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Tamm-Horsfall Protein Protects Against Urinary Tract Infection by *Proteus mirabilis*

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Abstract

Purpose—*Proteus mirabilis* is a frequent cause of urinary tract infection. We sought to determine the role of Tamm-Horsfall Protein as a host-defense factor against cystitis and pyelonephritis caused by *Proteus mirabilis*.

Materials and Methods—We generated Tamm-Horsfall Protein gene knockout mice by the technique of homologous recombination. We introduced *Proteus mirabilis* transurethrally into the bladders of the Tamm-Horsfall Protein-deficient (THP^{−/−}) and genetically similar wild-type (THP^{+/+}) mice. We cultured urine to quantitate the degree of bacteriuria. We examined bladders and kidneys grossly and histomorphometrically to determine the intensity of inflammation.

Results—The Tamm-Horsfall Protein-deficient mice had more severe bacteriuria, cystitis, and pyelonephritic abscesses than the wild-type mice. The difference in the severity of pyelonephritis by semiquantitative histomorphometric analysis neared but did not reach statistical significance (p=0.053).

Conclusion—Tamm-Horsfall Protein acts as a host defense factor against *Proteus mirabilis* induced urinary tract infection.

Keywords

knockout mice; *Proteus mirabilis*; cystitis; pyelonephritis

Urinary tract infection (UTI) is a common health problem in the community. It is also the most common nosocomial infection in North America, occurring at a rate of approximately one million episodes per year. UTI is caused by a variety of bacteria that ascend the urinary tract from the periurethral region. *P. mirabilis* is a motile gram-negative bacterium belonging to the family *Enterobacteriaceae*. It is a common cause of hospital-acquired UTI, and it can cause infection in non-hospitalized patients too, especially those with staghorn (struvite) calculi, structural abnormalities of the urinary tract, or indwelling catheters.^{1, 2}

Normal downward urine flow helps excrete planktonic bacteria in urine but may be less effective in removing bacteria adherent to the urothelial surface. Hence, there is interest in identifying soluble urinary factors that might bind bacteria, prevent bacterial adherence to urothelium and facilitate bacterial excretion from the urinary tract. Tamm Horsfall Protein (THP; also known as uromodulin) is the most abundant protein in normal urine.³ THP is

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conserved in evolution⁴ and synthesized only in the kidney.⁵ It is a polymeric glycoprotein with a monomeric molecular weight of 90 kDa. It is decorated with the most varied array of N-linked complex-type oligosaccharides found in any human glycoprotein.⁶ High-mannose and O-linked residues are also present.³ The wide variety of oligosaccharides in THP provides a potential for binding several different receptors. THP has been identified as a host defense factor against UTI by *Escherichia coli*,⁷ *Staphylococcus saprophyticus*,⁸ and *Klebsiella pneumoniae*.⁸ THP's role in the pathogenesis of *Proteus* UTI has not been explored previously *in vivo*, although two of three *in vitro* studies^{9–11} have demonstrated binding between THP and *P. mirabilis*.

In the present study, we examined the role of THP as a defense factor against UTI caused by *P. mirabilis*. We investigated whether the THP^{-/-} mice had difficulty clearing *P. mirabilis* from the urinary bladder compared to the THP^{+/+} mice. The bladder and kidneys were assessed for gross and histological evidence of cystitis and pyelonephritis.

