UROPATHOGENIC ESCHERICHIA COLI AS A MODEL OF HOST-PARASITE INTERACTION

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INTRODUCTION

Urinary tract infections (UTIs) provide an excellent model to study how the host recognises and deals with mucosal pathogens [1-3]. The infecting strain encounters a microbially naïve mucosal environment where the pathogenic strains may cause acute, potentially life-threatening infections. Chronic sequels are prevalent, and there is a link between acute infection and chronicity [4,5]. The mechanisms underlying commensalism may also be studied in the urinary tract, as asymptomatic bateriuria (ABU) occurs in at least 1% of the population and the patients may carry $>10^5$ cfu / ml of *Escherichia coli* in the urine for months or years with no or few symptoms [6,7]. Studies in the UTI model have identified molecular mechanisms that initiate tissue attack by mucosal pathogens and that trigger the innate host response [8]. Based on these mechanisms the genetics of disease susceptibility are beginning to be understood [9,10].

Virulence or symbiosis

The severity of UTI reflects the virulence of the infecting strain. In the 1940s, hemolysin was identified as a characteristic of *E. coli* causing extra-intestinal infections [11]. Uropathogenic *E. coli* (UPEC) strains were later shown to belong to a restricted set of serotypes or ‘’clones’’ [12], and acute pyelonephritis and ABU strains were found to differ in surface antigen repertoire [7]. In the 1970s this information was extended to involve attachment to the urinary tract mucosa [13]. High tissue attachment was shown to characterise the most virulent strains but not the asymptomatic carrier strains. Attachment was therefore proposed as the first step in the pathogenesis of UTI, and the epithelial cell was recognised as the first sensor of tissue attack [14-16]. The molecular basis of virulence has since then been extensively studied and a number of essential virulence factors have been identified [17]. The virulence genes are encoded on pathogenicity islands, and their expression, regulation and evolution have been elegantly characterised [18-20]. Recent studies have suggested that ABU strains may be attenuated pathogens, carrying deletions in the virulence genes involved in attachment and the tissue attack [21].

The virulence factors enable UPEC to trigger epithelial cell responses leading to inflammation, cell detachment and apoptosis, or invasion causing bacteremia [14,15,22-28]. The tissue response is lethal for the organism, however, and is unlikely that invasion offers an advantage that would drive the evolution of the virulent phenotype. The adaptation of uro-pathogenic clones occurs mainly in the large intestine, and it is more likely that virulence is "co-incidental" [29]. For example, the mucosal receptor for P fimbriated uro-pathogens are expressed both in the intestine and urinary tract, but ligand binding has different consequences at the two sites [30]. ABU, on the other hand, may represent a successful adaptation, as the bacteria can persist without competition in a niche with a rich nutrient source, often for several years. In this case, the host response may be advantageous by providing signals that attenuate bacterial virulence.

Responders or non-responders

The susceptibility to UTI varies greatly in the population, as does the severity of disease in susceptible individuals [31]. Studies in pyelonephritis prone children have identified ''*high responders*'' with abnormalities that exaggerate the damaging rather than protective aspects of innate immunity [3,32] and experimental infections in different mutant mice have identified a single gene defect, which causes the ''*high responder*'' phenotype [5]. Neutrophils are critical effectors of the host defence in the urinary tract and neutrophil dysfunctions, due to defective IL-8 receptor expression, cause acute pyelonephritis and renal scarring [10]. In ''*low responders,*'' on the other hand, bacteruria establishes without evoking a response, showing that suppression of inflammatory signals may be protective even though the infection remains. Also in this case, studies in mice have identified genetic control mechanisms that decide if the host will remain asymptomatic or develop disease. Mice carrying a mutation in the signalling domain of Toll like receptor 4 (Tlr4) were shown to develop an asymptomatic carrier state resembling human ABU [33-35].

This review focuses on these two steps in disease pathogenesis and their consequences for human disease.

Step 1: Mechanism of pathogen recognition and host response induction

Pathogen recognition by the mucosa is guided by molecular specificity. Attachment is an essential first step, which promotes bacterial persistence and activates the host defence signalling pathways (Figure 1; Step 1) [13,14,36]. The commensals mostly lack the virulence associated adhesive ligands and fail to bind to signalling receptors in the mucosa. The situation may be different in the gut, where pathogens have been proposed to actively inhibit the epithelial response by disrupting NF-κB dependent transcription mechanisms [37,38].

