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تأثير مكونات الدهون عديدة السكريات لميكروب عصويات الصديد الاخضر على احداث الحماية المناعية في فئران التجارب المصابة بالحروق

شعبان أحمد ، اسماعيل صديق، عبدالخالق الطماوي ، عماد نافع

لقد تضمنت هذه الدراسة أختيار فئران التجارب المعملية المصابة بالحروق وذلك لتقييم مقدرة مكونات الدهون عديدة السكريات الخاصة بميكروب عصويات الصديد الاخضر على احداث الحماية المناعية٠ وقد ثبت من هذه الدراسة أن لهذه المكونات كفاءة وقائية ومناعية عالية كما وجد أيضا من النتائج التي حصل عليها أن حقن حيوانات التجارب بثلاثة جرعات متتالية من هذه المكونات كل منها ١ر٠ من الميكروجرام يزيد معدل الجرعة الميتة بحوالي ٢٠٠ر٢٠ ضعف٠ كما وضح أيضا أن مستوى الحماية المناعية ضد الميكروب الحي المحقون له الصفة الخصوصية٠ حيث أن مقدرة مكونات الدهون عديدة السكريات لاحد عترات هذا الميكروب على أحداث الوقاية المناعية ضد عترات أخرى (مختلفة) لاتزيد عن ٢٢٠ ضعف٠ وقد خلصت النتائج الى اثبات القدرة العالية للقاح المحضر من خليط مكونات الدهون عديدة السكريات للعترات المختلفة لهذا الميكروب على أحداث حماية مناعية عالية ضد مختلف العترات٠

Dept. of Microbiology & Parasitology,
Fac. of Med., Assiut University,
Head of Dept. Prof. Dr. M.M. Zaki.

EFFECT OF LIPOPOLYSACCHARIDE (LPS) COMPONENT OF PSEUDOMONAS AERUGINOSA ON PROTECTIVE IMMUNITY IN EXPERIMENTAL MOUSE BURN INFECTION

(With 4 Tables)

By

S.H. AHMED; I. SEDDIK; A.M. EL-TIMAWY and E.K. NAFIE

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SUMMARY

Experimental mouse burn wound model was employed to evaluate the relative efficacy of Pseudomonas aeruginosa lipopolysaccharide as a protectives immunogen. LPS was found to be highly immunogenic and protective. Immunization of the animals with three doses of LPS (0.01 Ug each) increased the mean lethal dose more than 20,000-fold. The level of protection against a live challenge was proved to be serotype dependent. The fold-protection obtained by LPS of one strain against live challenges of other strains was not more than 220-fold. A multivalent LPS vaccine evoked high levels of protection against different challenge strains of various serotypes.

INTRODUCTION

Psudomonas aeruginosa is found on burn wounds more frequently as the postburn time increases. It is seldom encountered on burn wounds during the first 24 hours. The rate of colonization with Pseudomonas increases rapidly after 24 hours postburn. A considerable number of burn patients were colonized by pseudomonas organisms from their own lower gastrintestinal tract. The emergence of Ps.aeruginosa as a predominant member of the burn wound flora has been accompanied by the appearance of a distinctive clinical syndrome of invasive burn wound sepsis which is associated with a high rate of morbidity and mortality (NATHAN et al., 1973; SCHIMPFF et al., 1973; PRUITT, 1974; REYNOLDS et al., 1975 and HOIBY and OLLING, 1977).

Removal of the invading Ps.aeruginosa producing bacteraemia is dependent upon their phagocytosis and subsequent killing (YOUNG, 1972 and YOUNG and ARMSTRONG, 1972). Human antibody directed against lipopolysaccharide (LPS) has been shown to promote phagocytosis (PIER, 1982). Injection of mice with very small doses of purified LPS gives protection against a live challenge administered intraperitoneally (PIER et al., 1981). Burn wound sepsis as a model for evaluation of protective capacity of LPS against Ps.aeruginosa infection was used by SADOFF et al. (1982) and CRYZ et al. (1984).

The present investigation was planned to determine the protective immunity produced in experimental mouse burn wound sepsis against LPS of Ps.aeruginosa.

MATERIAL and METHODS

Bacterial strains :

Strains PA_1, PA_6 and PA_{10} were kindly obtained from J. Borst, National Institute of Public Health,P.O. Box 1,3720 BA, Bilthoven, the Netherland.

