Detection of IL-1β, IL-2, IL-4 and IL6 concentrations in serum of rabbits immunized with lipopolysaccharides

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Abstract

LPS of *P. rettgeri* was extracted and purified in our previous study, the present study found that the injection of 150μg/ Kg of LPS (intramuscularly and subcutaneously) was able to stimulate humoral immune response at systemic level. Some cytokines concentrations such as IL-2, IL-4, IL-6 and IL-1β were detected using ELISA kits. The study was observed that, the concentrations of IL-2 (3.21 pg/ml) , IL-4(5.84 pg/ml) and IL-6 (4831 pg/ml) were increased significantly in LPS immunized animals group compared with control group which were 2.05 , 2.34 and 3121.06 pg/ml for IL-2,IL-4 and IL-6 respectively, while the concentration of IL-1β (115.7 pg/ml) was decreased significantly in LPS immunized animal group compared with control group which was 177.24 pg/ml.

الكشف عن تراكيز IL-1، IL-2 و IL-4 و IL-6 في مصل الأرانب المنعمة بعدد (LPS) السكرى الشحمي

فيال جميل عبد
كلية العلوم/ جامعة بابل

الخاصة

تم في دراستنا السابقة استخلاص وتنقيح عدد السيركلأخشمي من عزلة بكتريا *Providencia rettgeri* ووجد من خلال هذه الدراسة أن عدد السيركلأخشمي عند حقنه في الأرانب بالجرعة 150 مايكروغرام/كم في الخلاصة وتحت الجلد قد حفز الاستجابة المناعية الخلطية على المستوى الاجهادي. تم قياس تركيز بعض السايتوكنات مثل IL-2 و IL-4 و IL-6 و IL-18 باستخدام تقنية ELISA. إن تركيز كل من السايتوكنات IL-2 و IL-4 و IL-6 و IL-18 قد ارتفعت معنويًا في الحيوانات المنعمة بعدد السيركلأخشمي وكانت (3.21 و 5.84 و 3112.06 بيكوغرام/مل على التوالي) مقابلة تراكيزها في حيوانات السيطرة والتي كانت 2.05 و 2.34 و 3121.06 بيكوغرام/مل لكل من السايتوكانات-2 و السايتوكانات-4 و السايتوكانات-18 على التوالي. في حين أن تركيز السايتوكانات IL-18 قد انخفض معنويًا في الحيوانات المنعمة بعدد السيركلأخشمي ليصل إلى 115.7 بيكوغرام/مل مقابلة تراكيزه في مجموعة السيطرة والذي كان 177.24 بيكوغرام/مل.
Introduction

The genus *Providencia* is a member of the *Enterobacteriaceae* family which consists of eight species [1]. *Providencia rettgeri* is one of five *Providencia* species that is known to cause various infections, especially the urinary tract infection (UTI) [2]. The importance of this species is associated with its tendency to cause nosocomial infections and with its marked resistance to numerous antibiotics [3]. Endotoxins, also called lipopolysaccharides (LPS) is the major component of the outer membrane of the cell wall of Gram-negative bacteria, which is considered as a virulence factor of pathogenic bacteria, including *Providencia* [4].

The lipopolysaccharide (LPS), as in other members of the family *Enterobacteriaceae*, consists of three domains, an endotoxic glycolipid (lipid A), an O-polysaccharide (O-PS) chain or O-antigen, and an intervening core oligosaccharide (OS) region, the O-antigen is the major surface antigen, and its serological O specificity, in contrast to that of other Gram-negative bacteria [5]. The lipid A is the most conserved part of endotoxin and is responsible for most of the biological activities of endotoxin, i.e. its toxicity and the lipid A form of *P. rettgeri* warrants maximal immunostimulatory activities [6].

Bacterial lipopolysaccharide play a critical role in the initiation of the proinflammatory events that contribute to the pathogenesis of the sepsis and the pathophysiologic mechanism(s) responsible for this illness are thought to result from the noncytotoxic interaction of LPS with mammalian host inflammatory mediator cells monocytes/macrophages constitutes a major mechanism responsible for innate immune response to Gram-negative infection [7]. LPS was a potent stimulator of macrophages, B-cell and T-cell activation in vivo were recognized at same time in both early and late responses to LPS and macrophages released various multifunctional cytokines, such as IL-1 and TNF-α [8].

Endotoxin elicits a wide variety of pathophysiological effects, such as endotoxin shock, tissue injury, fever, sickness behavior and death, animal studies indicate that these effects are mediated by cytokines, particularly IL-1β, IL-6, IL-2, and TNF-α [9,10]. Locally few studies about the *Providencia* especially, the virulence determinant like LPS. Therefore, the aim of this study was to demonstrate the role of LPS in activation of immunological cells and produce of cytokines.
Materials and methods

1-Bacterial strain:

P. rettgeri strains were isolated from urinary tract infections (UTIs). Traditional biochemical tests were used for final identification of bacterial isolates [11]. And the confirmed identifications to species level were also carried out by using Hi25 E system (Enterobacteriacea identification system, Himedia, India).

2-Extraction and purification of LPS:

An isolate of P. rettgeri from a patient with UTI were cultivated for extraction. Endotoxin extraction was carried out according to method of [12] and purification of LPS was carried out according to methods of [13], [7] and [14].

3-Bioassay of endotoxin:

This was carried out according to method of [15].

4-experimental animals:

Six (6) of local rabbits (Oryctylagus conniculus), each rabbit was about (1.5-2kg) of weight were selected as test experimental animals and left for two weeks for adapted conditions and kept at libitum and then divided into two groups.