UPEC use P fimbriae for epithelial cell adherence and P fimbriae are expressed by up to 80% of the strains causing acute pyelonephritis compared to <20% of ABU strains [39,40]. The host cell receptors for P fimbriae are glycosphingolipids, and the PapG tip adhesin binds to Galα1→4Galβ oligosaccharide receptor epitopes, which are abundantly expressed in the human urinary tract mucosa [41-43]. The glycosphingolipid receptors also play a central role in host response induction [27,36,44-46]. Infection studies in animal models and human patients have shown that P fimbrial expression is essential for the uropathogenic *E. coli* strains to trigger the innate host response in vivo. P fimbriae thus fulfil the molecular Koch postulates as an independent virulence factor in the human urinary tract [36].

The mucosal response to P fimbriated *E. coli* is controlled by TLR4 both *in vitro* and *in vivo* [28,47,48]. The extra-cellular, leucine-rich repeat domains of the TLRs recognize conserved microbial patterns such as LPS, but need co-receptors to function optimally [49]. TLR4 dimerisation by LPS in myeloid cells involves co-receptors CD14 and MD2 and leading to recruitment of IL-1R associated serine kinase 4 (IRAK-4) via the adaptors MyD88 and TIRAP [50,51]. As a consequence, LPS responses are severely decreased in Myd88, Irak and Tirap knock-out mice [51-53]. The uro-epithelial cells lack CD14, and respond poorly to LPS [28,45,54]. This inertia to LPS is probably essential to allow asymptomatic carrier state, but raises the question how TLR4 signalling may be triggered specifically by the pathogenic strains. We propose that

fimbrial lectins and their recognition receptors selectively activate mucosal TLR4 responses [48].

In view of the CD14 independence of the epithelial response to P fimbriated *E. coli*, we speculated that TLR4 signalling might involve different adaptor proteins. This hypothesis was supported by *in vivo* studies, in the murine UTI model [48]. The epithelial response to P fimbriated *E. coli* was controlled by the Trif/Tram adaptors and *Trif* -/- and *Tram* -/- mice showed no significant response to infection. *Myd88* -/- and the *Tirap* -/- mice were fully responsive. The results suggested that fimbriae and glycoconjugate receptors offer a mechanism of ''pathogen recognition'' that allows TLR4 to respond selectively to pathogens at mucosal surfaces. Recognition receptors for other bacterial ligands may work in a similar manner. This mechanism offers a solution to the paradox specificity with the convergence on a limited number of mucosal TLRs.

The mechanism of human TLR4 recruitment by the glycolipid receptors is not fully understood, but our studies have identified ceramide as a possible signalling intermediate. Ceramide is the membrane anchor of the receptors, and early studies showed an increase in free ceramide after P fimbrial binding. Sphingomyelinase, which releases ceramide by cleaving sphingomyelin was shown to stimulate a TLR4 dependent chemokine response and exogenous C2 and C6 ceramide triggered a TLR4 dependent cytokine response in CD14 and MD-2 negative HEK cells [55]. By confocal microscopy, the levels of membrane associated ceramide and TLR4 were shown to increase after stimulation with P fimbriated *E. coli,* and significant co-localization was observed in lipid rafts [47]. The results show that TLR4 signalling can be activated in the absence of CD14 and MD-2, by agonists that modify membrane glycolipids. Receptor cleavage may also be a useful defence strategy, as it would serve to release the ligand and to activate a host response.

Step 2: Neutrophil defects and genetics of disease susceptibility

The anti-bacterial defence of the urinary tract relies almost entirely on innate immunity. Following intra-vesical inoculation, bacteriuria is cleared within hours or days and neutrophils are the crucial effector cells. The infected uro-epithelial cells secrete chemotactic substances including chemokines [15,16,56]. A chemotactic gradient is created, and in response to the gradient, neutrophils leave the bloodstream, migrate through the tissues and cross the epithelial barrier into the lumen. These molecular and cellular interactions explain the emergence of leucocytes in urine, known as ‘’pyuria’’, which is a classical sign of UTI. IL-8 is one of the main driving forces for neutrophils to cross the human urinary tact epithelium, and MIP-2 plays a similar role in the murine urinary tract [56-58]. It should be noticed, that several different neutrophil chemoattractants are secreted by epithelial cells and that additional studies are needed to understand their function in the response to UTI (See Figure 1; Step 2) [59,60]. IL-8 and other neutrophil activating CXC chemokines exert their effects by binding to G protein coupled cell surface receptors [61-63]. Infection stimulates CXCR1 and CXCR2 expression by epithelial cells, and CXCR1 is essential for the increased neutrophil migration across infected cell layers *in vitro* [64].

