Lipopolysaccharide directly alters renal tubule transport through distinct TLR4-dependent pathways in basolateral and apical membranes

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Good DW, George T, Watts BA 3rd. Lipopolysaccharide directly alters renal tubule transport through distinct TLR4-dependent pathways in basolateral and apical membranes. Am J Physiol Renal Physiol 297: F866–F874, 2009. First published July 22, 2009; doi:10.1152/ajprenal.00335.2009.— Bacterial infection of the kidney is associated with renal tubule dysfunction and dysregulation of systemic electrolyte balance. Whether bacterial molecules directly affect renal tubule transport is unknown. We examined the effects of LPS on HCO3−/H+ exchange with amiloride eliminated inhibition of HCO3− absorption when added to bath or lumen. The MEK/ERK inhibitor U0126 eliminated inhibition by bath LPS but had no effect on inhibition by lumen LPS. Conversely, the mammalian target of rapamycin (mTOR) inhibitor rapamycin eliminated inhibition by lumen LPS but had no effect on inhibition by bath LPS. Inhibiting basolateral Na+/H+ exchange with amiloride eliminated inhibition of HCO3− absorption by lumen but not bath LPS. Confocal immunofluorescence showed expression of TLR4 in basolateral and apical membrane domains. Inhibition of HCO3− absorption by bath and lumen LPS was eliminated in MTALs from TLR4−/− mice. Thus LPS inhibits HCO3− absorption through distinct TLR4-dependent pathways in basolateral and apical membranes. These results establish that bacterial molecules can directly impair the transport function of renal tubules, identifying a new mechanism contributing to tubule dysfunction during bacterial infection. The LPS-induced reduction in luminal acidification may contribute to Gram-negative pathogenicity by promoting bacterial adherence and growth and impairing correction of infection-induced systemic acid-base disorders.

bacterial kidney infection; medullary thick ascending limb; acid-base transport; Na+/H+ exchange; sepsis

Ion transport processes in the renal thick ascending limb play a vital role in a number of important homeostatic functions, including maintenance of sodium and water balance and acid-base regulation (3, 29, 40). Recent studies suggest that the thick ascending limb also may play a role in the innate immune response of the kidney. TLR4 has been localized to the thick ascending limb (84). These findings suggest that the thick ascending limb may be a potential target within the nephron for LPS-induced transport modulation.

Acid-base transport is relevant to the pathogenesis of Gram-negative bacterial infection on several levels. These include elimination of invading bacteria but also can lead to inflammatory kidney injury (2, 10, 14, 19, 46). Whether LPS acts directly to modify the transport functions of renal tubules has not been investigated.

The Toll-like receptors (TLR) are a family of transmembrane pattern-recognition receptors that recognize structural components unique to different microbial pathogens. At least 11 mammalian TLRs (TLR1–TLR11) have been identified (1, 2, 10). TLR4, the signaling receptor for Gram-negative LPS (1, 35, 54, 56), is constitutively expressed in proximal and distal nephron segments in vivo (19, 20, 39, 43, 60, 82, 83, 86) and mediates LPS-induced production of proinflammatory cytokines by renal epithelial cell lines (9, 11, 52, 60, 70). Mice with loss-of-function mutations in the TLR4 gene have impaired bacterial clearance during Escherichia coli pyelonephritis (35, 54, 56), and TLR4 on renal epithelial cells was required for control of ascending UTI in bone marrow chimeric mice (52). Beyond its role in defense against bacterial infection, increasing evidence suggests that TLR4 plays a role in mediating inflammatory kidney injury caused by ascending E. coli infection and LPS-induced sepsis (16, 19, 20, 52, 70). In addition, there is rapidly growing evidence at the whole kidney level that activation of TLR4 by endogenous ligands contributes to inflammatory kidney injury in a variety of noninfectious conditions, including ischemia-reperfusion injury, diabetes, nephrotoxic injury, and transplant rejection (5, 19, 39, 43, 62, 67, 72, 82, 83, 86, 88). To date, however, there have been no direct studies of the mechanisms or functional effects of TLR4 signaling in any native nephron segment. Whether activation of TLR4 alters renal epithelial transport and can contribute to infection-induced renal tubule dysfunction is unknown.

