A study of the immune response of *Salmonella typhi* - LPS in rabbits

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**Summary**

The study included isolation of *S. typhi* from a diagnosed cases of typhoid patients, which dependent on the morphological and biochemical reactions in addition to the serological tests. LPS antigens were prepared and assauged, as well as cell free culture filtrate antigen beside the ready prepared *S. typhi* agglutinogens. Such antigens were made available for plotting the immune status of the rabbits. In animals, *S. typhi* specific agglutinins titer (S.TH, S.TO) were measured and found to be within the range of 160-640 in peripheral blood respectively. The cellular immune response were determined by performing, leukocyte inhibition factor LIF. In case of *S. typhi* infection the LIF percentage raised up to 40% in systemic LIF when CFCC were used as a sensitizer. *S. typhi* immune protection was determined in animals; the immune protection rate with LPS vaccine was 90% while the death rate was 10%.

**Introduction**

Endotoxins are part of the outer membrane of the cell wall of Gram negative bacteria. The lipopolysaccharide complex associated with the outer membrane of Gram – negative bacteria such as E. Coli, Salmonella, Shigella, Pseudomonas, Neisseria, Haemophilus, and other leading pathogens (Kenneth, 2002). The biological activity of endotoxin is associated with the lipopolysaccharide (LPS). Toxicity is associated with the lipid component (Lipid -A) and immunogenicity is associated with polysaccharide components. The cell wall antigens (O antigens) of Gram – negative bacteria are components of LPS. LPS elicits a variety of inflammatory responses in an animal. Because it activates complement by the alterantive (properdin) pathway, it is often part of the pathology of Gram – negative infections (Michetti, et al 1993). The function of the outer membrane of Gram – negative bacteria is to act as permeability barrier. The outer membrane is impermeable to large molecules and hydrophobic compounds from the environment. It may play several roles in the pathogenesis of Gram – negative infections. It is a permeability barrier that is permeable only to low molecular weight, hydrophilic molecules. It also a barrier to lysozyme and many antimicrobial agents. It impedes destruction of the bacterial cells by serum.
components and phagocytic cells. LPS may be involved in adherence (colonization), or resistance to phagocytosis (Robbins et al., 1994; Kenneth, 2002). LPS consists of three components or regions, Lipid A, R polysaccharide and O polysaccharide. Both lipid-A (the toxic component of LPS) and the polysaccharide side chains (the nontoxic but immunogenic portion of LPS) act as determinants of virulence in Gram – negative bacteria (Collee et al., 1996). It is thought that LPS released into the bloodstream by lysed– Gram negative bacteria is first bound by certain plasma proteins identified as LPS – binding proteins. The LPS – binding protein complex interacts with CD14 receptors on monocytes and macrophages and other types of receptors on endothelial cells. In monocytes and macrophages several types of events are triggered during their interaction with LPS (Kenneth, 2002).

Production of cytokines, including IL-1, IL-6, IL-8, tumor necrosis factor (TNF) and platelet – activating factor stimulate the production of prostaglandins and leukotrienes. These are powerful mediators of inflammation and septic shock that accompanies endotoxin toxemia. LPS activates macrophages to enhanced phagocytosis and cytotoxicity. Macrophages are stimulated to produce and release lysosomal enzymes, IL-1 (endogenous pyrogen) and tumor necrosis factor (TNF alpha) as well as other cytokines and mediators (Blanden et al., 1966; Kenneth, 2002). Activation of the complement cascade, C3a and C5 a cause histamine release (leading to vasodilation) and effect neutrophil chemotaxis and accumulation which result is inflammation (Blanden et al., 1966).

LPS also acts as a B cell mitogen stimulating the polyclonal differentiation and multiplication of B cells and the secretion of immunoglobulins, especially IgG and IgM (Schumonn et al., 1990; Michetti et al., 1993; Schiff et al., 1998; Kenneth, 2002).

The aims of this study included the purification (LPS) of S. typhi in addition to the role of LPS to elicited the immune response in rabbits.

