Folia Microbiol. 41 (4), 373–376 (1996)

Lipopolysaccharide-Induced Resistance in Mice against Ascending Urinary Tract Infection with *Klebsiella pneumoniæ*

U. CHOPRA NEE CHATHLEY, S. SHARMA and S. CHHIBBER*

Department of Microbiology, Panjab University, Chandigarh 160 014, India

Received September 28, 1995
Revised version April 4, 1996

ABSTRACT. Protective effect of the lipopolysaccharide (LPS) antigen of *Klebsiella pneumoniæ* was tested against ascending-mode urinary tract infection in BALB/c and LACA strains of mice. LPS was given by two different routes; LPS was found to be protective (whatever the application route) since colonization with the challenge organism was significantly lower in both cases as compared with unimmunized mice. A maximum decrease in bacterial count in the kidney of LPS-treated animals was observed on challenge after a 4-d treatment.

Urinary tract infection (UTI) is a common disease and most of the females suffer from this infection at one or the other time in their lifetime. *E. coli* is frequently encountered in these infections followed by other gram negative organisms. *Klebsiella* is an important pathogen in hospital-acquired UTI. The morbidity and mortality associated with *K. pneumoniæ* infections is considerable (Jarvis *et al.* 1985). The prevalence of multidrug resistant isolates of *K. pneumoniæ* in recent years has directed the attention of many scientists to the development of immunological means to prevent or treat these life-threatening infections (Chhiber *et al.* 1995; Cryz *et al.* 1984; Hoffman and Preston 1968; Rani *et al.* 1990).

The potential of the capsular polysaccharide (CPS) for vaccine development has been explored and success has been achieved in human trials (Cryz *et al.* 1984, 1985, 1986*b*). However, this antigen provides only type-specific protection, as observed in experimental animals and humans using vaccination with homologous CPS (Cryz *et al.* 1986*a*). Recently it has been observed that *Klebsiella* capsular polysaccharide does not present any permeability barrier to immunoglobulins (Williams *et al.* 1988); some of the strains have in fact been shown to express O antigen on the capsule itself. Attention is therefore being paid to developing a lipopolysaccharide antigen as an alternative to CPS. Only eight different O antigens have been reported so far in this genus (Mizuta *et al.* 1983) and out of these strains which predominantly cause clinical infections only a few are known to belong to serotype O1. Hence, LPS, an essential structural and immunopathogenic component of the outer cell membrane of all Gram-negative bacilli (Riestschel *et al.* 1982*b*), appears to be a promising antigen for vaccine development. Previously we have reported protection against the development of lobar pneumonia in rats on immunization with *K. pneumoniæ* LPS (Rani *et al.* 1990). The immunoprotective potential of *K. pneumoniæ* LPS was now evaluated to determine its potential use in the control of experimentally induced UTI in female mice.

MATERIALS AND METHODS

Bacterial strain. Klebsiella pneumoniæ strain NCTC 5050 (K_2^+, O_1^+), procured from Dr. P. Williams (*Microbiology Research Group*, Aston Triangle, Birmingham, UK) was used. The strain was maintained on nutrient agar stabs at 4 °C.

Infection procedure. Two different strains of female mice (LACA and BALB/c) were compared for their susceptibility to infection with *K. pneumoniæ* in the ascending model of UTI infection as described earlier (Sinha *et al.* 1988). The number of infecting bacteria introduced in the bladder *via* the intrauretheral way was 10^8 bacteria per 0.1 mL. The bacterial inoculum was prepared by growing *K. pneumoniæ* in nutrient broth for 18–24 h. The cells were centrifuged at 83 Hz for 15 min and washed twice with normal saline. The cell suspension absorbance was adjusted to 0.5 at 540 nm. The

*Corresponding author.

animals were anæsthesized with diethyl ether and a special catheter was inserted in the urethra for introduction of bacteria. The animals were then kept in clean polypropylene cages, given a regular diet (*Hindustan Levers* Bombay) and water *ad libitum*. The mice were sacrificed 5 d post challenge by a high dose of diethyl ether. Both kidneys were removed aseptically and weighed, homogenized in glass blenders and serial dilutions made in PBS were spread on MacConkey's agar plates. The plates were incubated at 37 °C for 1 d and bacterial viable counts were expressed as CFU/g of kidney tissue.

