

The cell-bound hemolysin of *Serratia marcescens* contributes to uropathogenicity

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(Received June 30, 1989; accepted July 3, 1989)

Marre, R. (Institut für Medizinische Mikrobiologie der Medizinischen Universität zu Lübeck, F.R.G.), J. Hacker and V. Braun. The cell-bound hemolysin of *Serratia marcescens* contributes to uropathogenicity. *Microbial Pathogenesis* 1989; 7: 153-156.

The contribution of the cell-bound hemolysin of *Serratia marcescens* to uropathogenicity was studied in an experimental urinary tract infection in rats. The strain carrying the *Serratia* hemolysin colonized the urinary tract more and lead to a stronger inflammatory response compared to the isogenic hemolysin negative strain.

Key words: *Serratia marcescens*; uropathogenicity; hemolysin; rat.

Introduction

Serratia marcescens is an important pathogen causing predominantly nosocomial infections in immunocompromised patients ranging from urinary tract infections to endocarditis, meningitis and bacteremia. Pathogenicity of this microorganism has been associated with a formation of fimbriae,^{1,2} the production of proteases, which degrade various serum proteins,^{1,3,4} the presence of different cell wall antigens⁵ and the ability to resist the bactericidal action of serum.⁶ Recently Braun *et al.*⁷ noticed that *Serratia marcescens* strains rapidly lyse human erythrocytes in solution. On blood agar *Serratia* colonies show a narrow zone of a faint hemolysis. The *Serratia* hemolysin has been shown to differ markedly from the well characterized *E. coli* hemolysin.⁸ It is predominantly cell associated, requires actively metabolising bacteria and does not need calcium ions for activity. The hemolysin determinant is contained on a 7.5 kb fragment of chromosomal DNA and shows two open reading frames designated *shIA* which encodes the hemolysin protein and *shIB* which somehow activates *shIA*.⁹ Since there has been no study on the role of the *Serratia* hemolysin in experimental infections, we investigated the contribution of this hemolysin to uropathogenicity in an experimental rat model.¹⁰

Results and discussion

As demonstrated in Fig. 1 renal colonization of the strain producing the *Serratia* hemolysin was more than five times higher than that of the Shl-negative recipient strain ($P < 0.05$). Bladder colonization was distinctly higher than renal colonization in both groups of rats with a tendency to higher colony counts in the group infected with 536/21 pES2. The mean bladder weights of animals infected with 536/21 pES2 were more than double as high as in the control group (0.30 vs 0.14 g). Numbers of leukocytes in the urine were 2.6 times higher in the animals colonized with *E. coli* 536/21 pES2.

It is obvious from the data presented here that the *Serratia* hemolysin contributes to uropathogenicity in an experimental rat pyelonephritis model. Shl-positive bacteria lead to higher renal colonization than their non-hemolytic counterparts. Bladder colonization was also affected by the *Serratia* hemolysin but to a lesser extent. The contribution of Shl to inflammatory responses was indicated by an increase of leukocyturia and thickening of the bladder walls. These results are in line with those of König *et al.*¹¹ who were able to demonstrate that Shl induces the release of leukotriene from polymorphonuclear leukocytes, the release of histamine from rat mast cells, and chemiluminescence of neutrophils.

The extent of contribution of Shl to uropathogenicity was similar to that of the secreted *E. coli* alpha hemolysin derived from an *E. coli* O18 strain.¹⁰ It shows that a hemolysin which is predominantly cell bound and rapidly inactivated in non-growing bacteria, also exerts biological effects. The hemolysin might facilitate the destruction of the epithelium of the urinary tract and the tubuli and thus facilitate invasion of urinary tract tissue.