MATERIALS AND METHODS

Generation of THP Gene Knockout Mice

THP gene knockout mice were generated in our laboratory by the technique of homologous recombination.⁷ Human THP cDNA probes⁵ were used to isolate mouse THP cDNA¹² that was used to probe a mouse 129/sv liver lambda Fix II genomic library (Stratagene, LaJolla, CA, USA) to obtain the full-length mouse *THP* gene. The *THP* gene was characterized by restriction analysis, partial sequencing, and comparison with human and rat gene restriction maps. An omega-type replacement targeting vector was constructed incorporating a 2 kb segment 5' of the cap site of the *THP* gene and the first four exons plus the intervening introns of the *THP* gene. The linearized vector was electroporated into embryonic stem cells, obtained from mouse strain 129/sv (brown coat). Successfully transfected embryonic stem cells were injected into developing blastocysts from C57Bl/6 (black coat) mice to obtain chimeric mice. Chimeric mice were bred with Black Swiss female mice and screened by polymerase chain reaction (PCR) of tail DNA to obtain mice heterozygous for THP deficiency. The heterozygous mice were bred with each other to obtain THP^{+/+} and THP^{-/-} offspring. The absence of THP was confirmed by Northern blot, Western blot, and by immunocytochemistry. The THP mutation was also backcrossed onto the 129/sv strain for seven generations to produce THP^{+/+} and THP^{-/-} offspring with a similar genetic background. The THP^{-/-} mice revealed no gross anomalies. They grew and bred normally. Serum electrolytes, urinalysis, and kidney histology was normal. There was no evidence of pyelonephritis. The gross appearance and weight of the bladders and kidneys of THP^{-/-} mice did not differ from THP^{+/+} mice.

The Bacteria and Preparation of Inoculum

P. mirabilis was obtained from the American Type Culture Collection (ATCC, Manassas, VA) (Cat # 49565, isolated from a patient with struvite urinary calculus), and propagated in trypticase soy broth. An overnight, stationary phase culture was made from the main culture, and the number of bacteria per ml was determined spectrophotometrically at 600 nm. The bacterial suspension was centrifuged at 3600 rpm for 20 minutes, and the pellet washed with PBS. A final suspension was made in PBS at 4×10⁶ bacteria per ml.

Experimental Procedure

Ten pairs of age and weight matched, female, THP^{+/+} and THP^{-/-} mice were selected. The mice were kept in separate cages and allowed to eat and drink ad lib. To obtain a urine specimen, the mouse was restrained in the left hand while pressure was applied with the right hand over the lower abdomen. Urine was allowed to drip on to a piece of sterile parafilm. Baseline urine specimens were obtained and cultured on trypticase soy agar (Becton Dickinson, Sparks, MD)

at 37° C overnight to exclude pre-existing infection. The mice were deprived of water for two hours, and anesthetized with xylazine (Butler, Columbus, OH), 8mg/kg and ketamine (Fort Dodge Animal Health, Fort Dodge, IA), 120 mg/kg. *P. mirabilis* (25 ul of a 4×10^6 bacteria per ml suspension) was introduced into the bladder transurethrally, under aseptic conditions, using a 50 ul syringe (Hamilton, Reno, NV) fitted with a 28 G needle and polyethylene tubing with an inner diameter of 0.28 mm and outer diameter of 0.61 mm (Becton Dickinson, Sparks, MD). Aliquots from a common bacterial suspension were used for each pair of THP^{+/+} and THP^{-/-} mice. The catheter was clamped for one hour and then released and removed. The mice were allowed to recover from anesthesia and allowed free access to food and water. Urine was collected aseptically on day 1, day 2 and day 6 after inoculation. The urine specimens were serially diluted and cultured on trypticase soy agar overnight at 37°C. Colony forming units (CFU) were counted the next day and the number of bacteria per ml of urine was calculated.

Mice were euthanized 8 days after inoculation using carbon dioxide narcosis. The bladders and kidneys were removed, weighed, and photographed for size comparison. Digital images of bladders were obtained (Figure 2). The contours of the bladder images were traced (Figure 3), and the area of traced bladders was calculated in μm^2 using an image analysis software (NeuroLucida, MBF Bioscience, Williston, VT). The kidneys were removed and inspected for discoloration and abscess formation. The bladders and kidneys were cut into half, fixed in formalin, embedded in paraffin, sectioned, and stained with H&E for histopathological examination. The slides were examined by a renal pathologist in a blinded manner. Histological changes of inflammation were assessed semiquantitatively on a scale of zero to 4+ for bladder and zero to 5+ for kidney (Table 1). The animal experiments were pre approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center.

Statistical Analysis

Student's t-test (paired), two-proportion z test and nonparametric Mann Whitney U test were used for comparison of groups using the SPSS statistical program (SPSS Inc., Chicago, IL). Data were expressed as means $\pm$ SE. Statistical significance was set at $P < 0.05$.