Purification of LPS. The LPS was extracted by the conventional phenol extraction method of Westphal and Jann (1965) as modified by Morrison and Leive (1975). It was further purified by sequential ultracentrifugation as described by Johnson an Perry (1976). The final material was analyzed for proteins, DNA (Burton 1956) and RNA (Munro and Fleck 1966). It was also tested for pyrogenicity and toxicity as described by Rani *et al.* (1990).

Protection studies. Female BALB/c mice about 90-d old and weighing 25–30 g were used. They were divided into two groups of 6 mice each. An equal number of animals was taken as control in each group. Animals in one group were treated with 100 μg of LPS i.m. while in the other group 100 μg of LPS was given intraurethrally. The animals in both groups were challenged at different time intervals (4 h, 4 d and 14 d) after acquiring LPS-induced resistance. The number of viable bacteria in each kidney was quantitated as mentioned above.

RESULTS

Fig. 1 shows the normal course of infection in LACA and BALB/c strains of mice with *K. pneumoniæ*. The maximum number of bacteria was isolated from the kidneys of infected mice on post-infection day 5. The difference in the number of bacteria isolated from the two strains of mice was not statistically significant ($p > 0.05$). The number of bacteria isolated increased significantly from the 2nd to the 5th day of infection ($p < 0.01$) but the difference was not significant between infection days 5 and 7 ($p > 0.05$). The number of isolated bacteria decreased significantly on the 14th day of infection ($p < 0.001$). Both strains of mice exhibited a similar trend. However, BALB/c mice were found to be more susceptible to infection since the number of bacteria isolated from kidneys of BALB/c mice was always higher than in the LACA strain.

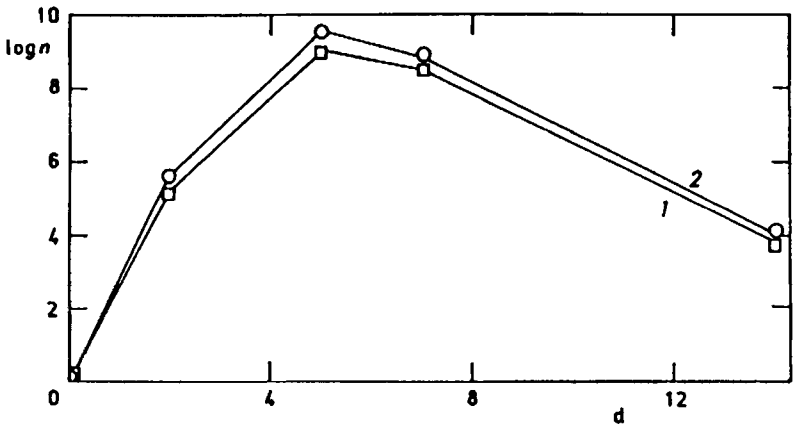

Fig. 1. Normal course of infection in LACA (*1*) and BALB/c (*2*) mice with *K. pneumoniæ*; *n* – bacterial count.

The LPS preparation was found to contain 40 % polysaccharide, 2.5 % protein, 19.6 % lipid and negligible amounts of DNA (0.1 %) and RNA (1.0 %).