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1) changes in urine pH influence the virulence of *E. coli*
through effects on bacterial adherence and growth (27, 37, 68); 2) the production of inflammatory mediators is affected by pH in several cell types (12, 23, 44, 48, 58); and 3) endotoxemia and Gram-negative sepsis are associated with systemic metabolic acidosis that contributes to the pathogenesis of organ dysfunction (8, 14, 38, 53, 66). The MTAL participates in urine acidification and acid-base regulation by reabsorbing most of the filtered HCO₃⁻ not reabsorbed by the proximal tubule (3, 29). Absorption of HCO₃⁻ by the MTAL depends on luminal H⁺ secretion mediated by the apical NHE3 Na⁺/H⁺ exchanger (4, 74, 75) and is regulated by a number of important physiological factors (28–30, 32, 74–76, 79). In the present study, we examined directly the effects of LPS on HCO₃⁻ absorption by the isolated, perfused MTAL. We show that LPS inhibits HCO₃⁻ absorption from either the basolateral or luminal cell surface. In addition, the results reveal a novel sidedness to LPS receptor signaling, whereby LPS alters HCO₃⁻ absorption through the activation of distinct TLR4-dependent signaling pathways in the basolateral and apical membranes. These studies provide the first evidence that bacterial molecules can act directly through innate immune receptors to modify the transport function of renal tubules, thereby identifying a new pathophysiologic mechanism contributing to tubule dysfunction during bacterial infection. They also reveal a novel, pathoadaptive mechanism through which Gram-negative bacteria can adversely affect the progression and severity of urinary tract infection and endotoxemia, namely, through LPS-induced impairment of renal tubule acid secretion.

METHODS

Animals. Male Sprague-Dawley rats (50–100 g body wt) were purchased from Taconic (Germantown, NY). Male C57BL/10SnJ (wild-type) and C57BL/10ScNJ (TLR4−/−) mice (7–8 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/10ScNJ mice do not express TLR4 due to a Tlr4 gene deletion (54, 55). Male Sprague-Dawley rats (50–100 g body wt) were purchased from Taconic (Germantown, NY). Male C57BL/10SnJ (wild-type) and C57BL/10ScNJ (TLR4−/−) mice (7–8 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/10ScNJ mice do not express TLR4 due to a Tlr4 gene deletion (54, 55).

Tubule perfusion and measurement of net HCO₃⁻ absorption. MTALs were isolated and perfused in vitro as previously described (28, 33, 79). Tubules were dissected from the inner stripe of the outer medulla at 10°C in control bath solution (see below), transferred to a bath chamber on the stage of an inverted microscope, and mounted on a 1°C circulation bath. The tubules were then incubated for 15 min at 37°C in a flowing bath using the same control solution as in HCO₃⁻ transport experiments. Following incubation, the tubules were washed with phosphate-buffered saline (PBS) and fixed and permeabilized in ice-cold acetone for 10 min. The tubules were incubated in Image-iT FX signal enhancer (Invitrogen) for 30 min at room temperature, washed, and blocked in 10% donkey serum in PBS for 1 h at room temperature. The tubules were then incubated overnight at 4°C with a 1:100 dilution of goat anti-mouse TLR4 polyclonal antibody (L-14, Santa Cruz Biotechnology), washed, and then incubated for 1 h at room temperature in Alexa 488-conjugated donkey anti-goat IgG antibody (1:100; Invitrogen) in blocking buffer. In some experiments, the anti-TLR4 antibody was incubated in the absence and presence of a five-fold excess by weight of blocking peptide (Santa Cruz) for 2 h at room temperature prior to tubule staining. Fluorescence staining was examined using a Zeiss laser-scanning confocal microscope (LSM510 UV META), as described (32, 78). Tubules were imaged longitudinally and z-axis optical sections (0.4 μm) were obtained along a plane at the center of the tubule, which provides a cross-sectional view of cells in the lateral tubule walls. For individual experiments, two to four tubules from the same kidney for each experimental condition, or from wild-type and TLR4−/− mice, were fixed and stained identically and imaged in a single session at identical settings of illumination, gain, and exposure time.

