resistances (106).

Acute pyelonephritis may include or progress into permanent damage to the tissue, organ failure, and urosepsis. Urosepsis is treated by a combination of approaches, which include removal of the infection source, eradication of the existing infection, application of countermeasures against complications, and life-supportive care (106).
Folk remedies have also been used widely as a treatment for UTI infection. However, application is likely to be more suited to the category of uncomplicated UTI. Among the wide range in the market, one common folk remedy is cranberry juice. Also available in tablet form, cranberries contain fructose and proanthocyanidins, which inhibits bacterial adherence via type 1 and P fimbriae, respectively (108–110). However, clinical trials have yet to show definitive evidence supporting its efficacy as UTI treatment.

**FUTURE PROJECTIONS**

The pathophysiology of pyelonephritis is evidently a complex scenario, which changes dynamically over time. It involves a combination of factors originating from the infection as well as from physiological injuries, such as ischemia and obstruction. The latter are both well-studied physiological injuries, known to cause inflammation and tissue destruction in their own right (67–70, 77, 78, 111–114). Adding the tissue alterations associated with bacterial colonization, such as coagulation, epithelial breakdown, vascular leakage, immune cell recruitment, and tissue destruction, further complicates the picture. The homeostatic imbalance at the local site of infection, as well as involvement of distal signaling and engagement of multiple cell types, acts in concert over time to manage the infection. The pathophysiological changes occurring during infection can be schematically summarized in a ‘pathophysiogram of pyelonephritis.’ As Fig. 5 illustrates, the multi-faceted host response will dramatically alter the local microenvironment, forcing bacteria to adapt physiologically in order to maintain themselves. The integrated pathophysiology of pyelonephritis thus constitutes a number of integrated events in which the host and the microbes mutually influence each other over time. Full understanding of these events is required to coherently define relevant interactions and identify new potential angles for disease intervention.

In future microbial pathogenesis research, it can be predicted that focus will be placed on monitoring and mimicking host-pathogen interaction within the dynamic micro-ecology significant for infectious niches.
in the live host. This all-inclusive approach, which integrates all elements of ‘cellular microbiology’, ‘histology’, and ‘physiology’ when studying infections in real-time, is termed ‘tissue microbiology’ (Fig. 6) (17, 115, 116). During the time-course of infection, the assayed organ acts as the test tube in which the experimental parameters are set by the tissue’s own response to infection. In this changing environment, the roles certain bacterial virulence factors play in vivo can be addressed, either as individual factors or as multiple factors acting in synergy. This strategy will allow for identification of any subtle effects virulence or fitness factors may exert on bacterial-colonization kinetics, or effects on the kinetics of the host response. Furthermore, this will allow for more precise definitions of the infectious niche. Instead of an organ-based definition, i.e., bladder versus kidney, these niches will be precisely defined as sub-compartments within the tissue. In pyelonephritis, this is exemplified by the center or periphery of a single tubule lumen (see previous section ‘Synergistic effect of fimbriae to cope with urine shear stress’).

Successful implementation of the novel area of tissue microbiology will, however, require the cooperation between different research disciplines, ranging from engineering sciences, pre-clinical research, and clinical research. Nanomedicine is an expanding field that is likely to produce novel tools with great promise to propel intravital studies into a new era. Also, we are likely to see the development of advanced biomimetic in vitro systems that closely resemble the in vivo microenvironment. Using these integrated in vivo and in vitro approaches, our understanding of host-pathogen interactions will advance, and so will clinical diagnostics, treatment, and patient care.

**FIGURE 6** Schematic representation of classical research disciplines that coalesce to form ‘tissue microbiology’, a recently proposed concept that integrates a range of disciplines and expertise. At the base of the pyramid are microbiology, which represents the in vitro study of microbial pathogens, and cellular biology, which focuses on the study of host cell types. Cellular microbiology was a discipline formed where microbiology and cellular biology overlapped. Coined in 1996, the approach was aimed at studying host–pathogen interactions using another’s perspectives, tools, and competences. Histology and physiology remained individual and required separate analysis. As knowledge and technological advancements make for unprecedented tools for intravital studies, tissue microbiology can now be established, advancing infection biology with an all-inclusive approach to generate an integrated view of the pathophysiology of infection. doi:10.1128/microbiolspec.UTI-0014-2012.f6
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