ABSTRACT
The smooth lipopolysaccharide preparations of Aeromonas hydrophila, the human and animal gut pathogen were previously extracted and partially purified as fraction 1 (F1) and fraction 2 (F2). F1 and F2 were assessed for: Mitogenicity, immunogenicity and immune protectivity. F1 and F2 were mitogenic in mouse food pad model giving skin induration and bone marrow lymphocyte blastogenicity, rise up of humoral antibody response specific for A. hydrophila, cytokine network activator IL4, IL6 and TNF alpha, and partially immune protective through death percentages. Thus, the smooth F1, F2, LPS of A. hydrophila were mitogenic, immunogenic and partially immune protective in mouse model.

Key Words: Cytokine network, humoral response, mitogenicity, protection lipopolysacchride.

INTRODUCTION
The immune response to an immunogen can be as an immunogenic but non immune protective and be immunogenic, immune protective as well. The smooth lipopolysaccharide as an immunogen derived from Aeromonas hydrophila, the human and animal gut pathogen, in small mammalian model to which class immunogen mentioned above can we put them. Knowing that gram negative smooth LPS were; Mitogenic, polyclonal B lymphocyte activator cytokine network activator and what remains to be mentioned if crude smooth LPS purified and fractionated, could different fractions give different immune potentials. The objective of the present work was to report on the nature of the immune protectivity mediated by two fractions of the smooth A. hydrophila LPS in postchallenge mouse model.

MATERIALS AND METHODS
Bacterial strains: A. hydrophila was obtained from Advance genetic engineering lab. Department of Biology Faculty of Science, University of Babylon. The isolate identification was confirmed biochemical tests.

Method of LPS isolation: LPS Extraction according to Westphal et al. partial purification in accordance with Boyer RF.

Methods for in vivo mitogenicity:
In mice: Twenty mice, their weight (20-25 gm) divided into four groups each one of groups included 5 mice:
Group I - (A. hydrophila F1) Included 5 mice which inoculated with LPS as 2.5 mcg/gm of mouse in pad at dose 0.2 ml / mouse.
Group II - (A. hydrophila F2) Included 5 mice which inoculated with LPS as 2.5 mcg/gm of mouse in pad at dose 0.2 ml / mouse.
Group III and IV: - were positive and negative group respectively.
Blastogenicity assay in vivo: the indurations were
mice.

decimal double dilution of the sera of immunized between through microtiteration bacterial agglutination assay The anti LPS specific antibody titer were assessed Microtiteration direct bacterial agglutination test.

A.hydrophila

Bacterin preparation:

Antibody

Group II and

A.hydrophila

15 days gave booster dose, after that challenge by A.hydrophila

15 days gave booster dose left for two week then blood was collected.

Group III and IV:

Post immunization challenge:

Twenty mice, their weight (20-25gm) divided into four groups each one of groups included 5 mice: Group I- (A.hydrophila F1) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group II- (A.hydrophila F2) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group IIIandIV: - were positive and negative group respectively.

Immunization protocol:

Twenty mice, their weight (20-25gm) divided into four groups each one of groups included 5 mice:

Group I- (A.hydrophila F1) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group II- (A.hydrophila F2) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group III and IV:

Antibody assessment:

Bacterin preparation: Heat killed bacterin from A.hydrophila fresh cultures were made as suspensions and subjected to heat treatment at 100C° for 1 hour then bacterin densities were adjusted using 0.5 McFarland tube.

Microtiteration direct bacterial agglutination test. The anti LPS specific antibody titer were assessed through microtiteration bacterial agglutination assay between A.hydrophila heat killed bacterins with decimal double dilution of the sera of immunized mice.

Cytokine assessment:- Interleukin-4, Interleukin-6 and TNFα Assay Procedure according to manufacturer's instructions (Boster’s –Korea).

RESULTS

Mitogenicity:- The mitogenicity of A.hydrophila LPS in mice was assessed through skin induration and blastogenicity for both fraction 1and fraction 2. The skin indurations were 1.95±0.4 ,1.87±0.4 for A.hydrophila fraction 1 and fraction 2 LPS respectively ,While blastogenicitie were 3.975±0.8 , 2.55±0.6 for A.hydrophila fraction 1 and fraction 2 LPS accordingly. Table (1).

