Evaluation of interleukin-10 production in
*Pseudomonas aeruginosa* induced acute pyelonephritis

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Summary *Pseudomonas aeruginosa* is an opportunistic pathogen of immunocompromised hosts. This pathogen has a tendency to form biofilms on the surface of indwelling catheters leading to acute and chronic urinary tract infections that result in significant morbidity and mortality. In the present study, kinetics of interleukin-10 (IL-10) production in mouse renal tissue was studied employing experimental mouse model of acute pyelonephritis induced with planktonic and biofilm cells of *P. aeruginosa*. IL-10 production was found to be significantly lower in biofilm cell instilled mice compared to planktonic cell infected animals, which corroborated with higher bacterial load and tissue damage. The data suggests that downregulation of IL-10 production may be novel strategy employed by biofilm cells to cause tissue damage and hence bacterial persistence. The results of the present study may open up avenues of research that will ultimately provide the foundation for the development of preventative measures and therapeutic strategies to successfully treat *P. aeruginosa* biofilm infections based on the administration of anti-inflammatory agents.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting humans throughout their life span. UTIs account for about 8.3 million doctor visits each year at a cost of over $1 billion [1]. Up to 40% of women will develop UTI at least once during their lives, and a significant number of these women will have recurrent UTIs. *Pseudomonas aeruginosa* is the third most common pathogen causing hospital acquired catheter associated UTIs [2]. Despite the use of antibiotic therapy, the mortality and morbidity associated with *P. aeruginosa* induced acute pyelonephritis still remains high. This poor outcome is due to our incomplete understanding about the patho-
IL-10 and pyelonephritis

A large number of studies have been performed targeted at the bacterial biofilms. However, little attention has been paid to *P. aeruginosa* biofilms on urinary catheters. Apart from catheter removal, there is currently no treatment proven successful for completely eradicating a biofilm-related infection caused by *P. aeruginosa*, highlighting the need for research in this area. Transplantation procedures, immunosuppression and the use of urinary catheters have increased the prevalence of *P. aeruginosa* induced pyelonephritis. Formation of biofilms on urinary catheters offers survival advantage to *P. aeruginosa* [3]. Biofilm infections are important clinically because bacteria in biofilms exhibit recalcitrance to antimicrobial compounds and persistence in spite of sustained host defenses [4–6].

The interplay between the proinflammatory and anti-inflammatory mechanisms/mediators is essential in fight of the host against the invading pathogen in limiting its effect and resolving inflammatory process. In this context, interleukin-10 (IL-10), an anti-inflammatory cytokine, appears relatively late following induction of immune responses [7]. This cytokine has been reported to be produced by T cells, B cells and macrophages/monocytes [8]. IL-10 pathway is a critical regulator of immune responses to microorganisms. The challenge faced by the immune system of an infected host is to respond with sufficient intensity and duration to control and eliminate the infection while minimizing nonspecific injury to host tissue. Within the context of infectious disease, IL-10 plays an important role in the balance between protective immunity and the development of immune pathology. IL-10 modulates expression of cytokines and soluble mediators by immune cells like monocytes, with important consequences for their ability to activate and sustain immune and inflammatory responses [9]. However, the role of IL-10 in UTIs has not been elucidated. In the present investigation, we studied the production of IL-10 during *P. aeruginosa* induced acute pyelonephritis and its correlation with bacterial load and tissue damage. The results of this study may provide a window of opportunity for the potential use of anti-inflammatory intervention against UTIs.

**Methods**

**Organisms**

A urinary isolate of *P. aeruginosa*, employed in earlier studies [10–12], isolated from a hospitalized patient having complicated UTI and serotyped as O11 by the Laboratory of HealthCare Associated Infection, London was used. In addition, a standard strain of *P. aeruginosa*, PAO was obtained from Dr. Barbara H. Iglewski, University of Rochester, Rochester, New York. Both the strains were grown overnight in nutrient broth at 37°C and harvested by centrifugation. Cells were washed three times with phosphate-buffer saline (PBS), resuspended to a concentration of $1 \times 10^8$ organisms per ml in PBS (confirmed by viable cell counting) and were used as planktonic cells for induction of infection in mice.

**Generation of biofilm cells**

The method of Mittal et al. [10] was followed for generation of biofilms. Both the uroisolate and PAO strain of *P. aeruginosa* were used to generate biofilm cells. Foley’s catheter (Bardia) was cut into 1.0 cm pieces, inoculated with 100 µl of overnight grown culture and incubated at 37°C. Every 24 h catheter pieces were removed from each flask, rinsed three times with PBS and transferred to the new flask containing fresh medium until day 4. On day 4, catheter pieces were rinsed three times with PBS to remove adherent bacteria and sliced longitudinally into equal halves. Cells were removed from the surface of catheter pieces by scraping the inner surface with a sterile scalpel blade. The dispersed sample was then centrifuged, and the biofilm cells were suspended in 1 ml PBS. Bacterial concentration was confirmed by viable counts.