* Material and methods
*Culture media and reagents

Culture media and reagents are mentioned in table (1)

<table>
<thead>
<tr>
<th>Seq</th>
<th>Name</th>
<th>Class</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brain heart infusion</td>
<td>Enrichment</td>
<td>Cultivation of bacteria for dry weight</td>
</tr>
<tr>
<td>2.</td>
<td>Tetrathionet broth</td>
<td>Selective media</td>
<td>Enriches S. typhi</td>
</tr>
<tr>
<td>3.</td>
<td>MacConky agar</td>
<td>Differential media</td>
<td>Cultivation of enteric bacteria</td>
</tr>
<tr>
<td>4.</td>
<td>S.S agar</td>
<td>Selective media</td>
<td>Isolation of salmonella</td>
</tr>
<tr>
<td>5.</td>
<td>Mast agar</td>
<td>Agar</td>
<td>For LIF test</td>
</tr>
<tr>
<td>6.</td>
<td>Normal saline</td>
<td>Solvent</td>
<td>Titration of antibody</td>
</tr>
<tr>
<td>7.</td>
<td>Basal medium Eagle</td>
<td>solution</td>
<td>As cell nutritive of LIF</td>
</tr>
<tr>
<td>8.</td>
<td>Methyl red</td>
<td>reagent</td>
<td>Acid production</td>
</tr>
<tr>
<td>9.</td>
<td>H2O2 product</td>
<td>reagent</td>
<td>Gas production</td>
</tr>
<tr>
<td>10.</td>
<td>Oxidase</td>
<td>reagent</td>
<td>Electron transport</td>
</tr>
<tr>
<td>11.</td>
<td>ONPG test</td>
<td>reagent</td>
<td>Late lactose ferment</td>
</tr>
</tbody>
</table>

* Determination of S. typhi infection dose
The bacteria cells (from the culture) were emulsified in normal saline to a visible density approximately $1.5 \times 10^5$ cell/ml bacteria, compared with McFarland standard tube (0.5) (Shnawa and Thwaini, 2002).

* **Lab animal inoculation.**

Rabbits (1000-1500 g) were orally infected using orogaoline tube (Shnawa and Thwaini, 2002).

* **Ready prepared agglutinogens.**

Standard bacterial suspension of known agglutinogens were used for the two types of antigens involving *S. typhi* O. Ag and *S. typhi* H antigens (Plasmetic company).

* **Preparation of leukocyte sensitizer.**

Cell free culture filtrate (CFCF) was prepared by cultivation of *S. typhi* in brain heart infusion broth for 24 hr; and the liquid media was centrifuged at 5000 Rpm for 15 min.

The culture supernatant was membrane filtered through millipore filter in syringe device, the filtrate was collected and distributed in sterile bottles and preserved in refrigerator (4C) until use as sensitizer (Shnawa and Thwaini, 2002).

* **preparation of bacterial dry weight**

Brain - heart infusion broth was inoculated by *S. typhi* using shaking incubator for overnight to obtain high density growth. The culture was cooled - centrifuged at 5000 Rpm for 20 min., the precipitate which represents the cells of bacteria was dried in an incubator (37 C) for 48 hr.

* **Preparation of LPS-antigen**

LPS suspension prepared according the (Westphal's et al, 1952) method (Adoos 2005).

* **Bacteriology**

Laboratory diagnosis of salmonella infection depends mainly on the isolation and identification of salmonella from the specimens of patients blood and faeces (Collee et al 1996 MacFadin, 2000).

* **Blood culture**

Blood samples were collected from patients using sterile disposable syring immediately cultured on blood agar, MacConkey and S.S. agar for isolation and biochemical identification of the bacteria (Collee et al, 1996).

* **Agglutination**
Direct semi quantitative slide as well as standard tube agglutination using serial double dilution for sera, solutions were reacted with laboratory prepared antigens (Garvey et al., 1977).

**Peripheral blood LIF**

Measurement of migration inhibition factor in systemic blood according to (Soberg, 1968):
*Preparation of agar-A medium in sterile plastic plates and 2 wells were made 2cm in diameter.
*Capillary tube containing systemic blood from rabbits infected was placed in each well after being centrifuged by haematocrite centrifuge for 10 min. (Leucocytes and Buffy coat).
*0.1ml of Eagle basal medium was put in each well; one of the wells kept as control.
*0.1ml of antigen (CFCF) of the causative bacteria was added in one well.
*Incubation was done at 37°C for 24 hr in Jar humid environment.
*Measurement of LIF by ocoulometer, same steps were used for control and normal saline was added instead of CFCF as mentioned (Soberg, 1968).

**LPS-immunization**

Five rabbits, exo-endo and haemo parasite free as well as anti. S. typhi antibodies pre rabbits were kept adlibitum condition and assigned in three groups (Table- 2).

LPS-suspension was administrated in five doses respectively at four week intervals, while the saline injected once, blood was collected for immunological investigation. (Shnawa and Thwaini, 2002).