The syndrome of acute pyelonephritis and renal scarring is precipitated by a single gene defect. We have used the murine UTI model to define the *in vivo* importance of chemokines and chemokine receptor and to study how neutrophil defects influence disease susceptibility [9,35,65]. A deletion of the murine IL-8 receptor was shown to precipitate the syndrome of acute pyelonephritis and renal scarring, by perturbing neutrophil exit across the epithelial barrier and the innate host defence. In control mice, neutrophils appeared in the kidneys within a few hours after infection, and were seen crossing the epithelial barrier into the lumen. In the process, infection was cleared with no evidence of tissue damage. The mIL-8Rh-/- mice showed neutrophil accumulation under the epithelial barrier until abscesses were formed throughout the kidney parenchyma. In parallel, there was an increase in bacterial tissue counts and the mice developed bacteremia. In surviving mice, the kidneys shrunk in size, and

histology revealed tissue damage with fibrosis and other signs of renal scarring [5,10,64].

Genetics of human disease susceptibility

The results described above suggest at least three ways in which genetic variation may influence human disease susceptibility.

a) Recognition receptors for bacterial fimbriae. Glycolipid receptor expression varies with the P blood group. The results predict that an individual lacking receptors would be resistant to P fimbriaed *E. coli*, but there are too few receptor negative individuals to investigate this hypothesis. Patients prone to UTI show a higher density of epithelial cell receptors, however, and individuals of blood-group P_1 run an increased risk of developing recurrent pyelonephritis [66]. Furthermore, the receptor repertoire influences which fimbrial type can cause infection. Individuals of blood group A_1P_1 express the globoA structure on their epithelial cells and strains expressing the *prs* type of P fimbriae preferentially infect these individuals [67].

Glycolipid receptors expression may be modified by pharmacological inhibitors. The glucose analogue *N*-butyldeoxynojirimycin (*N*B-DNJ) blocks the ceramide specific glycosyl transferase involved in epithelial receptor expression [68]. Receptor inhibition was shown to be protective against colonisation and inflammation in the murine UTI model, confirming that the primary receptor is one essential component in the host response [46]. This approach should be pursued also in man.

b) TLR4 expression and signalling. The Tlr4 signalling deficiency in C3H / HeJ or Tlr4 -/- mice disrupts the inflammatory response. The unresponsiveness has two main consequences. The mice are unable to clear the infection, but develop a carrier state resembling ABU. The human *TLR4* gene has been mapped to chromosome 9 (9q32-q33) [69,70]. The extra-cellular domain combines with microbial ligands, while the cytoplasmic Toll / IL-1 receptor (TIR)-domain controls signalling through interaction with the adaptor proteins. Based on the analogy to the C3H / HeJ mouse [71], we

obtained DNA sequences from children with ABU and healthy controls. No sequence variation was detected in the TIR domain. Further clinical studies of TLR4 and adaptor proteins in patients with ABU and acute pyelonephritis are currently being performed [72].

c) *Low surface expression of CXCR1 in UTI-prone children and associated genetic polymorphisms.* The progression from acute disease to renal scarring in the mIL-8Rh KO mice suggested that a CXC chemokine receptor deficiency might underlie the susceptibility to UTI also in man. In a prospective clinical study, CXCR1 expression was found to be significantly lower in pyelonephritis prone children than in age-matched controls [10]. The low CXCR1 surface expression was also reflected in lower mRNA levels [10]. DNA sequencing revealed five single nucleotide polymorphisms, SNPs, unique to the UTI-prone children. Two of the SNPs were found at a low frequency in adult controls, but three were unique to the UTI prone children [73].

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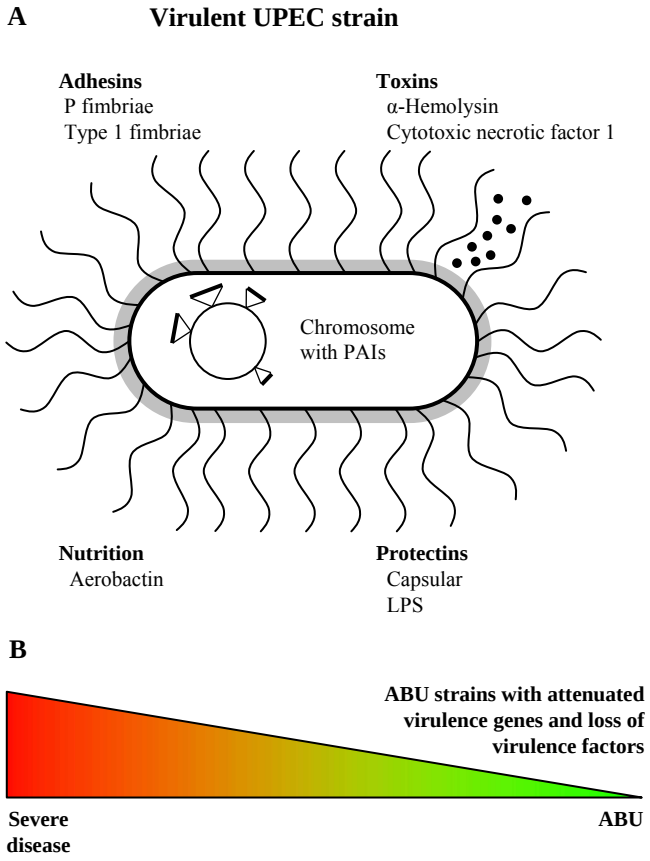
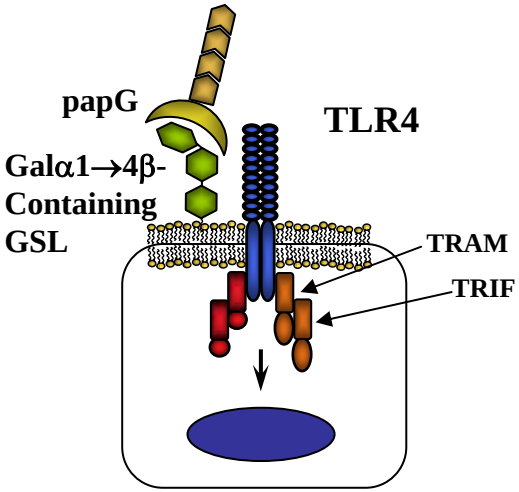


