An endogenous ribonuclease inhibitor regulates the antimicrobial activity of ribonuclease 7 in the human urinary tract

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Recent studies stress the importance of antimicrobial peptides in protecting the urinary tract from infection. Previously, we have shown that ribonuclease 7 (RNase 7) is a potent antimicrobial peptide that has a broad-spectrum antimicrobial activity against uropathogenic bacteria. The urothelium of the lower urinary tract and intercalated cells of the kidney produce RNase 7, but regulation of its antimicrobial activity has not been well defined. Here, we characterize the expression of an endogenous inhibitor, ribonuclease inhibitor (RI), in the urinary tract and evaluate its effect on the antimicrobial activity of RNase 7. Using RNA isolated from non-infected human bladder and kidney tissue, quantitative real-time polymerase chain reaction showed that *RNH1*, the gene encoding RI, is constitutively expressed throughout the urinary tract. With pyelonephritis, *RNH1* expression and RI peptide production significantly decrease. Immunostaining localized RI production to the umbrella cells of the bladder and intercalated cells of the renal collecting tubule. *In vitro* assays showed that RI bound to RNase 7 and suppressed its antimicrobial activity by blocking its ability to bind the cell wall of uropathogenic bacteria. Thus, these results demonstrate a new immunomodulatory role for RI and identified a unique regulatory pathway that may affect how RNase 7 maintains urinary tract sterility.

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Urinary tract infections are one of the most common and serious bacterial diseases in humans. Although the urine is considered sterile, little is known how the body maintains sterility. Recent evidence suggests that antimicrobial peptides (AMPs) have an important role in maintaining urinary tract sterility.[1–3] AMPs have been identified as key components of the innate host defense that are important contributors to maintaining health at mucosal barriers. AMPs, which serve as natural antibiotics, are cationic molecules expressed by leukocytes and epithelial cells. AMPs may be constitutively expressed and/or induced by invading pathogens. AMPs possess a broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, enveloped viruses, fungi, and some protozoa.

Recently, our research group has shown that ribonuclease 7 (RNase 7) is an epithelial-derived AMP that helps maintain sterility in the human kidney and urinary tract.[4–6] RNase 7 is a member of the ribonuclease A superfamily that has a broad-spectrum antimicrobial activity against uropathogenic bacteria at micromolar concentrations.[5,7] The uroepithelium of the lower urinary tract and the intercalated cells of the renal collecting tubules constitutively express RNase 7 and secretes it into the urinary stream.[5,6] In addition to maintaining urinary tract sterility, RNase 7 contributes to the sterility in other organ systems—including the skin, hair follicles, and oral cavity.[8–12] It has been stated that, on a per molar basis, RNase 7 is the most potent human AMP.[13]

The mechanisms that regulate the antimicrobial properties of RNase 7 are not well defined. The bactericidal activity of RNase 7 has been linked to its capacity to bind and permeate the bacterial cell membrane, independent of its ribonuclease activity.[14,15] Recently, Abtin *et al.*[16] identified an endogenous ribonuclease inhibitor (RI) in human epidermal keratinocytes that suppresses the antimicrobial activity of RNase 7 against yeast and *Enterococcus faecium*. RI is a 49-kDa cytosolic protein that was first isolated from human placenta.[17] It has also been identified by northern blot in

other human tissues—including brain, liver, testis, and peripheral blood leukocytes.[18] RI is a horseshoe-shaped peptide that binds with femtomolar affinity to members of the ribonuclease A superfamily. Its unique structure, consisting of a repetitive amino-acid sequence that is rich in leucine residues, helps foster protein–protein interactions.[19,20] Complexes formed between RI and target ribonuclease are among the tightest biomolecular interactions. When complexed with RI, ribonucleases cannot bind or degrade RNA. RI's affinity to ribonucleases has been shown to be the primary determinant of ribonuclease cytotoxicity.[21,22] To date, the biological role of RI is not entirely understood.

In this study, we provide the initial evaluation of RI expression in the human kidney and urinary tract during states of sterility and infection. We investigate whether RI binds RNase 7 or influences the antimicrobial properties of RNase 7 against uropathogenic bacteria. This work provides the basis for a better understanding of the potential roles of the RI-RNase 7 complex in maintaining urinary tract sterility.

