Evaluation of Cross Protection Conferred by Human Antisera against Septic Shock Caused by Endotoxins

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Abstract
Objectives
To evaluate the cross protection conferred by pooled anti-lipopolysaccharide antibodies in human sera against a challenge with a specific lipopolysaccharide (LPS) endotoxin.

Methods
Three groups, each comprising of fifteen weight and sex matched Tuck-ordinary (T.O) mice were passively immunized intraperitoneally with 0.25 ml of 0.15M sterile PBS saline, 0.25 ml of high titer J5 (mutant E.coli 0111:B4) LPS antiserum, and 0.25 ml of high titer concoction LPS antiserum containing a mixture of antibodies against four different endotoxins. After one hour all mice received intraperitoneal injection of 5 µg (LD-100) LPS from Salmonella abortus equi in sterile PBS containing 10 mg D-galactosamine. Survival of mice was assessed at four hourly intervals for 24 hours. Mice surviving 24 hours after administration of LPS were considered as protected.

Conclusion
Concoction LPS antiserum was able to provide 100% protection since all the mice in that group survived at the end of 24 hours. E.coli J5-LPS antiserum could protect only nine out of fifteen mice (60%), whereas none of the mice survived in the group that received normal saline (p<0.0001, χ² =30.53).

Key words: Endotoxin, Lipopolysaccharide, Core region, Oligosaccharide, E.coli-J5, Concoction-LPS

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Introduction

Infections caused by gram negative bacteria constitute one of the major causes of the sepsis syndrome, characterized by hypotension, tachycardia, tachypnea, disseminated intra vascular coagulation, and multiple organ system failure. Gram-negative bacterial sepsis is a serious complication especially in patients with burns, trauma and post-operative cases of abdominal surgery in intensive care units. Although antibiotic therapy plays an important role in limiting the incidence of this complication, there has been a little change in the mortality of this condition once developed. The mortality rate for septic shock patients continues to be unacceptably high, in spite of the therapeutic intervention and rigorous supportive care.

It is generally acknowledged that the systemic inflammatory response associated with gram-negative bacterial infection is triggered by the release of lipopolysaccharide (LPS) from bacterial cell wall. LPS is the main component of the outer membrane of the cell wall of gram-negative bacteria and is believed to be responsible for the initiation of endotoxic shock. The systemic administration of LPS has been commonly used in experimental animals to reproduce the typical feature of clinical septic shock, including the production of pro-inflammatory mediators. Therefore this molecule has been targeted for development of new preventive and therapeutic strategy.

It has been found that endotoxins derived from different bacterial species share a common basic structure. LPS consists of a polysaccharide part that includes an O-specific chain and core oligosaccharide (OS) linked in a covalent bond to lipid A. Most of the antibodies produced during immunization with bacterial cells are directed against the O-specific part of endotoxin. The variability of O-sero-types among bacteria of one species prevents the use of anti-LPS antibodies as broad-protective agents for therapeutic intervention in gram negative bacterial infections. The core oligosaccharide is a conserved part of endotoxins. Structural analysis of LPS isolated from different strains of bacteria show less variability among the core oligosaccharide part in comparison with O-specific chain.

In order to address the issue, this study was performed to evaluate cross protection conferred by antibodies against LPS from E.coli J5 rough core oligosaccharide and against LPSs from a concoction of four different gram negative bacteria (E.coli EH100, Salmonella minnesota Re595, Salmonella typhimurium SL684 and Shigella flexneri).

Materials and Methods

Screening for High Titer Human Antisera

Antisera were obtained from the routine blood samples sent to the laboratory for other investigations. All the sera were screened for E.coli J5 LPS and the concoction LPS antibodies using enzyme-linked immunosorbent assay (ELISA) technique. Briefly, ELISA plates (Immuno M129, Dynatech) were coated with 20 μg/ml of either E.coli O111:B4 J5 mutant Rc chemotype LPS at 1mg/ml in pyrogen free water or E.coli EH100, Salmonella minnesota Re595, Salmonella typhimurium SL684 and Shigella flexneri were diluted 1mg/ml in pyrogen free water and used as a combination of LPS (Sigma) overnight at room temperature. The plates were incubated for 1 hour with 1% pyrogen-free BSA at 37 to block nonspecific binding and washed three times with washing buffer (0.1% Tween20 containing 0.05 % Sodium azide and 0.02M Magnesium Chloride (MgCl)). Following this, 100 μl of test sera were dispensed in the micro-titer plate and incubated (1 hour at 37 °C). Plates were then washed five times in washing buffer (0.1% Tween20 containing 0.05 % Sodium azide and 0.02M Magnesium Chloride (MgCl)). After washing, 100 μl alkaline phosphatase conjugate anti human IgG was added to plates and incubated (1 hour at 37 °C). Plates were then washed five times in washing buffer then 100 μl alkaline phosphatase substrate (Sigma) was added to all the wells and optical density was obtained using 405nm
filter. Sera containing IgG to *E.coli* J5 LPS and the cocktail LPS either equal to or more than a titer of 1:2560 were collected and used in the experiment.