RESULTS

LPS inhibits HCO₃⁻ absorption from bath or lumen. To determine whether LPS directly alters renal tubule transport, transepithelial HCO₃⁻ absorption was measured in rat MTALs isolated and perfused in vitro. Addition of LPS (500 ng/ml) to the bath decreased HCO₃⁻ absorption by 33%, from 16.7 ± 0.5 to 11.2 ± 0.5 pmol·min⁻¹·mm⁻¹ (Fig. 1A). Addition of LPS (250 ng/ml) to the tubule lumen decreased HCO₃⁻ absorption by 26%, from 16.9 ± 0.4 to 12.5 ± 0.4 pmol·min⁻¹·mm⁻¹ (Fig. 1B). Quantitatively similar inhibition was obtained in response to the addition of either *E. coli* O111:B4 or ultra-pure K12 LPS, indicating that the inhibitory effects are induced by...
LPS itself and not by contaminating products in the standard bacterial LPS preparation (11, 17, 36). Similar inhibition also was observed using purified R515 LPS (Alexis, data not shown). The inhibition by LPS is rapid (<15 min), sustained for up to 60 min, and reversible. These results demonstrate that LPS is able to modify the transport function of renal tubules directly from either the basolateral or luminal surface.

**Lipid A inhibits HCO\(_3\)\(^-\) absorption from bath or lumen.** Lipid A is the bioactive component of LPS responsible for its immunoregulatory activity (2, 46). Addition of lipid A (500 ng/ml) to the bath decreased HCO\(_3\)\(^-\) absorption by 25%, from 17.0 ± 0.5 to 12.8 ± 0.5 pmol·min\(^{-1}\)·mm\(^{-1}\) (Fig. 2A). Addition of lipid A (250 ng/ml) to the lumen decreased HCO\(_3\)\(^-\) absorption by 24%, from 17.3 ± 0.4 to 13.1 ± 0.2 pmol·min\(^{-1}\)·mm\(^{-1}\) (Fig. 2B). The time course for inhibition by lipid A was similar to that of LPS. Similar inhibition was observed using lipid A from *E. coli* F583 or synthetic lipid A, indicating that the transport inhibition is triggered by lipid A itself and not by contaminants in the bacterial lipid A preparation (11, 17, 70). These results demonstrate that the inhibitory effects of LPS are reproduced by lipid A and support further the specificity of LPS-induced transport inhibition.

**Serum enhances the sensitivity to LPS.** The sensitivity of target cells to LPS is enhanced by serum due to circulating proteins (LPS-binding proteins) that facilitate binding of LPS to its membrane receptors (24). To test this in the MTAL, tubules were studied with and without 10% heat-inactivated, low-endotoxin fetal bovine serum in the bath solution. In the absence of serum, adding 100 ng/ml LPS to the bath had no effect on HCO\(_3\)\(^-\) absorption (Fig. 3A). In tubules bathed with 10% serum, adding 100 ng/ml LPS to the bath decreased HCO\(_3\)\(^-\) absorption by 20%, from 15.4 ± 0.8 to 12.3 ± 0.6 pmol·min\(^{-1}\)·mm\(^{-1}\) (Fig. 3B). The enhanced response in the presence of serum is consistent with interaction of LPS with the TLR4 receptor complex (24, 46; see below). All other experiments in this study were carried out in the absence of serum: 1) to study the effects of LPS on HCO\(_3\)\(^-\) absorption under the same conditions used previously for other regulatory factors (28–30, 76, 79); and 2) to avoid the potential confounding effects of serum on the activity of Na\(^+\)/H\(^+\) exchangers and other transport proteins.

