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ATG16L1 and pathogenesis of urinary tract infections

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Autophagy is generally considered to be antipathogenic. The autophagy gene *ATG16L1* has a commonly occurring mutation associated with Crohn disease (CD) and intestinal cell abnormalities. Mice hypomorphic for *ATG16L1* (*ATG16L1^{HM}*) recreate specific features of CD. Our recent study shows that the same *ATG16L1^{HM}* mice that are susceptible to intestinal inflammatory disease are protected from urinary tract infections (UTI), a common and important human disease primarily caused by uropathogenic *E. coli* (UPEC). UPEC colonize the bladder and exhibit both luminal and intra-epithelial stages. The host responds by recruiting innate immune cells and shedding infected epithelial cells to clear infection. Despite these countermeasures, UPEC can persist within the bladder epithelium as membrane-enclosed quiescent intracellular reservoirs (QIRs) that can seed recurrent UTI. The mechanisms of persistence remain unknown. In this study, we show that *ATG16L1* deficiency protects the host against acute UTI and UPEC latency. *ATG16L1^{HM}* mice clear urinary bacterial loads more rapidly and thoroughly due to *ATG16L1*-deficient innate immune components. Furthermore, *ATG16L1^{HM}* mice exhibit superficial urothelial cell-autonomous architectural aberrations that also result in significantly reduced QIR numbers. Our findings reveal a host-protective effect of *ATG16L1* deficiency in vivo against a common pathogen.

degradation of intracellular pathogens is a significant host defense. However, pathogens employ many strategies to evade or subvert the autophagy machinery for survival including retarding the maturation of autophagosomes, impairing fusion with lysosomes, escaping to the cytosol, and adapting to survive and replicate within the autophagosomal or lysosomal compartment. Recently, for example, Kim and coworkers demonstrated the importance of host autophagy in modulating effective antimicrobial responses to *Mycobacterium tuberculosis*, and the Celli group showed that *Brucella* subverts autophagy complexes to facilitate its intracellular cycle. However, little is known about how UPEC interact with autophagy. We previously showed that UPEC are enclosed in LAMP1-positive vesicles (QIRs) that resemble LAMP1-positive spacious *Listeria*-containing phagosomes (SLAPs) described by Brumell's group. In this study, we show that UPEC are also targeted by the *ATG16L1*, LC3, and SQSTM1/p62 proteins. Our findings for UPEC, thus, resemble other studies on intracellular recognition of *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, and *Listeria monocytogenes*. We also find UPEC in double-membranous structures in transmission electron micrographs of infected bladders (Fig. 1).

The Virgin group had previously demonstrated that *ATG16L1^{HM}* mice develop intestinal abnormalities in Paneth cells. Our work shows that *ATG16L1* deficiency induces multiple, baseline abnormalities in cellular components that UPEC encounter during infection. *ATG16L1*-deficient cells dramatically accumulate multivesicular bodies, lysosomes and the

Keywords: Atg16L1, autophagy, urinary tract infections, uropathogenic *E. coli*, bladder, Atg5, Crohn disease

Abbreviations: UTI, urinary tract infections; UPEC, uropathogenic *E. coli*; *ATG16L1^{HM}* mice, *ATG16L1* hypomorphic mice; CD, Crohn disease; QIRs, quiescent intracellular reservoirs

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Recent reviews highlight the complex interplay between autophagy and microbial adaptations governing host-pathogen interaction outcomes. Autophagic

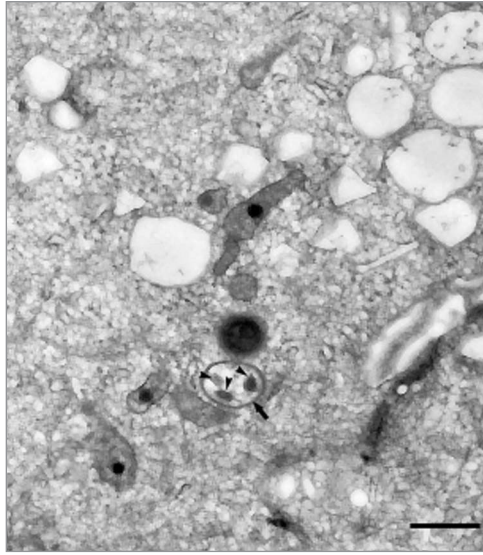


Figure 1. UPEC enclosed within a double-membraned autophagosomal structure. TEM of bladder tissue from *ATG16L1^{HM}* mice 14 d post-infection, depicting UPEC (arrowheads) enclosed in a double-membraned autophagosomal structure (arrow). Bar: 1 μ m.