The preparation was found to be non-pyrogenic and non-toxic when injected in the experimental animals in an amount of 100 μg. This dose was used for treating the mice in protection studies. Both intramuscular and intrauretheral routes used for LPS administration were found to be equally effective in reducing the number of viable bacteria in the kidney of treated animals relative to controls. This decrease in bacterial counts in the kidneys of both BALB/c and LACA mice was statistically significant ($p < 0.01$) when the bacterial challenge was given after 4 d of LPS treatment (Table I). Bacterial challenge of BALB/c mice after 4 h of treatment resulted in significant protection ($p < 0.01$) while

in LACA mice the significance was lower ($p < 0.05$). The protection was also observed in mice challenged after 14 d of LPS treatment but the level of significance was less in both strain ($p < 0.05$).

Table I. Bacterial counts ($-\log c$) in the kidneys of BALB/c and LACA mice challenged at different time intervals after LPS application

Time	Control (IU)	Treated (IU)	Control (IM)	Treated (IM)
		BALB/c		
4 h	9.0	7.0	9.3	6.6
4 d	9.0	6.6	9.3	6.0
14 d	9.5	7.5	9.4	7.5
		LACA		
4 h	7.0	6.2	7.5	5.8
4 d	7.0	4.5	7.6	5.2
14 d	7.6	6.2	7.4	5.6

DISCUSSION

To study the protective ability of LPS antigen of *K. pneumoniæ* in an ascending model of urinary tract infection, the susceptibility of BALB/c and LACA strains of mice to infection with *K. pneumoniæ* was compared. In both mouse strains the infection was introduced by the ascending route and the numbers of bacteria isolated from the kidneys of infected animals were comparable ($p > 0.05$). Our earlier studies with *E. coli* have indicated that the susceptibility of BALB/c mice for infection may vary in comparison with LACA mice. By contrast, the results of this study indicate a similar receptor distribution for *K. pneumoniæ* in BALB/c and LACA mice.

Protection experiments indicate that the bacterial counts in the kidneys of BALB/c mice were higher than in LACA mice, yet the protection provided by LPS in both mouse strains was comparable. The route used for LPS treatment had no effect on the protection afforded since a similar drop in bacterial count was observed following both intraurethral and intramuscular application. An important factor appeared to be the time interval between the LPS treatment and the challenge with bacteria. The bacterial count drop was the highest when the time interval between the LPS treatment and the bacterial challenge was 4 d. The protection observed 4 h and 4 d after LPS treatment indicates a nonspecific response. Vuopio-Varikla *et al.* (1988) suggested that protection against bacterial challenge observed at a site away from the site where LPS is injected is due to a systemic nonspecific cell activation. Activation of macrophages following LPS treatment (Galelli *et al.* 1977; Rani *et al.* 1990) has been shown to be a contributory factor in restriction of bacterial proliferation and survival of the animals.

The results of this study corroborate our earlier finding that LPS of *K. pneumoniæ* has a protective potential which may be utilized especially in hospitalized patients who may acquire *K. pneumoniæ*-induced UTI during their stay in hospital.

REFERENCES

Burton K.: Diphenylamine method for DNA estimation. *Biochem J.* **62**, 315–322 (1956).

Chhibber S., Bajaj J.: Polysaccharide-iron regulated cell surface protein conjugate vaccine: its role in protection against *Klebsiella pneumoniæ* induced lobar pneumonia. *Vaccine* **13**, 179–184 (1995).

Cryz S.J. Jr, Furer E., Germanier F.R.: Protection against Klebsiella pneumoniae burn wound sepsis by passive transfer of anticapsular polysaccharide. *Infect.Immun.* **45**, 139–142 (1984).

Cryz S.J. Jr., Furer E., Germanier F.R.: Safety and immunogenicity of *Klebsiella pneumoniæ* K1 capsular polysaccharide vaccine in humans. *J.Infect.Dis.* **151**, 665–671 (1985).

Cryz S.J. Jr., Mortimer P.M., Mausfield V., Germanier R.: Seroepidemiology of *Klebsiella pneumoniæ* bacteremic isolates and implications for vaccine development. *J.Clin.Microbiol.* **23**, 687–690 (1986*a*).