**U0126 blocks inhibition by bath but not lumen LPS.** To determine whether bath and lumen LPS regulate MTAL transport through a common pathway, we examined the role of signaling pathways shown previously to inhibit HCO\(_3\)\(^-\) absorption in the MTAL. The MEK1/2 inhibitor U0126 selectively blocks ERK1/2 activation and ERK-mediated inhibition of Na\(^+\)/H\(^+\) exchange and HCO\(_3\)\(^-\) absorption in the MTAL (32, 76, 79). U0126 (15 μM in the bath) completely eliminated inhibition of HCO\(_3\)\(^-\) absorption by bath LPS but had no effect on inhibition by lumen LPS (Fig. 4, A and B). These results support an essential role for the ERK pathway in mediating the basolateral LPS response.

**Rapamycin blocks inhibition by lumen but not bath LPS.** Inhibition of Na\(^+\)/H\(^+\) exchange and HCO\(_3\)\(^-\) absorption via PI3K-mammalian target of rapamycin (mTOR) in the MTAL is blocked by the specific mTOR inhibitor rapamycin (32). In direct contrast to the preceding results with U0126, rapamycin

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**Fig. 1.** LPS inhibits HCO\(_3\)\(^-\) absorption from bath or lumen. Medullary thick ascending limbs (MTALs) from rats were isolated and perfused in vitro in control solution, and then LPS was added to and removed from the bath (500 ng/ml; A) or lumen (250 ng/ml; B) solution. The tubules were exposed to either LPS or synthetic lipid A from *E. coli* K12 LPS. Absolute rates of HCO\(_3\)\(^-\) absorption (JHCO\(_3\)\(^-\)) were measured as described in METHODS. Data points are average values for single tubules. Lines connect paired measurements made in the same tubule. P values are for paired t-test. Mean values are given in RESULTS.

**Fig. 2.** Lipid A inhibits HCO\(_3\)\(^-\) absorption from bath or lumen. MTALs from rats were perfused in vitro in control solution, and then lipid A was added to and removed from the bath (500 ng/ml; A) or lumen (250 ng/ml; B) solution. The tubules were exposed to either lipid A from *E. coli* F583 or synthetic lipid A. JHCO\(_3\)\(^-\), data points, lines, and P values are as in Fig. 1. Mean values are given in RESULTS.

**Fig. 3.** Serum enhances the sensitivity to LPS. MTALs from rats were studied in the absence (A) and presence (B) of 10% low-endotoxin fetal bovine serum in the bath solution. LPS (O111:B4, 100 ng/ml) was then added to and removed from the bath solution. JHCO\(_3\)\(^-\), data points, lines, and P values are as in Fig. 1. NS, not significant. Mean values are given in RESULTS.
(20 nM in the bath) had no effect on inhibition of HCO₃⁻ absorption by bath LPS but completely eliminated inhibition by lumen LPS (Fig. 5, A and B). These results suggest that PI3K-mTOR plays an important role in mediating the luminal response to LPS and, together with the experiments in Fig. 4, establish that basolateral and luminal LPS inhibit HCO₃⁻ absorption through different signal transduction pathways.

**Bath amiloride blocks inhibition by lumen but not bath LPS.** In previous studies, we demonstrated that both the ERK and mTOR signaling pathways inhibit HCO₃⁻ absorption in the MTAL through inhibition of the basolateral NHE1 Na⁺/H⁺ exchanger (32, 33, 76). To test whether NHE1 may be involved in mediating inhibition by LPS, we examined the effects of LPS on HCO₃⁻ absorption in tubules bathed with 10 μM amiloride, which selectively prevents inhibition of HCO₃⁻ absorption mediated through NHE1 (31, 33, 77, 78). Bath amiloride had no effect on inhibition of HCO₃⁻ absorption by bath LPS but eliminated inhibition by lumen LPS (Fig. 6, A and B). These results indicate that bath and lumen LPS inhibit HCO₃⁻ absorption through different transport mechanisms and suggest that NHE1 plays a key role in mediating the inhibition by lumen LPS.