RESULTS

Twenty four hours after transurethral inoculation of *P. mirabilis* the number of bacteria in urine were significantly higher in THP^{-/-} mice (Figure 1). Bacterial concentrations, expressed as $\log_{10}$ CFU/ml +1, were THP^{-/-}, 5.25 ± 0.58 versus THP^{+/+}, 3.38 ± 0.89 , $p = 0.01$ on day 1, THP^{-/-}, 5.62 ± 0.93 versus THP^{+/+}, 3.67 ± 0.85 , $p = 0.03$ on day 2, and THP^{-/-}, 5.98 ± 1.14 versus THP^{+/+}, 3.84 ± 0.92 , $p = 0.03$ on day 6. These results are consistent with a more intense colonization of bladder in the THP^{-/-} mice.

The gross appearance of the bladders is shown in Figure 2. The measured areas of bladder images from THP^{-/-} mice was greater than those from THP^{+/+} mice (Bladder area ($\mu\text{m}^2 \times 10^{-3}$), THP^{-/-} 46.6 ± 8.5 versus THP^{+/+} 30.7 ± 5.8 , $p = 0.005$, Figure 4). The bladder weights from THP^{-/-} mice were significantly higher in comparison with weights of the THP^{+/+} mice bladders (Bladder weight (mg), THP^{-/-} 68.6 ± 14.9 versus THP^{+/+} 41.6 ± 9.8 , $p = 0.019$, Figure 5). Upon examination of kidneys, gross discoloration and abscess formation (Figure 6) was present in 9 out of 20 kidneys from THP^{-/-} mice and 3 out of 20 kidneys from THP^{+/+} mice ($p = 0.038$).

Examination of the histopathological sections (Figure 7) showed generally more severe inflammation in the bladders and kidneys of THP^{-/-} mice than of THP^{+/+} mice. In 9 out of 10 cases, the THP^{+/+} bladder showed well-preserved mucosa with intact transitional epithelium. No acute or chronic inflammatory cells infiltrating the surface transitional epithelium were

seen. In 6 out of 10 cases, the THP^{-/-} bladder showed varying degrees of acute inflammation with acute inflammatory cell infiltrates, partial destruction of the surface transitional epithelium, and surface ulceration. Kidneys from 7 out of 10 THP^{+/+} mice showed well-preserved architecture with all four renal compartments (glomeruli, tubules, interstitium, vessels) intact, while kidneys from 6 out of 10 mice from THP^{-/-} mice revealed varying degrees of acute tubulointerstitial nephritis with abscess formation. Semiquantitative histomorphometry of bladder sections for acute cystitis (on a score of zero to ++++), showed more severe cystitis in THP^{-/-} mice (Mann-Whitney U test: mean ranks THP^{+/+}, 8.15; THP^{-/-}, 12.85, $p=0.02$) than in THP^{+/+} mice (Figure 8A). Kidney sections graded for inflammation (on a score of zero to +++) showed a trend towards more intense pyelonephritis in THP^{-/-} mice but the difference did not reach statistical significance (Mann-Whitney U test: mean ranks THP^{+/+}, 8.55; THP^{-/-}, 12.45, $p=0.053$, Figure 8B).

DISCUSSION

We found more bacteria in the urine of the THP^{-/-} mice than in the urine of the THP^{+/+} mice, suggesting more intense colonization of the urinary tract by *P. mirabilis* in the THP^{-/-} mice. The bladders from the THP^{-/-} mice were larger, and histological examination confirmed more intense cystitis in the THP^{-/-} mice. The kidneys from the THP^{-/-} mice showed more frequent discoloration and abscess formation. Histological examination of the kidneys showed a trend towards more intense pyelonephritis in comparison with the THP^{+/+} mice. Overall, these data provide evidence of increased susceptibility of THP^{-/-} mice to urinary tract infection with *P. mirabilis*.

