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Short Communication

Contribution of Exotoxin A of *Pseudomonas aeruginosa* in Acute and Chronic Experimental Renal Infection

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SUMMARY: The role of *Pseudomonas aeruginosa* exotoxin A was studied in acute and chronic pyelonephritis models employing an exotoxin A-producing strain PAO, and its toxin deficient mutant PAOT1. Interestingly, the mutant strain was found to be at an advantage in its ability to induce acute pyelonephritis and it induced severer renal pathology. No significant differences were observed in the ability of the parent strain and its mutant to induce chronic renal inflammation.

The pathogenesis of *Pseudomonas aeruginosa* infections is multifactorial based on the number of virulence factors (1). A growing line of evidence suggests that *P. aeruginosa* exotoxin A is an important virulence factor in infections such as septicemia (2), corneal infections (3), and lung infections (4,5). The contribution of this virulence factor in acute and chronic lung infections has also been well studied (6-8). However, no information exists regarding the contribution of exotoxin A in relation to urinary tact infections (UTIs) caused by *P. aeruginosa*. The importance of this organism is of special relevance since it is UTIs' third leading cause, accounting for about 11% of nosocomial UTIs (9). In the present study, an exotoxin A-producing strain of *P. aeruginosa* PAO, and its mutant lacking this ability were employed to study the possible role of exotoxin A in acute as well as in chronic pyelonephritis. *P. aeruginosa* PAO and its exotoxin A-deficient mutant PAOT1 were obtained from Dr. Barbara H. Iglewski, University of Rochester, N.Y., USA, who produced exotoxin A-deficient mutant PAOT1 by the chemical mutagenesis of the parent strain PAO. The organisms were introduced in planktonic cell form transuretherally via an ascending route (10,11). A total of eight mice were infected in each set of experiments (repeated three times). Kidneys were removed aseptically and examined for bacterial load and their pathology was assessed. Grading of the severity of pathological lesions was done according to the method of Garg et al. (12). Kidney sections were evaluated on a semi-quantitative scale of 0 to 4. Total score indicative of the lesions' overall severity was determined by adding each of the individual scores.

The results of the present study revealed that both the toxigenic parent strain and its mutant were able to induce renal infection in mice, but the toxin-deficient mutant was found to be more virulent since it induced severer renal pathology (Table 1). The precise reason for this observation is not clear, and for direct comparison no studies are available in relation to UTIs. However, studies regarding respiratory infections (RTIs) has shown that *P. aeruginosa* exotoxin A inhibits the production of pro-inflammatory cytokines (8,13,14). This down-regulatory effect on these proteins could provide a possible explanation for the lower severity scores of renal lesions in mice infected with the exotoxin A-producing parent strain. This explanation has relevance in that a common factor would be mucosal site involvement in both types of acute inflammatory responses.

The results of chronic renal infection, however, differed from those observed in the acute form of pyelonephritis. No significant differences were observed in the severity scores of the renal lesions induced by the parent strain and its mutant. For comparison, again a RTI-related study is available. Woods et al. (6) employed a rat model to assess the role of *P. aeruginosa* exotoxin A in chronic lung infection, and demonstrated that active toxin A is required for maximum virulence in the model. They observed that chronic inflammatory changes could be induced only with toxigenic parent strain. It appears that the virulence potential of exotoxin A may vary with the site and tissue involved. In relation to elastase, an enzyme produced by this organism, its virulence for different tissues has been

Table 1. Comparison of renal bacterial load and severity scores of *Pseudomonas aeruginosa* PAO and PAOT1 in acute and chronic pyelonephritis models

		PAO	PAOT1
Acute pyelonephritis	Renal bacterial load	5.455 ± 0.302	5.126 ± 0.121
	Severity score	3	6
Chronic pyelonephritis	Renal bacterial load	Sterile kidneys	Sterile kidneys
	Severity score	4	5

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shown to vary with organ site (7). The pathogenicity of an opportunistic pathogen such as *P. aeruginosa*, which is armed with multiple virulence factors, thus needs to be studied by employing mutants of different virulence factors with regard to UTIs. Further, there is a paucity of available literature regarding the role of different cytokines in the evolution of the UTIs caused by this organism. The results of the present study should lead to future research concerning this issue. The model being reported here is of special relevance in this regard.