**TLR4 is expressed in basolateral and apical membrane domains.** TLR4 is the signaling receptor for Gram-negative LPS in mammalian cells (1, 35, 54, 56). To determine its role in the MTAL, tubules microdissected from normal rats (Fig. 7, A and B) and from wild-type control (C57BL/10SnJ) and TLR4⁻/⁻ (C57BL/10ScNJ) mice (54, 56) (Fig. 7, C and D) were stained with anti-TLR4 antibody and analyzed by confocal immunofluorescence. In both rat and wild-type mouse...
MTALs, staining for TLR4 was present in the basolateral and apical membrane domains as well as in the cytoplasm (Fig. 7, A and C). The TLR4 staining was absent in the presence of specific blocking peptide (Fig. 7B) and in MTALs from the TLR4<sup>−/−</sup> mice (Fig. 7D). TLR4 staining in basolateral and apical membrane domains was confirmed using a second anti-TLR4 antibody raised against a different epitope (Abcam; data not shown). These results are consistent with the view that TLR4 is the receptor responsible for mediating MTAL responses to basolateral and luminal LPS.

Inhibition of HCO₃<sup>−</sup> absorption by LPS is eliminated in MTALs from TLR4<sup>−/−</sup> mice. To determine the functional significance of TLR4 for ion transport regulation, we examined the effects of LPS on HCO₃<sup>−</sup> absorption in tubules from wild-type control and TLR4<sup>−/−</sup> mice. The results of these experiments are shown in Figs. 8 and 9. The basal (control) rate of HCO₃<sup>−</sup> absorption did not differ in MTALs from wild-type and TLR4<sup>−/−</sup> mice. Similar to results obtained in the rat, addition of LPS to the bath or lumen decreased HCO₃<sup>−</sup> absorption in MTALs from wild-type mice (Figs. 8A and 9A). In contrast, addition of LPS to the bath or lumen had no effect on HCO₃<sup>−</sup> absorption in MTALs from TLR4<sup>−/−</sup> mice (Figs. 8B and 9B). These results support the conclusion that TLR4 is the signaling receptor that mediates inhibition of HCO₃<sup>−</sup> absorption by both basolateral and luminal LPS.

**DISCUSSION**

Gram-negative bacterial infection of the kidney due to ascending infection or via the bloodstream is frequently associated with tubule dysfunction that includes reduced urinary concentrating ability, increased fractional excretion of sodium and glucose, and impaired urinary acidification with reduced excretion of ammonium (6, 8, 13, 14, 34, 53, 64–66, 73). The pathophysiological mechanisms responsible for these functional defects are not understood. Evaluation of the effects of endotoxin on the kidney in vivo is complicated by the presence of factors such as changes in local or circulating cytokine and endotoxin on the kidney in vivo.

The direct action of LPS to impair HCO₃<sup>−</sup> absorption in the MTAL may contribute to the pathogenesis of Gram-negative infection on several levels. First, the relative alkalization of the luminal fluid may influence a number of infective processes. Recent studies show that physiological increases in urine pH over the range 5.0–7.0 increase the transcription of fimbrae genes in *E. coli* (68). This effect is observed within 20 min and results in increased expression of type 1 pili on the bacterial surface that are necessary for bacterial adherence. Consequently, the LPS-induced increase in lumen pH would promote attachment of bacteria to tubule cells that is critical for bacterial colonization and induction of the inflammatory response (15, 47, 68). LPS-induced alkalization of the luminal fluid would promote other pathogenic processes, including bacterial cell growth (27, 37, 68), the formation of Gram-negative-induced phosphate and calcium stones (57), and resistance to certain antibiotics (87). Second, absorption of HCO₃<sup>−</sup> by the MTAL depends on H<sup>+</sup> secretion mediated by the apical membrane NHE3 Na<sup>+</sup>/H<sup>+</sup> exchanger (4, 74, 75). Thus it is virtually certain that LPS decreases Na<sup>+</sup>/H<sup>+</sup> exchange activity in the MTAL (see below). Inhibition of Na<sup>+</sup>/H<sup>+</sup> exchange and the associated intracellular acidification impairs the induction of proinflammatory cytokines in several cell types, indicating that inflammatory processes may require a fully functional NHE (12, 23, 44, 48, 58). Inhibition of Na<sup>+</sup>/H<sup>+</sup> exchange activity and H<sup>+</sup> secretion by LPS could thus impair the renal tubule innate immune response. Third, endo-
Toxemia and sepsis are associated clinically with systemic metabolic acidosis, which contributes to the pathogenesis of multiorgan dysfunction (8, 14, 38, 53, 66). The direct action of LPS to inhibit renal tubule 