Table 1

<table>
<thead>
<tr>
<th>Test modulant</th>
<th>Skin induration</th>
<th>blastogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.hydrophila F1 LPS</td>
<td>1.95±0.4</td>
<td>3.975±0.8</td>
</tr>
<tr>
<td>A.hydrophila F2 LPS</td>
<td>1.87±0.4</td>
<td>2.55±0.6</td>
</tr>
<tr>
<td>Control tuberculin</td>
<td>4</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Immunogenicity

Humoral immune response

Direct microfiltration test:- Immunized mice groups (5mice from each group) were rising humoral antibody response . The antibody titers were upto (35 ± 7.8) group immunized with A. hydrophila LPS (table 2).

Table 2

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Animal group</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>F2</td>
<td>32</td>
</tr>
<tr>
<td>16</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>64</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>Mean ±SE</td>
<td>35 ± 7.8</td>
<td>35 ± 7.8</td>
</tr>
</tbody>
</table>

Cytokine profile:- A.hydrophila LPS immunized mice were subjected to determination of IL_4, IL-6 and TNFα. IL-4 concentrations were 39.4198 ± 15.5848 for both fraction 1 and 2 respectively. While, IL_6 concentrations were 23.3462 ± 1.308, 43.6993 ± 7.8 for both fraction 1 and 2

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accordingly. TNFα concentrations were 127.1946 ± 3.310, 159.6818 ± 6.697 for both fraction 1 and 2 respectively, table (3).

Table 3
Cytokine profile of A. hydrophila immunized mice

<table>
<thead>
<tr>
<th>Group</th>
<th>IL 4</th>
<th>IL 6</th>
<th>TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 LPS</td>
<td>39.4198±2.960</td>
<td>23.3462±1.308</td>
<td>127.1946±3.310</td>
</tr>
<tr>
<td>F2 LPS</td>
<td>43.6993±2.343</td>
<td>15.5848±1.537</td>
<td>159.6818±6.697</td>
</tr>
<tr>
<td>Control</td>
<td>3±0.1</td>
<td>1.3±0.1</td>
<td>19.5±0.6</td>
</tr>
</tbody>
</table>

Immune protection:-
The protection rates were 80% for each of F1 and F2 Table4.

Table 4
The rate of protection

<table>
<thead>
<tr>
<th>A. hydrophila</th>
<th>Live percentage</th>
<th>Death percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>80%</td>
<td>20%</td>
</tr>
</tbody>
</table>

DISCUSSION
The mechanisms involved in postchallenge immune protective rates may be viewed as a collective of the immune cell functions that include, mitogenic ability, B lymphocyte polyclonal activating ability, T lymphocyte helping and for regulatory up and down pathways through activation and/or inhibition of the cytokine network as well as survivors records3,4. Postchallenge immune promitogenic of smooth LPS of A.hydrophila in a mouse model is based upon a sort of balance between the pathogen virulence vajor and the potency limits for the host immune defense mechanisms. Three possible cases can be expected as an outcome of the balance. The first the pathogen virulence output weight the host immune defense mechanisms which stands as no protection, the second the pathogen equate the the immune defence mechanisms for host it holds as immune protection, while the third case the pathogen abilities is slightly out weight the host immune defense mechanisms a finding indicate partial immune protection which can be assured through death percentages3,11. LPS was found mitogenic in mouse Table1 in accordance with workers9,10,11. It was good B lymphocyte mitogen as well as activated macrophage to secrete IL4 which in turn enhance Th2 lymphocyte to release IL4 andIL5 to provoke B, lymphocyte to proliferate and differentiated to plasma cells producing A.hydrophila specific antibodies Table2.4,12,13. The events suggest a parallelism between mitogenic assessment through lectin-like LPS skin tests Table 1 and T lymphocyte potency13. F1,F2 LPS of A.hydrophila increase IL4,IL6 and TNF alpha as compared to the control mouse Table3 with no shift in the cytokine balance and partial immune protection Table 4.

CONCLUSIONS
1. A.hydrophila smooth LPS F1, F2 are being lymphocyte mitogen in mouse model.
2. A.hydrophila smooth LPS F1, F2 were proved inducing humoral antibody production.
3. F1 and F2 triggers the cytokine network leading to increase in IL4, IL6 and TNF alpha as compared to normal control mouse.
4. F1 and F2 were being partially immune protective in postchallenge mouse model.

REFERENCES