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REFERENCES

1. Woods, D. E. and Vasil, M. L. (1993): Pathogenesis of *Pseudomonas aeruginosa* infections. p.21-50 *In* A. L. Baltch and R. P. Smith (eds.), *Pseudomonas aeruginosa* Infections and Treatment. Marcel Dekker, New York.
2. Hirakata, Y., Furuya, N., Tateda, K., Kaku, M. and Yamaguchi, K. (1993): In vivo production of exotoxin A and its role in endogenous *Pseudomonas aeruginosa* septicemia in mice. Infect. Immun., 61, 2468-2473.
3. Pillar, C. M. and Hobden, J. A. (2002): *Pseudomonas aeruginosa* exotoxin A and keratitis in mice. Invest. Ophthalmol. Vis. Sci., 43, 1437-1444.
4. Miyazaki, S., Matsumoto, T., Tateda, K., Ohno, A. and Yamaguchi, K. (1995): Role of exotoxin A in inducing severe *Pseudomonas aeruginosa* infections in mice. J. Med. Microbiol., 43, 169-175.
5. Armstrong, S., Yates, S. P. and Merrill, R. (2002): Insight into the catalytic mechanism of *Pseudomonas aeruginosa* exotoxin A. J. Biol. Chem., 277, 46669-46675.
6. Woods, D. E., Cryz, S. J., Friedmass, R. L. and Iglewski, B. H. (1982): Contribution of toxin A and elastase to virulence of *Pseudomonas aeruginosa* in chronic lung infections of rats. Infect. Immun., 37, 1223-1228.
7. Blackwood, L. L., Stone, R. M., Iglewski, B. H. and Pennington, J. E. (1983): Evaluation of *Pseudomonas aeruginosa* exotoxin and elastase as virulence factors in acute lung infection. Infect. Immun., 39, 198-201.
8. Schultz, M. J., Rijneveld, A. W., Florquins, S., Speelman, P., van Deventer, S. J. H. and Paul, T. V. D. (2001): Impairment of host defence by exotoxin A in *Pseudomonas aeruginosa* pneumonia in mice. J. Med. Microbiol., 50, 820-827.
9. Centres for Disease Control (1983): Nosocomial infection surveillance (1980 - 1982). CDC Surveillance Summaries. 32 (No. 455). Centres for Disease Control, Atlanta.
10. Hagberg, L., Enberg, I., Feter, R., Lam, J., Olling, S. and Svanberg Eden, C. (1983): Ascending unobstructed urinary tract infection in mice caused by pyelonephritogenic *Escherichia coli* of human origin. Infect. Immun., 40, 273-283.
11. Gupta, R., Ganguly, N. K., Ahuja, V., Joshi, K. and Sharma, S. (1995): An ascending non-obstructive model for chronic pyelonephritis in BALB/c mice. J. Med. Microbiol., 43, 33-36.
12. Garg, U. C., Ganguly, N. K., Sharma, S., Chakravarti, R. N. and Bhatnagar, R. K. (1987): Quantitative histopathological methods for the evaluation of experimental ascending pyelonephritis. Med. Sci. Res., 15, 367-368.
13. Staugas, R. M., Harvey, D. P., Ferrante, A., Nandoskar, M. and Allison, A. C. (1992): Induction of tumor necrosis factor (TNF) and interleukin-1 (IL-1) by *Pseudomonas aeruginosa* and exotoxin A - induced separation of lymphoproliferation and TNF, lymphotoxin, gamma interferon, and IL-1 production in human leukocytes. Infect. Immun., 60, 3162-3168.
14. Wieland, C. W., Siegmund, B., Senaldi, G., Vasil, M. L., Dinarello, C. A. and Fantuzzi, G. (2002): Pulmonary inflammation induced by *Pseudomonas aeruginosa* lipopolysaccharide, phospholipase C, and exotoxin A: role of interferon regulatory factor 1. Infect. Immun., 70, 1352-1358.