5-Immunization Protocol:

First group of animals was injected with 1 ml of LPS dose (300μg/ml) intramuscularly and subcutaneously, while the other group was injected with 1 ml of normal saline and considered as control group. LPS and normal saline doses were administrated weekly for four weeks, on the fifth week all groups were left for one week then they were ready for immunological investigations [16]. The sera were separated from the whole blood, then divided into 0.5 ml small tubes, and stored at -20°C till testing time.

6- Detection of secreted cytokines:

To determine the concentration of systemic cytokines: IL-1β, IL-6, IL-2 and IL-4, ELISAs were conducted using commercial assay kits (IL-1β Stress gen.com, USA; IL-6, Bender Med.system, USA; IL-2 and IL-4, eBioscience, USA) according to the manufacturers’ protocols. All experiments were performed in triplicate. Results are shown as mean ± standard deviation (SD).
Results

Detection of cytokines:

Systemic IL-2 and IL-4 concentrations showed significant increase (P<0.05) in rabbits which were immunized with LPS antigen than control rabbits group, and the mean values of IL-2 and IL-4 concentrations for LPS group were (3.21±1.02 pg/ml) and (5.84 ±0.9190 pg/ml) respectively, while the mean values for control groups were (2.05±0.101 pg/ml) and (2.34±0.103 pg/ml), respectively (Table 1). IL-6 concentration showed significant increase (P<0.05) in LPS rabbits group (4831.57±181.03 pg/ml) than control rabbits group (3121.06±438.56 pg/ml) as shown in table 1.

On the other hand, IL-1ß was showed significant decrease (P<0.05) in rabbits group which were immunized with LPS antigen compared with control rabbits group and the mean values of concentrations were (177.24±54.65 pg/ml) for control group and (115.7±6.612 pg/ml) for LPS group (Table 1).

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>IL-2 (pg/ml) Mean ±S.D</th>
<th>P-value</th>
<th>IL-4(pg/ml) Mean ±S.D</th>
<th>P-value</th>
<th>IL-6(pg/ml) Mean ±S.D</th>
<th>P-value</th>
<th>II-1ß(pg/ml) Mean ±S.D</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>2.05±0.101</td>
<td>0.074*</td>
<td>2.34±0.103</td>
<td>0.0007*</td>
<td>3121.06±4</td>
<td>0.01*</td>
<td>177.24±54.6</td>
<td>0.058*</td>
</tr>
<tr>
<td>Immunized group with LPS (150µg/kg)</td>
<td>3.21±1.02</td>
<td></td>
<td>5.84±0.919</td>
<td></td>
<td>4831.57±1</td>
<td></td>
<td>115.7±6.62</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 is significance † Values of P are for control group compared with LPS group. (S.D) standard deviation.
Discussion

The result in table 1 showed that there was significance increase in the concentrations of IL-2 and IL-4 in rabbits which were immunized with LPS antigen compared with control group. This result was converged with other previous studies such as Bagchi and Sinha (2005) [10] which demonstrated that mice immunized with the 57 kDa outer membrane protein of Shigella dysenteriae it was found increasing of the level of IL-2 significantly and Bhatia and Basu (2007) [17] who observed that, LPS-activated macrophage secretes inflammatory mediators like IL-2.

The lower level of IL-2 in serum could be due to the fact that production of cytokines is transient at early time points after the microbial encounter [18].

IL-2-receptor stimulation via IL-2 did not significantly induce the release of IL-2 with consistent intracellular Ca+2 production, this indicate that phosphotidylinositol-3 kinase-mediated signals are up-regulated through intracellular free [(Ca+2)i], which is essential for Th1-type responses, the TCR up-regulates the protein kinase C (PKC)-mediated phosphorylation in CD4T cells, which in turn leads to IL-2 production [10].

The Th2 cells which produce IL-4, are primarily responsible for antibody-mediated immunity, IL-4 is one of the key cytokines regulating Th2 immune activities and helps drive antibody responses in a variety of diseases [19].

Our result also showed that IL-6 concentration was significantly increased in rabbits immunized with LPS compared with control rabbits group. On the other hand the result in table 2 was showed that there was significant decrease in the concentration of IL-1ß in LPS group than control group. This result agree with other study such as Ranallo et al. (2010) [20] who showed that the level of IL-6 was elevated in mice inoculated with Shigella flexneri strains while variable concentrations of IL-1ß were found in mice inoculated with different strains and Jansky et al. (2003) [21] who found that, the production of IL-1ß and IL-6 was lower than its level produced from peripheral blood mononuclear cells (PBMC) stimulated by Borrelia.

The low level of IL-1ß concentration in animal group immunized with LPS may be due to the effect of IL-6 which was at higher concentration in this group and this result was in agreement with Tilg et al. (1994) [22] study which showed that IL-6 inhibits LPS-induced TNF-α and IL-1ß production in cultured human monocytes and in mice in vivo and this may be due, in part, to the induction of IL-1 receptor antagonist (IL-1 Ra) synthesis and the release of soluble TNF receptors result in low plasma levels of IL-1ß and TNF-α plasma in all samples tested. Nandi et al. (2012) [23] demonstrated that IL-6 activate IL-10 production
in serum of endotoxic group and then IL-10 inhibit the synthesis of proinflammatory cytokines and also has a suppressive effect on proinflammatory cytokines like IL-1β,TNF-α,INF-γ and IL-12. In humans, circulating concentrations of IL-6 are highly correlated with increased mortality, a finding that has been attributed to its ability to persist in the circulation for a longer period of time than other proinflammatory cytokines [24].

Several lines of evidence indicate that the primary target of LPS on monocytes/macrophages is the mCD14 molecule, which triggers, together with TLRs intracellular signaling pathways leading to cytokine production [8].

References