Figure 1. Differences between virulent uropathogenic *E. coli* (UPEC) strains and asymptomatic bacteriuria (ABU) strains

A. Fully virulent UPEC possess a wide arsenal of virulence factors that include adherence factors (fimbriae) and toxins (LPS, hemolysin) and cause severe infections such as pyelonephritis and bacteraemia.

B. Attenuated ABU strains acquire mutations in fimbrial genes, pathogenicity islands (PAIs).

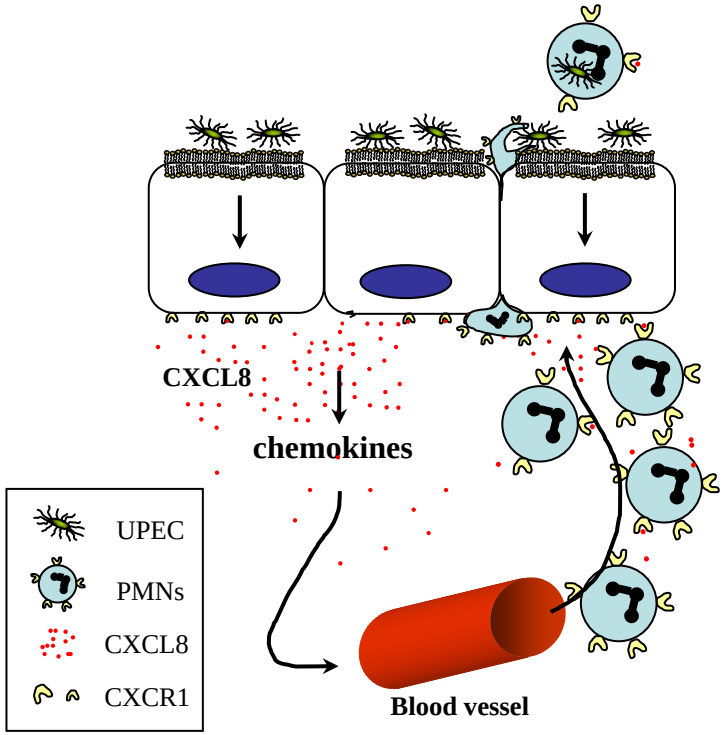
STEP 1



Tlr4 -/- → Asymptomatic bacteriuria

Low TLR4 → Asymptomatic bacteriuria?

STEP 2



mIL-8Rh -/- → Acute pyelonephritis, renal scarring

Low CXCR1 expression, SNPs in *CXCR1* → Recurrent pyelonephritis

Figure 2 Host response induction by adhering bacteria
Step 1: P-fimbriated *E. coli* adhere to the Galα1-4Galβ receptor epitope in the globoseries of glycosphingolipids (GSLs) on the uroepithelium and activate epithelial cells through TLR4 and the adaptor proteins, TRIF/TRAM. If Tlr4 signalling is abrogated, mice develop an asymptomatic carrier state. These findings predict that ABU patients may have modified TLR4 function.
Step 2: Activated epithelial cells respond by secretion of CXCL8 and by expression of CXCR1. Neutrophils are recruited to the mucosa and eliminate the bacteria after migrating across the epithelial barrier. If CXCR1 (mIL-8Rh) is absent, mice develop acute septic pyelonephritis and renal scarring. Patients prone to pyelonephritis have reduced level of CXCR1 and new polymorphisms in the *CXCR1* gene.