Pathogenetic mechanisms involved in UTI induced by *P. mirabilis* have not been fully elucidated.^{1, 2} The colon serves as a reservoir for *P. mirabilis* from which the bacteria migrate to colonize the perineum and peri-urethral space.² In the absence of a urinary catheter, the organisms ascend the urethra as short, vegetative, swimmer cells.¹³ In catheterized patients, the organisms attach to the catheter surface and undergo transformation to the elongated, highly flagellated, swarmer cell form that can migrate over the catheter surface¹⁴ and enter the bladder. The flagella help the organism to migrate from the bladder to the kidney, resulting in pyelonephritis.¹⁵

P. mirabilis adheres to the urothelial surface with the aid of hair-like structures known as fimbriae. Several fimbrial types have been described from *P. mirabilis*, including the MR/P fimbriae, MR/K fimbriae, PMF, NAF, ATF and PMP fimbriae.^{1, 2} Of the six major fimbrial types of *P. mirabilis*, the molecular structure of the binding site has been characterized only for NAF. It has been shown to recognize a GalNAc β 1-4 Gal β 1-4 GlcNAc β moiety present in the glycolipid, asialo-GM2.¹⁶ Therefore, it is interesting that THP contains abundant terminal GalNAc β 1-4 Gal β 1-4 GlcNAc β in its N-linked glycan structures that could potentially mediate binding of THP to this uropathogenic bacterium.⁶ Several non-fimbrial, virulence factors have also been postulated to play a role in the pathogenesis of *P. mirabilis* infection, including the enzymes urease¹⁷ and the IgA-degrading metalloprotease ZapA.¹⁸ In addition, *P. mirabilis* expresses lipopolysaccharide² and capsular proteins with capacity for antigenic variation, such as Flagellin.¹⁹

The exact mechanism by which THP might prevent colonization and infection of the urinary tract by *P. mirabilis* is not clear. We found only three studies that have previously examined interaction between THP and *P. mirabilis*. In one study, THP was shown to bind *P. mirabilis* and prevent its adherence to polymer surfaces.¹⁰ In a second study, *P. mirabilis* was shown to bind renal cells expressing THP. This binding was inhibited by exogenous THP.¹¹ A third study, however, showed that the high-molecular-weight fraction of urine, which contains THP, did not inhibit hemagglutination by *P. mirabilis*.⁹ We extend these studies by demonstrating

new *in vivo* evidence for a potential protective role of THP against UTI caused by *P. mirabilis*. Further mechanistic studies are necessary to define the molecular mechanisms involved in the interaction between THP and *P. mirabilis*. It is possible that NAF of *P. mirabilis* binds the GalNAc β 1-4Gal glycans in THP. In the future, as the binding sites of MR/P, MR/K, and other fimbriae are revealed, it would be interesting to note if these sites are contained in the glycan structures of THP. THP may also stabilize host protective factors like IgA by preventing cleavage by bacterial proteases. Also, because of its diverse array of glycans, THP may bind to toxins, such as the hemolysins secreted by *P. mirabilis* similar to THP binding to *Bordetella pertussis* toxin.²⁰

Human mutations in the uromodulin/THP gene have been found to be associated with the clinical syndrome of medullary cystic kidney disease/familial juvenile hyperuricemic nephropathy (MCKD/FJHN).²¹ Patients with MCKD/FJHN have not been shown to have increased susceptibility for UTI.²² It appears that mutant THP causes MCKD/FJHN and absence of THP predisposes to UTI. Further work is needed to clarify the mechanisms underlying this observation.

CONCLUSION

THP acts as a host defense factor against urinary tract infection induced by *P. mirabilis*.

Acknowledgments

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Abbreviations and Acronyms

UTI	Urinary tract infection
<i>P. mirabilis</i>	<i>Proteus mirabilis</i>
THP	Tamm-Horsfall protein
THP ^{-/-}	Tamm-Horsfall protein-deficient
THP ^{+/+}	Wild-type
PBS	Phosphate buffered saline
CFU	Colony forming units
H&E	Hematoxylin and eosin
MR/P	Mannose-resistant <i>Proteus</i> -like
MR/K	Mannose-resistant <i>Klebsiella</i> -like
PMF	<i>P. mirabilis</i> fimbriae
NAF	Nonagglutinating fimbriae
ATF	Ambient-temperature fimbriae
PMP	<i>P. mirabilis</i> P-like

