Immunological effects of *Citrobacter freundii* cell free culture in rabbit

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**Abstract**

*Citrobacter freundii* is usually considered a commensal species of the human gut, although some isolates have acquired specific virulence traits that enable them to cause *diarrhea*. *C. freundii* cell free culture (CFC) antigen was used in this study. Three rabbits of 2-3 months age (Newzealant, *Oryctolagus cuniculus*) were immunized by CFC in addition to complete Freunds adjuvant. After one month immunization period some of systemic immune response parameter (cellular and humoral) were studies and compared with control animal.

The results of test animals showed that CFC antigen lead to stimulate systemic cellular immune response which determined by using E-rosette test, (nitroblue tetrazolium) reduction test (NBT), leucocyte migration inhibitory factors (LIF) test. The results were appeared that higher number of E-rosette (0.45±0.02) compared with control animals (0.28± 0.01), also there was increasing in the percentage of neutrophils phagocytic activity (0.59±0.03 ) compared with control animals(0.48±0.02). The LIF in test animals was significantly produced in contrast to the control animals which not produce these factors.

The humoral immune response was studied. The results of hemagglutination test was appeared higher titer of test animals (128 ±0) compared with control group titer (1) and this true for immunoglobulin, complement and total protein concentrations. The concentration of IgM (424.1±.92)mg/dl, IgG concentration was (2365.3±34.2) mg/dl and IgA concentration (494.4±0)mg/dl. The concentration of C3 was (241.0±.54) mg/dl, C4 concentration was (55.4±2.4) mg/dl and total protein concentration (9.5±0.34) mg/dl. All these results for test animals were higher significant at (P<0.05) compared with control animals.

We conclude that CFC antigen of *C. freundii* stimulate humoral immune response in rabbits

**Key words:** *Citrobacter freundii*, cell free culture, LIF, E-rosette test, NBT.

فيما المقدمة

*Citrobacter freundii* هو نوع يُعتبر عادةً من依法ن اليونيغس، على الرغم من أن بعض العزمات لها*

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للتقليل من عدد الخلايا البائية في مصل الدم، ينبغي استخدام المواد المناعية الدقيقة في المصل الدم البشري. بغض النظر عن استخدام المواد المناعية الدقيقة في المصل الدم البشري، ينبغي استخدام المواد المناعية الدقيقة في المصل الدم البشري.

الخلاصة

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Introduction

Members of genus *Citrobacter* are Gram-negative, non-spore-forming rods belonging to the family *Enterobacteriaceae* and, as the name suggests, usually utilize citrate as a sole carbon source. These facultative anaerobes typically are motile by means of peritrichous flagella. They ferment glucose and other carbohydrates with the productions of acid and gas. They are oxidase negative, catalase and methyl red positive, Voges-Proskauer negative, and do not decarboxylate lysine. They are differentiated by their ability to convert tryptophan to indole. Of the dozen species, *C. freundii*, *C. diversus*, and *C. amalonaticus* are linked to human disease (Levinson, 2008; Samonis et al., 2009; Reddy, 2010; Keevil et al., 1997). In patients with a suppressed immune system, *Citrobacter* species are known to cause a wide variety of nosocomial infections of the respiratory tract, urinary tract, and the blood. Hepatic, biliary and pancreatic disease are also common diseases that are caused by *C. freundii*. *C. freundii* represents approximately 29% of all opportunistic infections (Whalen et al., 2007). The aim of this study was the effect of extracellular toxin of *Citrobacter* bacteria on some immunological parameters in rabbits.

Materials and Methods

1- *Citrobacter freundii* bacterium isolated from children's diarrhea specimens' and this bacteria diagnostic according to (MacFaddin, 2000)

2- Animals

Rabbit were used as experimental animals ,healthy Newzland rabbit (*Oryctolagus cuniculus*) about 1-1.5 Kg. they hands at room temperature in labium condition during experimental conditions (Schnider et al., 1990).

3- Cell free culture antigen preparation according to(Shnawa and Thewaini, 2002).

4- Immunization program

Animals were divided into two groups, three animals for each group . First group were given o.5 of CFC mixed with 0.5 of complete Freund’s adjuvant through injection intramuscular and subcutaneous equally for first week then they given 1 ml of CFC only through the same way for the second and third week while the second group given normal saline as a control for three weeks by using the same way of immunization (AL-Thahab, 2006).

5- Blood samples

Five ml of blood was collected from each rabbit by using sterile disposable syringes from heart , 3 ml was put into AFMA disposable tubes without anticoagulant ,then the serum was collected after centrifugation at 2500 rpm for 5 minutes and it was stored at freezing temperature (-4) , other 2 ml of blood was put in AFMA disposable tubes with anticoagulant for LIF, E-rosette and phagocyte activity tests.

6- Immune function tests

A- Hemagglutination test was done as in (Boyden, 1951).