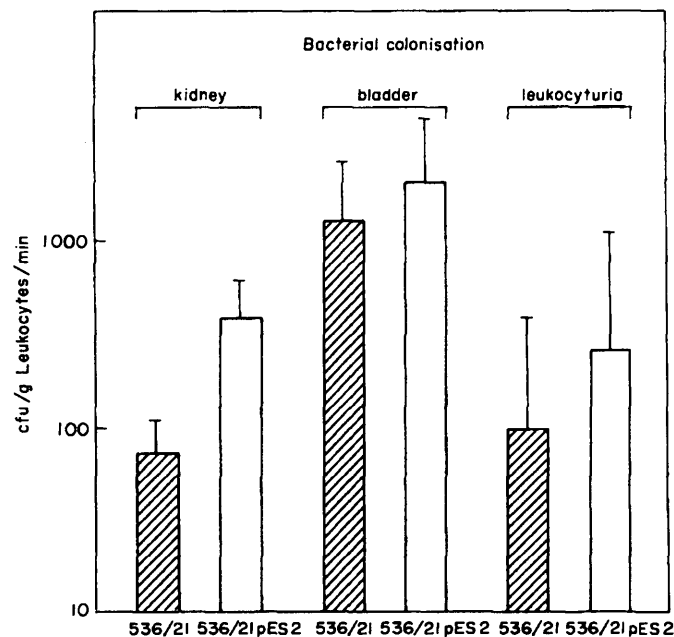


Fig. 1. Bacterial colonization of kidney and bladder by *E. coli* strain 536/21 (number of rats 36) and the transformant 536/21 pES2 (number of rats 40), which carried the determinant of the *Serratia* hemolysin. Each column gives the mean value of cfu/g of tissue. Bars indicate the standard error. Leukocyturia (number of rats per strain = 5) was calculated as number of cells eliminated per min (median+standard error). Each column represents the median of leukocytes eliminated per min, the bars indicate the standard error.

In this study the role of *Serratia* hemolysin in infection was demonstrated after transformation of a ShI-coding plasmid into an *E. coli* carrier strain which is well adapted to the animal system used. Studies are underway to demonstrate whether or not the *Serratia* hemolysin also plays a role in the virulence of its natural host bacteria.

Materials and methods

Strains. Since it was at first easier to construct isogenic pairs of *E. coli* than of *Serratia* which only differed in hemolytic activity, the well-characterized *E. coli* strain 536/21 06 K15 was used as a carrier strain for the recombinant *Serratia* hemolysin. This strain was previously used in this model of infection for studies on uropathogenicity and was able to colonize the rat urinary tract. The strain has been described in detail.¹⁰ Strain 536/21 was transformed with plasmid pES2, which carried the cloned chromosomal hemolysin determinant of *Serratia marcescens*.⁸ The transformant showed a narrow zone of faint hemolysis on sheep blood agar similar to that of *Serratia marcescens*. With respect to other possible virulence factors such as serum resistance, fimbriae, cell wall antigens and growth rates strain 536/21 and transformant 536/21 pES2 were identical.

Methods. For initiation of a urinary tract infection 1.5 ml of a suspension of the bacteria to be tested (concentration 10^7 cfu/ml) was injected via urethra into the bladder of the rats (strain: Han WIST).¹⁰ This resulted in a vesicoureteral reflux and subsequent colonization of the kidneys. Depending on the virulence of the strains used, a persisting pyelonephritis might develop. One week after the infection the rats were sacrificed, kidneys and bladder removed aseptically, the bladders were opened and rinsed in sterile saline. Both organs were homogenized, appropriately diluted and viable counts were made on McConkey agar. The identity of the isolated colonies with the *E. coli* strains injected was tested serologically and by the presence of the chromosomally encoded streptomycin resistance. The majority of the reisolated bacteria still contained the plasmid with the hemolysin gene. The mean number of cfu/g of tissue of each group was calculated and served as a measure of uropathogenicity. Forty rats were used in each group. In order to evaluate leukocyturia, five rats of each group received 10 ml of 0.9% saline and furosemide (10 mg/kg) intraperitoneally on the sixth day of infection. The rats were placed singly into metabolic cages for 2 h. The urine was collected and the number of leukocytes determined. The cell elimination per min was calculated. The statistical evaluation was performed according to Kruskal-Wallis followed by the Nemenyi-test, if statistically significant differences ($P < 0.05$) were found.¹²

This work was supported by the Deutsche Forschungsgemeinschaft.

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