References

1. Coker C, Poore CA, Li X, Mobley HL. Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes Infect* 2000;2:1497. [PubMed: 11099936]

2. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin Microbiol Rev 2008;21:26. [PubMed: 18202436]
3. Serafini-Cessi F, Malagolini N, Cavallone D. Tamm-Horsfall glycoprotein: biology and clinical relevance. Am J Kidney Dis 2003;42:658. [PubMed: 14520616]
4. Badgett A, Kumar S. Phylogeny of Tamm-Horsfall protein. Urol Int 1998;61:72. [PubMed: 9873244]
5. Hession C, Decker JM, Sherblom AP, Kumar S, Yue CC, Mattaliano RJ, et al. Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines. Science 1987;237:1479. [PubMed: 3498215]
6. Hard K, Van Zadelhoff G, Moonen P, Kamerling JP, Vliegenthart FG. The Asn-linked carbohydrate chains of human Tamm-Horsfall glycoprotein of one male. Novel sulfated and novel N-acetylgalactosamine-containing N-linked carbohydrate chains. Eur J Biochem 1992;209:895. [PubMed: 1425697]
7. Bates JM, Raffi HM, Prasad K, Mascarenhas R, Laszik Z, Maeda N, et al. Tamm-Horsfall protein knockout mice are more prone to urinary tract infection: rapid communication. Kidney Int 2004;65:791. [PubMed: 14871399]
8. Raffi HS, Bates JM Jr, Laszik Z, Kumar S. Tamm-Horsfall Protein Acts as a General Host-Defense Factor against Bacterial Cystitis. Am J Nephrol 2005;25:570. [PubMed: 16244464]
9. Sareneva T, Holthofer H, Korhonen TK. Tissue-binding affinity of *Proteus mirabilis* fimbriae in the human urinary tract. Infect Immun 1990;58:3330. [PubMed: 1976113]
10. Hawthorn L, Reid G. The effect of protein and urine on uropathogen adhesion to polymer substrata. J Biomed Mater Res 1990;24:1325. [PubMed: 2283351]
11. Hawthorn LA, Bruce AW, Reid G. Ability of uropathogens to bind to Tamm Horsfall protein-coated renal tubular cells. Urol Res 1991;19:301. [PubMed: 1683041]
12. Prasad K, Bates J, Badgett A, et al. Nucleotide sequence and peptide motifs of mouse uromodulin (Tamm-Horsfall protein)--the most abundant protein in mammalian urine. Biochim Biophys Acta 1995;1260:328. [PubMed: 7873609]
13. Jansen AM, Lockett CV, Johnson DE, Mobley HL. Visualization of *Proteus mirabilis* morphotypes in the urinary tract: the elongated swarmer cell is rarely observed in ascending urinary tract infection. Infect Immun 2003;71:3607. [PubMed: 12761147]
14. Jones BV, Young R, Mahenthalingam E, Stickler DJ. Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. Infect Immun 2004;72:3941. [PubMed: 15213138]
15. Mobley HL, Belas R, Lockett V, Chippendale G, Trifillis AL, Johnson DE, et al. Construction of a flagellum-negative mutant of *Proteus mirabilis*: effect on internalization by human renal epithelial cells and virulence in a mouse model of ascending urinary tract infection. Infect Immun 1996;64:5332. [PubMed: 8945585]
16. Lee KK, Harrison BA, Latta R, Altman E. The binding of *Proteus mirabilis* nonagglutinating fimbriae to ganglio-series asialoglycolipids and lactosyl ceramide. Can J Microbiol 2000;46:961. [PubMed: 11068685]
17. Johnson DE, Russell RG, Lockett CV, Zulty JC, Warren JW, Mobley HL. Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. Infect Immun 1993;61:2748. [PubMed: 8514376]
18. Walker KE, Moghaddame-Jafari S, Lockett CV, Johnson D, Belas R. ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. Mol Microbiol 1999;32:825. [PubMed: 10361285]
19. Belas R. Expression of multiple flagellin-encoding genes of *Proteus mirabilis*. J Bacteriol 1994;176:7169. [PubMed: 7961488]
20. Menozzi FD, Debie AS, Tissier JP, Loch C, Pethe K, Raze D. Interaction of human Tamm-Horsfall glycoprotein with *Bordetella pertussis* toxin. Microbiology 2002;148:1193. [PubMed: 11932463]
21. Hart TC, Gorry MC, Hart PS, Woodard AS, Shihabi Z, Sandhu J, et al. Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. J Med Genet 2002;39:882. [PubMed: 12471200]
22. Devuyt O, Dahan K, Pirson Y. Tamm-Horsfall protein or uromodulin: new ideas about an old molecule. Nephrol Dial Transplant 2005;20:1290. [PubMed: 15840660]