B- Total protein test was done as in (Tietz et al., 1999) by using biuret solution (colorimetric method).

C- Radial immunodiffusion test was done as in (Mancini et al., 1965) in which equal volumes of control and test serum samples were added to wells in an agarous gel-containing a mono-specific antiserum. The sample diffuses radically through this gel and
the substance being assayed (antigen) forms a precipitation ring with the mono-specific antiserum. Ring diameters were measured by viewing device (ocular). Unknown concentrations were determined from the tables supplemented with each type of endoplate which contains 12 wells.

D- LIF test was done as in (Soberg. 1969) the results calculated after 24 h incubation period by the flowing equation:

\[ \text{Migration cofactor} = \frac{X_1}{X_2} \]

\[ X_1 = \text{Diameter of migration circle with antigen} \]
\[ X_2 = \text{Diameter of migration circle without antigen} \]

E- E-rosette test was done as in (Gengozian et al. 2002). After preparation of slides, each lymphocyte combined with three to five sheep red blood cells was consider positive result.

F- Phagocyte activity test was done as in (Park et al. 1968). In this test nitroblue tetrazolium pigment was used in blood slid preparation and the results were calculated according to the flowing equation:

\[ \text{Phagocyte activity} = \frac{N_1}{N_2} \times 100 \]

\[ N_1 = \text{Number of phagocytes reducer to pigment.} \]
\[ N_2 = \text{Number of total phagocytes.} \]

G- Statistical analysis

Statistical analysis (Mean ± Standard Deviation) was done as in (Dawed and Al-Yas. 1991).

Results

The rabbits that immunized with CFC antigens of *C. freundii* were showed higher results in all of specific antibody titer, total protein, Immunoglobulins and complement (C3, C4) concentration compared with control animals (table 1, 2).

Also the effect of CFC antigens' on leukocyte migration inhibitory factor was shown significant compared with control and the test animals showed increased in both E-rosette and phagocyte activity tests compared with control animals (table 3).

Discussion

Diarrheal disease caused by enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* is a significant worldwide health problem (Nataro and Kaper. 1998). Insights into the pathogenesis of these infections are of great importance for improving prevention and treatment strategies, yet availability of reliable small animal models is limited. Bacterial pathogen, *C. freundii* shares functional and structural features with human EPEC strains, most important of which is the ability to attach intimately to the apical surface of the intestinal epithelium and induce localized microvillus destruction, a characteristic feature known as attaching effacing lesion (Nougayrede et al. 2003).

In this study the effects of CFC of *C. freundii* to induced immunity in rabbit was clearly by increasing the antibodies titer and antibodies concentrations of test animals compared with control animals and that was occurred by activation T cells by this antigens (Bry and Brenner. 2004; Maaser et al. 2004), this cell activated B cells to produced the large amount of these antibodies like (IgM, IgA, IgG) which play important role in opsonization to these antigen in phagocytosis process and that mean activation the phagocytic cells and this agree with others (Maaser et al. 2004; Bry and Brenner. 2004) also T cells produced cytokines like IL-6, IL-12, IFN-γ, TNF-α and IL-17 (Bry...
and Brenner. 2004; Shiomi et al. 2010) all that led to increased protein concentration (Maaser et al.2004).

**Table 1: Specific Antibody Titer and Total Protein of *Citrobacter freundii* Cell Free Culture in Rabbit**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>Hemagglutination titer</th>
<th>Total protein(gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test animals(3 rabbits)</td>
<td>128 ±0</td>
<td>9.5±0.34</td>
</tr>
<tr>
<td>Control animals(3 rabbits)</td>
<td>1±0</td>
<td>5.6±0.12</td>
</tr>
</tbody>
</table>

M :Mean S.D :Standard Deviation

**Table 2: Mean of Systemic Immunoglobulin's and Complement (C3,C4) in Rabbit Concentrations mg/dl**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testanimals(3 rabbits)</td>
<td>424.1±9.2</td>
<td>2365.3±34.2</td>
<td>494.4±0</td>
<td>241.0±5.4</td>
<td>55.4±2.4</td>
</tr>
<tr>
<td>Control animals(3rabbits)</td>
<td>236.5±7.3</td>
<td>1419.9±25.1</td>
<td>179.8±5.1</td>
<td>139.3±4.4</td>
<td>36.4±2</td>
</tr>
</tbody>
</table>

**Table 3: Cellular Immune Response in Rabbits that Immunized with *Citrobacter freundii* Cell Free Culture**

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>LIF</th>
<th>E-rosette (%)</th>
<th>Phagocyte activity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test animals(3 rabbits)</td>
<td>0.59±0.05</td>
<td>0.45±0.02</td>
<td>0.59±0.03</td>
</tr>
<tr>
<td>Control animals(3 rabbits)</td>
<td>0.90±0</td>
<td>0.28±0.01</td>
<td>0.48±0.02</td>
</tr>
</tbody>
</table>

**References**