**Results and Dissection**

**Bacteriological diagnosis**

The result showed that the salmonella like character. Some isolate of bacteria showing character of some biochemically (table .3)
Table (4) S. typhi immune protection against live S. typhi challenge

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rabbits</th>
<th>Infection dose</th>
<th>Symptoms</th>
<th>Death ratio</th>
<th>Protection ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS-Primed</td>
<td>5</td>
<td>S.typhi</td>
<td>-</td>
<td>0/4</td>
<td>90 %</td>
</tr>
<tr>
<td>Non-LPS primed</td>
<td>5</td>
<td>S.typhi</td>
<td>Fever and death</td>
<td>5/5</td>
<td>0 %</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>Saline</td>
<td>-</td>
<td>-</td>
<td>100 %</td>
</tr>
</tbody>
</table>

The findings of evolution of the LPS-immuno protection revealed the similarity between the

The clinically diagnosis typhoid fever patients were proved by culture and serological diagnosis (Collee et al., 1996; Gianella, 2001).

The extracted and proved S. typhi-LPS antigen was assaged biochemically and immunologically (Kenneth, 2002).

* LPS-immune protection.

Lab animals were injected subcutaneously with IFA, then immunogen in saline containing 3 mg S. typhi-LPS suspension was dosaged orally; the symptoms were scored as mild, the death ratio was 0/5, but the protection rate was 100%.

Animals with non-LPS-Vaccine treatment had, fever and diarrhea, then death. The death ratio was 0/4, while the protection rate was 90%, in compared with control group (Table - 4).
systemic humoral immune response in pre and post challenge. The antibody titer in serum ranged between (160-320). However, at the cellular level, \( S. \text{typhi} \) induced cellular immunity, since the LIF value ranged between 0.40 _ 0.41 at systemic immunity in pre and post challenge (Table - 5).

<table>
<thead>
<tr>
<th>seq.</th>
<th>Pre-challenge</th>
<th>Post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine titers</td>
<td>Vaccine titers</td>
</tr>
<tr>
<td></td>
<td>STO</td>
<td>STH</td>
</tr>
<tr>
<td>1</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
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</tr>
<tr>
<td>5</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>6.control</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Rang</td>
<td>160-</td>
<td>160-</td>
</tr>
</tbody>
</table>

**LPS - Immunology**

The results of post vaccine and post challenge immune state evaluations, death rate and protective rate indicate that LPS antigen were immunoprotective against live - \( S. \text{typhi} \) challenging in a lapin model (Kenneth, 2002). Immunity to \( S. \text{typhi} \) requires both cell mediated and humoral immune responses and is achievable by vaccination (Forrest et al., 1991). \( S. \text{typhi} \). LPS-Antigen used as oral vaccine elicits immune response (Table-4) and the protection rate of LPS - vaccine was 90 %, while the death rate was 1/5. The results demonstrated that the oral immunization with LPS-antigen elicits strong systemic cell mediated immunity to live infection bacteria (Michetti et al, 1993 Copra etal, 1994).

The data presented in (Table-5) showed that the titer of antiserum to LPS- Antigen was the highest in both pre and post challenge with -\( S. \text{typhi} \). Then inducing an antibody and cellular immune response, significant LIF were 40 % and the mean value were 40 for both challenge. Very effective protection was observed when LPS vaccine administered within 24 hr after challenge. This effect could be increased with repeated injections. The result showed that the LPS-antigen with IFA, could be more effective than LPS-antigen alone. This finding agrees with (Robbins et al., 1994; Sharn, 2004). Vaccine studies indicate that LPS- Lipopolysaccharide antigen should be an important
immune target, since parental immunization with this antigen has lead to increased protection against S-typhi (Schumonn, 1990; Schiff et al, 1998).

**Cellular immunology**

The cell produces and releases a variety of effectors molecules called lymphokines (Weinberg, 1984; Weir et al, 1994). There are biochemical mediators of a number of widely studied cases in vitro phenomena, but it is believed that a similar activity is responsible for the immune response seen in tissues, the best characterized lymphokin is LIF the subject of present assay (Table 5). Upon release from lymphocytes, LIF can be identified by this ability to trap macrophage and inhibitor their migration. The macrophage is the target cell responding to the product LIF of lymphocyte which is the effectors cell (Adoose, 2005).

**LIF test**

The data presented in (Table 5) indicate that S. typhi induce specific cellular immunity when culture filtrate were used as a sensitizer, the mean values of LIF were 40% and 41% at systemic level, pre and post challenge respectively. The results showed that the LIF formation increased peripheral blood leukocyte. (Weinberg, 1984; Weir et al, 1994; Adoose, 2005).

**References**