**Induction of ascending pyelonephritis**

For induction of ascending pyelonephritis, female LACA mice, 6–8-week-old, weighing 25 ± 5 g obtained from Central Animal House, Panjab University, Chandigarh, India were used. Animals were kept in clean polypropylene cages under pathogen-free conditions and given food and water ad libitum. The bladder of animals was pressed to evacuate urine before instillation. Urine was checked for preinfection bacteriuria by culturing on selective media. A soft intramedic polyethylene catheter, non-radiopaque (outer diameter 0.61 mm, Clay Adams, USA) was inserted into the bladder through the urethral meatus, and 0.05 ml of inoculum containing $10^8$ CFU/ml was slowly injected into the bladder to avoid leakage [10–12]. The catheter was kept in place for 10 min after completion of instillation and then it was withdrawn carefully. No obstruction or further manipulation of the urinary tract was done. 8 mice were used for each strain and for each time interval separately for planktonic and biofilm cell.
forms. All animal experiments were carried out in two groups in triplicate. The study protocol was approved by the Institutional Ethical Committee for Animal Experimentation.

Bacteriological examination

Animals were sacrificed at 1, 3, 5, 7, 10 and 14 days post-infection. One-half of both the renal tissue was removed aseptically, weighed and homogenized in 1 ml of sterile PBS. This homogenized tissue was plated on cetrimide agar plates. Quantitative bacterial counts per gram of the renal tissue were calculated [10].

Histopathological examination

The other half of the renal tissue was fixed in 10% buffered formal saline and was dehydrated in ethanol gradient of 30–100%. Tissues were then embedded in wax, sectioned and stained with hemotoxylin and eosin (H&E) [11].

Cytokine response

IL-10 levels in renal tissue were quantitated by using an ELISA kit (R&D Systems, Minneapolis). ELISA was carried out according to the manufacturer’s instructions.

Assessment of neutrophil response

Tissue neutrophils were quantitated using a myeloperoxidase (MPO) assay [10]. Renal tissue was homogenized in 2 ml of 50 mM potassium phosphate, pH 6.0 with 5% hexadecyltrimethylammonium bromide and 5 mM EDTA. Homogenates were sonicated and centrifuged. Supernatants were mixed at the ratio of 1:15 with assay buffer and read at 490 nm. MPO units were calculated as the change in absorbance over time.

Malondialdehyde (MDA) estimation

MDA was estimated following method of Wills [13]. Briefly, tissue supernatant was added to equal amount of Tris–HCl (0.1 M, pH 7.4) and incubated at 37°C for 2 h. After incubation, trichloroacetic acid was added and centrifuged at 700 × g for 10 min. Supernatant was mixed with equal volume of thiobarbituric acid (0.67%, w/v) and kept in boiling water bath for 10 min. After cooling, volume was made to 3 ml with double distilled water and absorbance was taken at 532 nm. Amount of MDA formed was expressed in nanomoles per milligram protein (nmol/mg) using an extinction coefficient of 1.56 × 10^5 M⁻¹ cm⁻¹.

Statistical analysis

For statistical analysis of data, ANOVA, Fischer test and Student’s t-test was applied, and P values were calculated. P < 0.05 were considered statistically significant.

Results and discussion

In the present study, kinetics of IL-10 production was studied in P. aeruginosa induced acute pyelonephritis employing mouse model of ascending urinary tract infection. IL-10 production started to appear in kidneys on 3rd post-infection day and reached a peak on 7th post-infection day in case of both planktonic and biofilm cell infected mice (Fig. 1A). Thereafter gradual decrease in
production of IL-10 was observed till 14th post-infection day. IL-10 levels were significantly lower in biofilm cell instilled mice compared to planktonic cell infected animals ($P < 0.001$). Maximal production of IL-10 was accompanied with decrease in renal bacterial load leading to resolution of infection by 14th post-infection day in planktonic cell infected mice. However biofilm cells were able to persist in the renal tissue till 14th post-infection day (Fig. 1B). Significantly lower levels of IL-10 corroborated with higher neutrophil recruitment (assessed by MPO assay) (Fig. 2A) and hence tissue damage (assessed in terms of MDA assay) (Fig. 2B) in mice infected with biofilm cells as compared to planktonic cell instilled mice. Histopathological evaluation of the renal tissue revealed severe inflammation along with destruction of tubules in biofilm cell infected mice (Fig. 3A). In contrast mild inflammation was observable in renal tissue of planktonic cell infected mice (Fig. 3B). In this context, IL-10 has been reported to be an important regulator of host response with a complex role in models of *P. aeruginosa* infection. IL-10 deficiency was found to exacerbate lung damage whereas treatment with IL-10 was associated with attenuation of excessive inflammation in mouse model of chronic endobronchial *P. aeruginosa* infection [14]. These workers suggested use of IL-10 as a potential therapeutic agent for treatment of cystic fibrosis patient. Chmiel et al. [15] demonstrated prolonged inflammation, persistence of neutrophils and tissue damage in IL-10 deficient mice as compared to wild type mice following respiratory tract infection with *P. aeruginosa*. However, no studies are available regarding role of IL-10 in *P. aeruginosa* induced acute pyelonephritis. The findings of present study as well as available information from literature brings out that IL-10 has a potential for dampening inflammation resulting in resolution of urinary tract infections. The data also suggests that down-regulation of IL-10 production may be a novel strategy employed by biofilm cells to persist inside host by causing inflammation and hence tissue damage. This may help in
forming a nidus where pathogen can grow and multiply leading to recurrent and chronic infections. This needs to be addressed to in future investigations especially with different strains of \textit{P. aeruginosa} and other uropathogens which may help in developing alternative preventive strategies against pyelonephritis based on administration of anti-inflammatory agents like IL-10.

**Conflict of interest**

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\textbf{Competing interests}: None declared.

\textbf{Ethical approval}: The study protocol was approved by the institutional ethical committee for animal experimentation.

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**References**