Growth environment:

Cultures for LPS production were grown on Trypticase Soya broth (TSB) containing 1% (vol/vol) glycerol in 500 ml. flasks at 37ºC to stationary phase. Challenge inocula were cultured on TSB medium to mid-log phase at 37ºC.

Preparation of LPS:

Lipopolysaccharide of different strains was prepared using the method of WESTPHAL and JANN (1965). The principle of this method is that bacteria after being suspended in a hot phenol-water mixture and then cooled, are separated into a water-soluble lipopolysaccharide and nucleic acid layer and a phenol-soluble protein layer.

Burned mouse model:

The burn wound sepsis model described by STIERITZ and HOLDER (1975) was employed. Phosphate buffered saline (PBS) were used for dilution of the challenge inocula, the number of viable organisms per challenge dose was determined for each experiment by plate counts. Groups of six mice were used per challenge dose to determine the mean lethal dose required to kill 50% of animals (LD_{50}).

Bacterial quantitation in tissues and blood:

Mice were challenged with approximately 10^2-bacteria. At various times post-challenge, groups of three mice were sacrificed, and the number of bacteria per milliliter of blood and per gram of skin at the challenge site was determined.

Immunization of mice:

White mice, 18 to 20 gm weight were immunized intramuscularly with 100 ul volumes containing the antigen in 0.5% aluminum hydroxide gel. Control groups were immunized only with aluminum hydroxide gel. Animals were challenged 14 days after the last immunization.

Polyvalent vaccine:

A polyvalent LPS vaccine was prepared by mixing equal amounts of LPS from strains PA_1, PA_6 and PA_{10}. For immunization of the animals, the vaccine was appropriately diluted in PBS and mixed with AL $(oH)_3$ suspension to yield a final concentration of 0.5% AL $(oH)_3$ in the vaccine.

RESULTS

A priliminary experiment was done to demonstrate the LD50 of different strains of Ps. aeruginosa; PA_1, PA_6 and PA_{10}; they were found to be $0.5X10^2$, $1.5X10^2$ and $2.5X10^2$ respectively (Table 1). In this study we compared LPS of the same serotype for its ability to produce a protective immuneresponse. The level of protection was dose dependent. Immunization with one dose of 0.1 Ug of LPS increased the LD50 value over 8000-fold as compared with control mice. Immunization of the animals with two and three doses of 0.1 Ug LPS showed marked increase in the fold protection if it is compared with the fold-protection obtained with single dose of LPS (Table 1).

The effect of previous immunization with LPS on the course of infection was studied by tracing the multiplication of bacteria with skin and the appearance of bacterimia. Bacterial growth in skin of control mice was extremely rapid, reaching levels of greater than 10^9 CFU/g of tissue, and 3 X 10^2 CFU/ml of blood at 24 hrs. postinfection. Immunization with 0.01 ug of LPS resulted in a marked reduction of bacterial multiplication in the skin and clearing of the infecting organisms by 72 hrs. post-challenge. Bacteraemia was not developed.

From Table (2) it can be observed that the protective immune response exhibited by LPS vaccination is a function of the dose injected as well as the number of immunizing dose. As the dose increases the fold-protection increases. Also, the number of immunizing dose increases the fold protection. Maximum immunization and protection were seen with the three dose schedule.

Since protection was found to be a serotype dependent, it was observed that immunization with three doses (0.1 Ug) of PA_1 LPS could not evoke a protection more than 220 times when the animals were challenged with the other two serotypes PA_6 and PA_{10} (Table 3). A multivalent vaccine prepared from the available three serotpes of LPS was used to immunize mice (Table 4). Protection was found to vary depending on the challenge strain. The LD_{50} of immunized mice with the poly-valent vaccine was markedly increased by more than 16.000-fold compared with control group.

DISCUSSION

In this investigation, murine burn wound model, was used to evaluate the efficiency of LPS as an active immunizing agent for protection against fatal Ps.aeruginosa sepsis. Many investigators proved the protective efficacy of LPS vaccination against an intraperitoneal or pulmonary challenge (PENNINGTON, 1979; PENNINGTON and KUCHMY,1980; PIER et al., 1981 and PIER, 1982). Uptill now there is a few published data about the value of LPS immunization in a burn model (SADOFF et al. 1982).

In the present study, we found that LPS isolated from several strains of Ps.aeruginosa, was highly immunogenic and protective in mice. The level of protection correlated with the dose and immunizing schedule. This comes in agreement with the findings of BJORNSON and MICHAEL (1970).