$\text{H}^{+}$ secretion would exacerbate and prevent the correction of sepsis-induced acidosis. LPS-induced impairment of luminal acidification also could contribute to the urine acidifying defect and failure to conserve HCO$_3^-$ impairment of luminal acidification also could contribute to the urine acidifying defect and failure to conserve HCO$_3^-$ reported during urinary tract infections (13) and to reduced urinary ammonium excretion in endotoxemia (6). Thus our study identifies a novel, pathoadaptive mechanism through which Gram-negative bacteria can adversely affect the progression and severity of urinary tract infections and endotoxemia at the cellular, epithelial, and systemic levels.

TLR4, the signaling receptor for LPS in mammalian cells, mediates LPS-induced production of proinflammatory cytokines by renal epithelial cell lines (9, 11, 52, 60, 70) and plays a critical role in the renal defense against ascending bacterial infection (35, 52, 54, 56). In addition, there is rapidly growing evidence that TLR4 plays a key role in mediating inflammatory kidney injury in a variety of conditions, including sepsis (16, 19, 20), ischemia-reperfusion injury (39, 82, 83), diabetes (62, 72), and nephrotic injury (43, 86, 88). Our study provides the first evidence that TLR4 signaling is directly involved in mediating alterations in renal tubule transport. TLR4 is expressed constitutively throughout the nephron, including segments of the proximal tubule, thick ascending limb, and collecting duct (9, 19, 20, 39, 43, 60, 82, 83, 86, 88). TLR4 has been localized to the apical membrane of the rat cortical thick ascending limb (20), and TLR4 expression in this segment is increased in response to sepsis and ischemia-reperfusion injury (20, 39, 82). In the present study, we found that TLR4 is present in both the basolateral and apical membrane domains of rat and mouse MTAL. The inhibition of HCO$_3^-$ absorption by basolateral or luminal LPS is eliminated in MTALs from TLR4$^{-/-}$ mice, indicating that TLR4 is the receptor responsible for mediating transport inhibition. These results establish that TLRs play a direct role in regulating ion transport in renal tubules and identify a previously unrecognized mechanism that can contribute to endotoxemia-induced defects in renal tubule function. In addition, our study provides the first direct evidence that renal tubules are able to recognize and respond to bacterial molecules through basolateral TLRs. Constitutive expression of basolateral TLR4 in the MTAL would enable this segment to monitor the composition of the interstitial fluid and initiate rapid innate immune defense mechanisms in response to the spread of bloodborne bacteria from the vascular compartment into the interstitial space (5, 14, 22, 61). In addition, endogenous TLR4 ligands such as heat shock proteins and the extracellular matrix components hyaluronin, fibronectin, and heparin sulfate are released into the interstitium by damaged cells during ischemic or toxin-induced kidney injury (5, 19, 67, 69, 83, 88). Interaction of these ligands with basolateral TLR4 in the MTAL would promote inflammatory responses that lead to tubule dysfunction and renal damage in these noninfectious conditions. Recent studies show that TLR2, which recognizes Gram-positive bacterial molecules, also is constitutively expressed at the basolateral surface of renal tubule cells in the outer medulla of mouse and human kidney (69). TLR2, in addition to TLR4, has been implicated in mediating ischemic renal injury (39, 41, 69, 82). Whether TLR2 plays a role in modulating renal tubule transport remains to be determined.