UPEC receptor (UPK1A/UP1a). The aberrations are intrinsic to *ATG16L1*-deficient epithelial cells, because transferring *ATG16L1*-deficient hematopoietic cells does not induce them in wild-type epithelium, and even newly regenerating *ATG16L1*-deficient superficial cells show the same accumulations. Our study shows that UPEC are less able to occupy their intracellular niches to persist as QIRs in the *ATG16L1^{HM}* epithelium. We propose, thus, that UPEC may normally utilize *ATG16L1* and possibly other autophagy proteins to establish latency, thus *ATG16L1* deficiency can be protective in this regard. In other words, UPEC may need the normal autophagic machinery to persist. The mechanisms underlying how UPEC avoid degradation or survive in the autophagosomal niches remain to be elucidated.

Autophagy plays multiple roles in both innate and adaptive immunity. Recent studies have suggested that autophagy governs the balance between defending against pathogens and modulating innate immunity to prevent excessive inflammatory responses and inflammatory signaling. Lee et al., demonstrated that *ATG16L1* deficiency leads to a

hyperinflammatory response by removing the restriction on IL1B/IL-1 β signaling cascades and IL-6 production. Similarly, Saitoh et al., showed that *ATG16L1*-deficient macrophages produce high amounts of the inflammatory cytokines IL1B and IL18. In our study, we observed significantly increased levels of IL-6 and IL1A/IL-1 α in infected *ATG16L1^{HM}* mice relative to controls. Furthermore, our findings demonstrated that *ATG16L1*-deficient hematopoietic cells, especially neutrophils and macrophages, contribute critically to mounting a hypervigilant innate immune response, which likely promotes the rapid clearance of extracellular UPEC. Cadwell and Virgin previously demonstrated enhanced transcription of pro-inflammatory cytokines in aberrant Paneth cells of *ATG16L1^{HM}* mice. In the setting of CD and the presence of commensal bacteria, elevated pro-inflammatory cytokine levels induced by *ATG16L1* deficiency lead to intestinal pathology, which is detrimental to the host. However, in the *ATG16L1*-deficient urinary tract, the elevated proinflammatory cytokine levels may have a beneficial effect, as UPEC has been shown previously to inhibit proinflammatory

cytokine production. Thus, an *ATG16L1* deficiency-induced hyperinflammatory response may help clear the infection by countering UPEC's ability to dampen innate immune responses.

It is important to determine if the protection we observed is *ATG16L1*-specific or represents a general effect of the autophagy pathway on UTI pathogenesis. The autophagy machinery comprises many essential proteins including *ATG5*, *ATG7* and *ATG12*. We demonstrated that innate immune cell-specific knockdown of *Atg5* induces a similar protective phenotype as that induced by *ATG16L1* deficiency, suggesting that UPEC may hijack multiple autophagy components to colonize and persist in the urinary tract.

A polymorphism in *ATG16L1* associated with CD can be found in up to 50% of individuals in certain populations. It is unclear why a seemingly unfavorable allelic variant of *ATG16L1* would occur at such high frequency. One explanation is that the alleles were originally selected for a beneficial property such as protection against chronic or recurrent infectious diseases. Our results demonstrating that *ATG16L1* mutation can confer protection against UTIs could explain why mutant alleles are unexpectedly frequent: protection against UTIs could be a mechanism to counter the negative selection imposed by the inflammation-promoting effects of the *ATG16L1* mutation. A better understanding of the link between autophagy, latency, and inflammation could lead to therapeutic approaches, for example by targeting *ATG16L1* or autophagy in general to treat recurrent UTI. Thus, the use of inhibitors of autophagy or *ATG16L1* to eliminate latent bacterial reservoirs may have substantial clinical benefits to combat refractory and recurrent UTIs, because conventional antibiotics are unable to penetrate urothelial barriers to clear bacteria sequestered as QIRs.

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