CRYZ S.J. Jr., FURER E., GERMANIDER R.: Safety and immunogenicity of a polyvalent *Klebsiella* capsular polysaccharide vaccine in humans. *Vaccine* **4**, 15–20 (1986*b*).

GALELLI A., PARANT M., CHEDID L.: Role of radiosensitive and radioresistant cells in nonspecific resistance to infection of LPS-treated mice. *J.Reticulo-endothel.Soc.* **21**, 109–118 (1977).

HOFFMAN N.R., PRESTON F.S.: Friedlander's pneumonia. *Dis.Chest.* **53**, 481–486 (1968).

JARVIS W.R., MUNN V.P., HIGHSMITH A.K., CULVAR D.H., HUGHS J.M.: The epidemiology of nosocomial infections caused by *Klebsiella pneumoniæ*. *Infect Control (Thorofares)* **6**, 68–74 (1985).

JOHNSON K.G., PERRY M.B.: Improved techniques for the preparation of bacterial lipopolysaccharides. *Can.J.Microbiol.* **22**, 29–34 (1976).

KAPOOR N., CHHIBBER S., VADEHRA D.V.: Sensitivity of multidrug resistant *Klebsiella pneumoniæ* to silver nitrate. *Folia Microbiol.* **34**, 94–98 (1989).

LASFARGUES A., LEDUR A., CHARON, SZABO L., CHABY R.: Induction by lipopolysaccharide of intracellular and extracellular interleukin-1 production. Analysis with synthetic models. *J.Immunol.* **139**, 429–436 (1987).

MIZUTA K., OHTA M., MORI M., HASEGAWA T., NAKASHIMA N.T., KATO N.: Virulence for mice of *Klebsiella* strains belonging to the O1-group: Relationship to their capsular (k) types. *Infect.Immun.* **40**, 56–61 (1983).

MORRISON D.C., LEIVE L.: Fractions of lipopolysaccharide from *Escherichia coli* O111: B4 prepared by two extraction procedures. *J.Biol.Chem.* **250**, 2911–2919 (1975).

MUNRO H.N., FLECK A.: The determination of nucleic acids. *Methods Biochem.Anal.* **14**, 113–176 (1966).

RANI M., GUPTA R.K., CHHIBBER S.: Protection against *Klebsiella pneumoniæ* induced lobar pneumonia in rats with lipopolysaccharide and related antigens. *Can.J.Microbiol.* **36**, 885–890 (1990).

RIETSCHEL E.T., SCHADE V., JENSEN M., WOLLENWEBER H.W., LUDERITZ O., GREISMAN S.E.: Bacterial endotoxins: Chemical structure, biological activity and role in septicemia. *Scand.J.Infect.Dis.* **37**, 8–21 (1982).

SINHA N., HARJAL K., SHARMA S.: Ascending pyelonephritis model in Swiss Webster (LACA) mice. *Ind.J.Med.Res.* **88**, 541–544 (1988).

VUOPIO-VARKILA J., NURMINEN M., PHYHALA L., MAKELA P.H.: Lipopolysaccharide induced nonspecific resistance to systemic *Escherichia coli* infection in mice. *J.Med.Microbiol.* **25**, 197–204 (1988).

WESTPHAL O., JANN K.: Bacterial lipopolysaccharides. Extraction with phenol–water and further applications of the procedure, pp. 83–91 in *Methods in Carbohydrate Chemistry, Vol. 5* (R.L. Whistler, Ed.). Academic Press, New York 1965.

WILLIAMS P., LAMBERT P.A., BROWN M.R.W.: Penetration of immunoglobulins through *Klebsiella* capsule and their effect of cell-surface hydrophobicity. *J.Med.Microbiol.* **26**, 29–36 (1988).

WRIGHT S.D., JONG M.T.C.: Adhesion-promoting receptors on human macrophages recognize *Escherichia coli* by binding to lipopolysaccharide. *J.Exp.Med.* **164**, 1876–1888 (1986).