قسم: الميكروبيولوجيا والطفيليات
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تأثير مكونات الدهون عديدة السكريات لميكروب عضويات الصيد الأخضر
على أحداث الحماية المناعية في فئران التجارب المصابة بالحروق

شعبان أحمد، اسماعيل صديق، عبد الخالق الطماوي، عادل نافع

لقد تضمنت هذه الدراسة اختيار فئران التجارب المتعلقة بالحروق وذلك
لتقييم مقايضة مكونات الدهون عديدة السكريات الخامة لميكروب توصيات الصيد
الأخضر على أحداث الحماية المناعية. وقد ثبت من هذه الدراسة أن هذه المكونات
كفاءة وقائية ومناعية عالية كما وجد أيضاً من النتائج التي حصل عليها أن حقن
حيوانات التجارب بثلاثة جرعة من متتالية من هذه المكونات كل منها 100 مغ
الميكروراجام يزيد معدل الجراحة المبكرة بحوالي 200% ضعف، كما وجد أيضاً أن
نسبة الحماية المناعية ضد الميكروب الحي المحروم لها الصفة الخصوصية حيث أن
مقدار مكونات الدهون عديدة السكريات لاحق عيّرات هذا الميكروب على أحداث
الوقاية المناعية ضد عيّرات أخرى (مختلفة) لا تزيد عن 25% ضعف. وقد خلصت
النتائج إلى اثبات القدرة العالية للقاح المحضر من خليط مكونات الدهون عديدة
السكريات للعوامل المختلفة لهذا الميكروب على أحداث حماية مناعية عالية ضد
مختلف العوامل.
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
(Received at 8/7/1987)

SUMMARY

Experimental mouse burn wound model was employed to evaluate the relative efficacy of Pseudomonas aeruginosa lipopolysaccharide as a protective 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

Pseudomonas 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 gastrointestinal tract. The emergence of P. 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 QLING, 1977).

Removal of the invading P. 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 P. 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 P. aeruginosa.

MATERIAL and METHODS

Bacterial strains:

Strains PA, PA, and PA were kindly obtained from J. Borst, National Institute of Public Health, P.O. Box 8, 3720 BA, Bilthoven, the Netherlands.

Growth environment:

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

Preparation of LPSs:

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 (LD₅₀).

Bacterial quantitation in tissues and blood:

Mice were challenged with approximately 10⁵ 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 PA₁, PA₆ and PA₁₀. 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 preliminary experiment was done to demonstrate the LD₅₀ of different strains of Ps. aeruginosa, PA₁, PA₆ and PA₁₀; they were found to be 0.5X10², 1.5X10² and 2.5X10² respectively (Table 1). In this study we compared LPS of the same serotype for its ability to produce a protective immune response. 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 bacteremia. Bacterial growth in skin of control mice was extremely rapid, reaching levels of greater than $10^9$ CFU/g of tissue, and $3 \times 10^2$ 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$_4$ 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 serotypes 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 *P. 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 *P. 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 *P. 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 high degree of fold protection. Such observation is consistent 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 *P. 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.

REFERENCES


Table (1): Active immunization of mice with LPS: effect of dose schedule on production against PA₁, PA₆, PA₁₀ in a burn wound sepsis model.

<table>
<thead>
<tr>
<th>Amt of LPS administered (Ug)</th>
<th>No of imm. (a)</th>
<th>PA₁</th>
<th>PA₆</th>
<th>PA₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td></td>
<td>0.5 X 10²</td>
<td>1</td>
<td>1 X 10²</td>
</tr>
<tr>
<td>[AL(gH)₃]</td>
<td></td>
<td>0.6 X 10⁶</td>
<td>12000</td>
<td>1.3 X 10⁶</td>
</tr>
<tr>
<td>0.1</td>
<td>1</td>
<td>7 X 10⁶</td>
<td>48,000</td>
<td>4.3 X 10⁶</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>5 X 10⁶</td>
<td>100,000</td>
<td>1.2 X 10⁷</td>
</tr>
</tbody>
</table>

(a) Immunizations were given intramuscularly in 100 Ul volume at 14 days intervals. Challenges with PA₁, PA₆, PA₁₀ were performed 14 days after the final immunization.

(b) LD₅₀ 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

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Serotype</th>
<th>Immunizing dose (Ug)</th>
<th>LD₅₀ (b)</th>
<th>Fold protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td></td>
<td>0.5 X 10²</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>[AL(gH)₃]</td>
<td>1</td>
<td>5 X 10⁶</td>
<td>100,000</td>
<td></td>
</tr>
<tr>
<td>PA₁ LPS</td>
<td>0.01</td>
<td>2 X 10⁶</td>
<td>40,000</td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td></td>
<td>1.5 X 10²</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PA₆ LPS</td>
<td>6</td>
<td>1.2 X 10⁷</td>
<td>80,000</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>3 X 10⁶</td>
<td>20,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td></td>
<td>2.5 X 10²</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PA₁₀ LPS</td>
<td>10</td>
<td>1.0 X 10⁷</td>
<td>40,000</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>7.5 X 10⁶</td>
<td>30,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(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 immunization.

(b) Expressed as number of viable bacterial count.
### Table (3)
Protection against *P. aeruginosa* PA₆ and PA₁₀ by active immunization with LPS₅(α) of PA₁

<table>
<thead>
<tr>
<th>Challenge strain (serotype)</th>
<th>LD₅₀ (b)</th>
<th>Fold protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Immunized</td>
</tr>
<tr>
<td>PA₆</td>
<td>1.5 x 10²</td>
<td>2.1 x 10⁶</td>
</tr>
<tr>
<td>PA₁₀</td>
<td>2.5 x 10²</td>
<td>5.5 x 10⁶</td>
</tr>
</tbody>
</table>

(a) Mice were immunized intramuscularly with 0.1 μg of PA₁ LPS in 100 μl volume at days 0, 14 and 28: challenges with PA₆ and PA₁₀ were performed 18 days after the final immunization.

(b) Expressed as number of viable bacterial count.

### Table (6)
Protection against *P. aeruginosa* challenge by vaccination with a polyvalent LPS Vaccine(α)

<table>
<thead>
<tr>
<th>Challenge strain (serotype)</th>
<th>LD₅₀(α)</th>
<th>Fold protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Immunized</td>
</tr>
<tr>
<td>PA₁</td>
<td>0.5 x 10²</td>
<td>1.6 x 10⁶</td>
</tr>
<tr>
<td>PA₆</td>
<td>1.5 x 10²</td>
<td>2.4 x 10⁶</td>
</tr>
<tr>
<td>PA₁₀</td>
<td>2.5 x 10²</td>
<td>6.0 x 10⁶</td>
</tr>
</tbody>
</table>

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

(b) Expressed as number of viable bacterial count.