RESULTS

With pyelonephritis, RNH1 mRNA expression decreases concomitantly with increased RNase 7 mRNA expression

Quantitative real-time polymerase chain reaction (PCR) demonstrates that *RNH1*, the gene encoding RI peptide, is basally expressed in all tested samples (Figure 1a). In the human bladder ($n=4$), the mean RI expression was 1692 ± 283 transcripts per 10 ng RNA. *RNH1* expression was significantly greater in the bladder than that within the kidney ($P=0.0018$). In human kidney tissue ($n=4$), *RNH1* expression was analyzed separately in the cortex, medulla, and pelvis (Figure 1a). *RNH1* expression was greatest in the renal pelvis with a mean expression of 1157 ± 208 transcripts per 10 ng RNA.

In human kidney specimens with pyelonephritis, *RNH1* mRNA expression was significantly lower as compared with non-infected kidney (Figure 1b). Non-infected kidney tissue ($n=7$) had a mean *RNH1* expression of 806 ± 68 transcripts per 10 ng RNA. Kidneys with the histological diagnosis of pyelonephritis ($n=8$) expressed 509 ± 42 transcripts *RNH1* per 10 ng RNA ($P=0.0015$). In contrast, with pyelonephritis, kidney *RNASE7* expression increased from 1028 ± 150 transcripts per 10 ng RNA ($n=7$) to 2602 ± 595 transcripts per 10 ng RNA ($n=7$, $P=0.035$) (Figure 1b).

RI peptide production decreases with pyelonephritis, whereas RNase 7 peptide production increases

To assess for concurrent decreases in kidney RI peptide production and increases in RNase 7 peptide production with pyelonephritis, enzyme-linked immunosorbent assay (ELISA) assay and immunoblot analysis were performed on the same kidney tissues used for quantitative real-time PCR analysis. Results confirm the mRNA expression results. Specifically, RI peptide production decreases and RNase 7 peptide production increases in kidney tissues with pyelonephritis ($n=6$) compared with non-infected kidney tissue ($n=6$; Figure 2 and Supplementary Figure S1 online).

RI peptide is produced by the uroepithelium of the lower urinary tract and renal collecting tubules

Immunohistochemical analysis (IHC) showed that RI immunoreactivity was greatest in the superficial umbrella cells lining the uroepithelium of the ureter and bladder. RI was not detected in the mesenchymal compartments of the ureter or bladder of all investigated specimens ($n=4$; Figure 3). In the kidney, immunohistochemistry demonstrated that RI was produced in tubules of the renal cortex and medulla of non-infected and infected kidney tissues. RI showed cell-specific expression in the cortical and medullary collecting tubules. RI-positive cells were more readily detectable in the inner medulla than in the cortex ($n=4$; Figure 4 and Supplementary Figure S2 online). In both states of sterility and infection, RI was not routinely detected in the glomeruli, proximal nephron, or interstitium. RI expression was noted in erythrocytes and infiltrating leukocytes (not shown). Negative controls showed no RI immunoreactivity (Supplementary Figure S2 online).

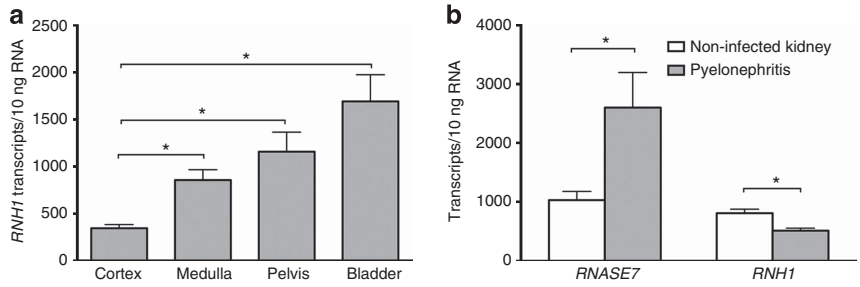

Figure 1 | *RNH1* and *RNASE7* mRNA expression in the human urinary tract during sterility and infection. (**a**) *RNH1*, the gene encoding ribonuclease inhibitor (RI), transcript levels were quantified by real-time polymerase chain reaction (PCR) in non-infected human bladder and kidney tissue. *RNH1* mRNA expression was significantly greater in the bladder than in the kidney. (**b**) *RNASE7* and *RNH1* mRNA transcript levels in non-infected human kidney tissue and in kidney tissue with pyelonephritis. *RNH1* expression significantly decreased with pyelonephritis ($P=0.0015$), whereas *RNASE7* expression increased ($P=0.0317$). Shown are the results of three independent experiments and the s.e.m. *Denotes statistical significance ($P<0.05$).