**Mouse Protection Studies**
Three groups of fifteen Tuck Ordinary (T.O) mice each matched for weight, age and sex (Harlan Olac, UK) were used in the study. Each group of mice received an intraperitoneal injection of either 0.25 ml of 0.15M sterile PBS, 0.25 ml (dose calculated earlier by series of titration) of high titer anti J5 serum or 0.25 ml of high titer anti concoction serum. An hour later, all mice were injected intraperitonially with a lethal dose of 5 µg of commercially obtained pure LPS (Sigma product, UK) from *Salmonella abortus equii* in sterile PBS containing 10 mg D-galactosamine lethal dose (LD100) in 24 hours. This dose was determined prior to experimenting on mice through series of dilutions. Mice were kept in separate cages and examined at four hourly intervals. Mice surviving 24 hours after administration of LPS were considered protected. Statistical significance was assessed by Chi square test.

**Results**

*Figure 1* shows the result of protection conferred by anti LPS sera against septic shock inflicted by the lethal dose challenge with *Salmonella abortus equii*. Over 24-hour period, none of the mice survived the challenge in the group immunized by PBS saline. Pooled human anti *E.coli* J5 sera, however, provided 60% protection (out of 15 mice 9 survived). On the other hand, pooled human anti concoction serum provided 100% protection (p<0.0001, $\chi^2 = 30.53$), where all the 15 mice had survived 24 hours after the challenge with *Salmonella abortus equii* LPS (*Table 1*).

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*Figure 1:* Survival chart for mice illustrating cross protection conferred by anti-LPS Sera
Table 1: Survival of mice given 0.25 ml anti-J5 sera, anti-concoction sera or 0.25 ml saline 60 minute before an LD-100 injection of LPS from *Salmonella abortus equi*

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Number</th>
<th>Surviving Mice After 24 Hrs N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-J5*</td>
<td>15</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>Anti-concoction**</td>
<td>15</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Saline</td>
<td>15</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

* Mutant *Escherichia coli* 0111: B4 J5-Rc chemotype LPS.

** (E.coli EH100, *Salmonella Minnesota* Re595, *Salmonella typhimurium* SL 684 and *Shigella flexneri*) mixture LPSs.

Discussion

This study shows that anti concoction LPS serum effectively provides protection against gram negative septic syndrome, whereas the anti *E.coli* J5 serum provided a less efficient protection. The idea of targeting rough mutant antibodies as broadly cross protective therapy gained popularity with the report that equine antiserum raised against *Salmonella* strain of the Ra chemo type protected mice against challenge with virulent strain of *Klebsiella pneumoniae* [13]. However, due to the fact that immunodominant epitope in *E.coli* J5 (Rc) chemotype is the most conserved among gram negative organisms, it has been used in most investigations of cross protection by rough mutant organism [14].

Passive immunization with J5 was reported to provide partial protection to experimental animals against toxic effects of LPS [15-18]. The same partial protection was reported against lethal gram negative bacteremia [19, 20]. Previous animal model studies conducted on a large scale showed that *E.coli* J5 antiserum could offer only 56-59% protection [15, 21]. Although a small number of mice were used in this study the protection offered by *E.coli*-J5 antibodies was similar (60%), suggesting that antiserum comprising of IgG against a single organism LPS provides partial cross protection against gram negative sepsis.

Since the sharing of bacterial antigens across the species is a well known phenomenon and in order to increase achieved cross protection using antisera, it would appear logical to use antiserum that contains immunoglobulins against LPS from more than one organism [11]. A pervious study using the antiserum comprising of immunoglobulins against two organisms failed to show any enhancement in the protection of animals against gram negative sepsis [22]. These finding are in contradiction to what was observed in the present study, where a concoction of immunoglobulins against four different organisms proved to be more effective compared to the antiserum containing IgG against a single organism.

The difference could be due to the fact that the anti serum used in the previous studies [18, 22] was derived from animal source whereas the antiserum used in the present study was of human origin. The difference in the ability of human and animal immune response to react to foreign antigens may be responsible for the discrepancy of results observed in the studies.

Despite some contradictions in previous experimental findings, there is evidence that anti core antibodies protect in clinical settings, as illustrated in recent studies which show that high levels of natural anti-LPS core antibodies correlate with reduced incidence of complications after surgery and better outcomes from infection [23-25].
In the study using concoction LPS was shown to confer complete cross-protection against lethal challenge with LPS. Despite of the fact that the concoction LPS did not contain anti LPS antibodies specific to the LPS used for the lethal challenge, the concoction LPS appeared to have the ability to protect the mice efficiently. Because of shared antigens among different gram negative LPS further studies are needed to elucidate the role of pooled human anti sera containing anti LPS antibodies against various organisms as a potential therapeutic agent. The fact that the anti human sera used in this study was collected from blood specimens would open a new venue in mass screening of blood donors for the presence of relevant protective antibodies that could provide effective protection in septic shock syndrome. Recent study has demonstrated that the cyanobacterial LPS analogue, CyP, has inhibited E.coli LPS-induced cytokines as an inflammatory agent26. Other studies have illustrated that a combined inhibition of complement and CD14 abolish E.coli-induced inflammatory response in human blood27. On the other hand, some have argued that the cooperative interplay between the LPS-binding protein (LBP), the membrane-bound of CD14 and TLRs are required to trigger a pro-inflammatory response which is crucial for keeping infection under control28. Finally, a combination therapy of anti bacterial drugs, anti concoction LPS and anti-inflammatory agents might be suggested for optimal treatment to overcome the complexity of sepsis.

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