Fatalities of Ps.aeruginosa were due to septicemia, anti LPS antibody was found to limit bacterial multiplication at the initial site of infection and to limit or prevent bacteraemia. Low immunizing dose (0.01 Ug) resulted in a substantial delay in the onset of bacteraemia and bacterial multiplication in the skin. When a higher immunizing dose (0.1 Ug) was employed, bacteraemia and bacterial multiplication in the skin was nearly prevented within a short period of time as it was observed from the hgih degree of fold protection. Such observation is consistant with the observation of CRYZ et al. (1984).

In this study we observed that the level of protection is a serotype dependent. This finding correlates well with previous studies showing that passively transferred anti-LPS provided uniformly high protection against challenge strains of the same serotype (CRYZ et al., 1983).

From our study it was observed that vaccination with a polyvalent LPS vaccine can provide substantial protection against highly virulent strains of Ps.aeruginosa in a burn wound model relevant to human disease. In this model, LPS antibody is of great value in preventing

lethal sepsis by limiting bacterial multiplication at the skin and consequently limiting or preventing bacteraemia.

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Table (I): Active immunization of mice with LPS: effect of dose schedule on production against PA_1, PA_6, PA_{10} in a burn wound sepsis model.

Amt of LPS administered (Ug)	No of immunization[(a)]	PA_1		PA_6		PA_{10}	
		LD_{50}[(b)]	Fold protection	LD_{50}	Fold protection	LD_{50}	Fold protection
None (control)	-	0.5×10^2	1	1.5×10^2	1	2.5×10^2	1
$[AL(oH)_3]$							
0.1	1	0.6×10^6	12000	1.5×10^6	10000	2×10^5	8000
0.1	2	2.4×10^6	48.000	4.5×10^6	3000	5×10^6	20.000
0.1	3	5×10^6	100.000	1.2×10^7	80.000	1.0×10^7	40.000

(a) Immunizations were given intramusculary in 100 Ul volume at 14 days intervals. Cnailenges with PA_1, PA_6, PA_{10} were performed 14 days after the final immunization.

(b) LD_{50} expressed in terms of viable bacterial count.

Table (2)

Protection against different strains of pseudomonas by active immunization in a burn wound sepsis model: Effect of immunizing dose

Immunogen	Serotype	Immunizing[(a)] dose (Ug)	LD_{50}[(b)]	Fold protection
None (control)		0	0.5×10^2	1
$[AL(oH)_3]$	1	0.1	5×10^6	100.000
PA_1 LPS		0.01	2×10^6	40.000
None (control)		0	1.5×10^2	1
PA_6 LPS	6	0.1	1.2×10^7	80.0000
		0.01	3×10^6	20.000
None (control)		0	2.5×10^2	1
PA_{10} LPS	10	0.1	1.0×10^7	40.0000
		0.1	7.5×10^6	30.000

(a) Mice were immunized intramuscularly at days 0, 14 and 28 with the indicated dose of LPS in 100 Ul volumes: challenges were performed 14 days after the final immunizaion.

(b) Expressed as number of viable bacterial count.

Table (3)

Protection against Ps.aeruginosa PA_6 and PA_{10} by active immunization with LPS[(a)] of PA_1

Challenge strain (serotype)	LD_{50}[(b)]		Fold protection
	Control	Immunized	
PA_6	1.5×10^2	2.1×10^4	140
PA_{10}	2.5×10^2	5.5×10^4	220

(a) Mice were immunized intramusularly with 0.1 Ug of PA_1 LPS in 100 Ul volume at days 0, 14 and 28: challenges with PA_6 and PA_{10} were performed 14 days after the final immunization.

(b) Expressed as number of viable bacterial count.

Table (4)

Protection against Ps.aeruginosa challenge by vaccination with a polyvalent LPS Vaccine[(a)]

Challenge strain (serotype)	LD_{50}(a)		Fold protection
	Control	Immunized	
PA_1	0.5×10^2	1.6×10^6	32.000
PA_6	1.5×10^2	2.4×10^6	16.000
PA_{10}	2.5×10^2	6.0×10^6	24.000

(a) Mice were vaccinated with the 3 valent LPS vaccine (0.03 Ug) on days 0, 14 and 28: challenges were performed on days 42.

(b) Expressed as number of viable bacterial count.