A surprising finding in this study is that LPS modifies MTAL function through the activation of distinct TLR4-dependent signaling pathways at the basolateral and apical membranes. The results of experiments using highly selective inhibitors suggest that basolateral LPS inhibits HCO$_3^-$ absorption through activation of the ERK pathway, whereas lumen LPS inhibits HCO$_3^-$ absorption through activation of the PI3K-mTOR pathway. Both pathways have been shown previously to inhibit HCO$_3^-$ absorption in the MTAL (32, 76, 79), and there is precedent for activation of these pathways by LPS in other systems. ERK is activated by LPS in inflammatory cells (1, 7, 18, 50, 59) and epithelial cell lines (9, 70) and plays a role in activating transcription factors that promote the expression of proinflammatory cytokines (1, 7, 9, 18, 59). The PI3K-mTOR pathway is activated by LPS in classic immune cells, where it is involved in controlling cell migration and survival and serves as a negative feedback system to prevent excessive and prolonged activation of innate immune responses (26, 42, 50, 80, 81). Our studies provide the first evidence that activation of mTOR is a component of TLR4-induced signaling in renal cells. Of potential clinical significance, we found that the signaling response of the MTAL to lumen LPS is blocked by the immunosuppressive drug rapamycin. This provides new insight into a mechanism that may contribute to the increased sensitivity of transplanted kidneys to ascending bacterial infection (45). Further detailed studies to examine the effects of LPS on the biochemical activities of proteins in the ERK and PI3K-mTOR pathways are required to confirm directly the importance of these pathways in the MTAL innate immune response. Our results suggest strongly that the ERK and PI3K-mTOR pathways play major roles in coupling innate immune receptors to alterations in renal tubule transport.

Our results demonstrate further that the basolateral and apical LPS signaling pathways inhibit HCO$_3^-$ absorption through effects on different ion transport proteins. Previously, we demonstrated that both the ERK and PI3K-mTOR pathways decrease HCO$_3^-$ absorption in the MTAL through inhibition of the basolateral NHE1 Na$^+/H^+$ exchanger, which results secondarily in inhibition of apical NHE3 and luminal H$^+$ secretion (32, 33, 76). In the present study, we found that inhibiting NHE1 with bath amiloride (31, 33, 77, 78) eliminates the inhibition of HCO$_3^-$ absorption by lumen LPS but does not prevent inhibition by bath LPS. These results suggest that NHE1 plays a critical role in mediating mTOR-dependent inhibition of HCO$_3^-$ absorption by lumen LPS but is not involved in mediating ERK-dependent inhibition by basolateral LPS. A potential alternative mechanism for the inhibition by bath LPS is direct coupling of the ERK pathway to inhibition of NHE3, as observed previously with aldosterone (79). These findings raise the possibility that the different TLR4 pathways activated by LPS at the basolateral and apical membranes are targeted to inhibit different Na$^+/H^+$ exchanger isoforms. Further studies examining NHE1 and NHE3 activities are needed to establish directly that these exchangers are targets for innate immune regulation through TLR4 in the kidney and to define the roles of these exchangers in mediating LPS-induced inhibition of HCO$_3^-$ absorption. Effects of LPS on additional transport proteins involved in HCO$_3^-$ absorption, including basolateral HCO$_3^-$ efflux pathways, also remain to be investigated.
that bacterial molecules can directly modify the transport
function of renal tubules through TLRs, thereby identifying a
new pathophysiologic mechanism contributing to renal tu-
bule dysfunction during bacterial infection. Our results reveal
a novel complexity of TLR4 signaling in MTAL cells, whereby
activation of TLR4 in the basolateral and apical membranes by
LPS leads to the induction of different intracellular signaling
pathways. Further studies aimed at understanding the mech-
anisms and functional significance of this differential TLR4
signaling may lead to new therapeutic strategies that target
specific TLR4-induced pathways to enhance host defense or
prevent maladaptive inflammatory responses that lead to kid-
ney injury in a variety of infectious and noninfectious condi-
tions.

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