Fig. 1.

Comparison of colony forming units (CFU) per milliliter of urine on day 1, day 2 and day 6 after transurethral inoculation of *P. mirabilis* (expressed as the log₁₀ CFU/ml + 1) in THP^{+/+} and THP^{-/-} mice. THP^{-/-} mice have more severe bacteriuria than THP^{+/+} mice. * Indicates the mean.

Fig. 2.
Gross appearance of nine pairs of bladders from THP^{+/+} and THP^{-/-} mice 8 days after transurethral inoculation of *P. mirabilis*.

Fig. 3. The contours of the bladders were traced on the gross photographs using Neurolucida program and the inner area measured in μm^2 .

Fig. 4. Distribution of bladder area of THP^{+/+} and THP^{-/-} mice 8 days after transurethral inoculation of *P. mirabilis*. * Indicates the mean.

Fig. 5. Distribution of bladder weight of THP^{+/+} and THP^{-/-} mice 8 days after transurethral inoculation of *P. mirabilis*. * Indicates the mean.

Fig. 6. Representative gross morphology of THP^{+/+} and THP^{-/-} mice bladders and kidneys 8 days after transurethral inoculation of *P. mirabilis*. The bladder is enlarged in THP^{-/-} mice (**B**) in comparison with THP^{+/+} mice (**A**). Both kidneys in the THP^{-/-} mice (**D**) are severely affected in comparison with the THP^{+/+} mice (**C**). Scale bar indicates 1 mm.

Fig. 7.

Representative histology (H&E) of THP^{+/+} and THP^{-/-} mice bladders and kidneys 8 days after transurethral inoculation of *P. mirabilis* (20X).

(A) The THP^{+/+} bladder shows well-preserved mucosa with intact transitional epithelium. No acute or chronic inflammatory cells are seen infiltrating the surface transitional epithelium.

(B) The THP^{-/-} bladder shows severe acute inflammation with prominent acute inflammatory cell infiltrates, partial destruction of the surface transitional epithelium and surface ulceration.

(C) The THP^{+/+} kidney shows well-preserved architecture with all four renal compartments (glomeruli, tubules, interstitium, vessels) being intact.

(D) The THP^{-/-} kidney reveals severe acute tubulointerstitial nephritis with abscess formation. Polymorphonuclear leukocytes are also seen throughout the tubulointerstitium outside the abscess.

Fig. 8. Histopathological grading of severity of inflammation for (A) bladder and (B) kidney from THP^{+/+} and THP^{-/-} mice 8 days after trans-urethral inoculation of *P. mirabilis* (non-parametric Mann-Whitney U test, N = 10).

Table 1

Histomorphometric grading scale for mouse bladder and kidney

Grade	Bladder	Kidney
0	Normal	Normal
+	Acute inflammation affecting less than 25% of the bladder inner circumference	Mild inflammation (pyelitis)
++	Acute inflammation affecting less than 50% of the bladder inner circumference	Acute pyelonephritis with focal medullary involvement
+++	Acute inflammatory cell infiltrates within the transitional epithelium involving the entire inner circumference of the bladder	Acute pyelonephritis with focal medullary and cortical involvement
++++	Severe acute inflammation with mucosal erosion(s) or ulceration(s).	Pyelonephritis with extensive medullary and cortical involvement (i.e., more than 50% of the medulla and cortex involved)
+++++		Most severe inflammation (acute pyelonephritis with abscesses)