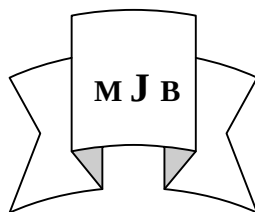


Humoral and cellular immune response against *Escherichia coli* in vivo

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Abstract

Enteropathogenic *E.coli* type I (EPEC) was isolated from feces of 8 months age infant baby. The bacterium was characterized according to the morphological, biochemical and serological properties. Their killed and formalized antigens (somatic and flagellar) were separated and mixed with complete and incomplete Freund's adjuvant (sunflower oil) which injected in rabbits (*Oryctolagus cuniculus*). The results were revealed that the effect of *E.coli* antigens in humoral immunity which expressed with immediate hypersensitivity were reached by single radical immunodiffusion assay (SRID) to 16.6, 15.4 and 19.4 mm in diameter for killed, somatic and flagellar antigens, respectively, while the control rabbits did not show such changes. The killed, somatic and flagellar antigens were explained the changes in cellular immune response by delayed hypersensitivity reaction and the results by SRID assay were 19.8, 17.8 and 26 mm in diameter, respectively, for the above mentioned antigens, while the control group did not show such changes. The concentration of total protein was appeared higher in rabbits which were immunized with killed antigen compared with other groups which reached to 87.3 g/L. The immunoglobulins (Ig) concentrations of rabbits primed with *E.coli* antigens were higher in treated groups compared with control group, but the treated group primed with somatic antigen was higher than other groups which reached to 2203, 326.9 and 577.5 mg/dL for IgG, IgM and IgA, respectively. The concentrations of complement components C₃ & C₄ were higher in treated groups primed with somatic antigen compared with other groups which reached to 266.5 and 60.4 mg/dL, respectively. These results suggest that the above antigens of EPEC were stimulated the humoral and cellular immune response.

الخلاصة

تم عزل بكتريا *E.coli* المعوية الممرضة النمط I من براز طفل رضيع بعمر ثمانية اشهر. شخصت البكتريا اعتماداً على الصفات المصلية، الكيمو حيوية والشكلية. عزل المستضد المقتول والمستضدين الجسمي والسوطي المعاملين بالفورمالين وتم مزجهم مع مساعد فرويند الكامل وغير الكامل (زيت زهرة الشمس) ومن ثم حقنت في الارانب المحلية (*Oryctolagus cuniculus*). اظهرت النتائج ان تأثير مستضدات بكتريا *E.coli* المقتولة والجسمية والسوطية على المناعة الخلطية والمعبر عنها بفطر الحساسية الانية قد بلغ قطرها بإعتماد فحص الانتشار المناعي الشعاعي المنفرد 16.6، 15.4 و 19.4 ملم على التوالي، في حين لم يلاحظ أي تغيرات في ارانب السيطرة. ان المستضدات المقتولة، الجسمية والسوطية والمعبر عنها بالتغيرات في الاستجابة المناعية الخلوية والمتمثلة بفطر الحساسية المتأخرة قد بلغ قطرها بإعتماد فحص SRID 19.8، 17.8 و 26 ملم على التوالي، في حين لم يلاحظ أي تغيرات في مجموعة السيطرة. كان تركيز البروتين الكلي عالياً في الارانب المنعة بالمستضد المقتول مقارنةً ببقية المجاميع، اذ بلغ ذلك التركيز 87.3 غم/لتر وان تراكيز الكلوبولينات المناعية في الارانب المنعة بمستضدات بكتريا *E.coli* كانت عالية مقارنةً مع مجموعة السيطرة، لكن المجموعة المعاملة بالمستضد الجسمي كان التركيز فيها عالياً مقارنةً بالمجاميع الاخرى، اذ بلغت التراكيز 2203، 326.9 و 577.5 ملغم/لتر للاضداد IgG، IgM و IgA على التوالي. كانت تراكيز مكوئي المتممات C₃ و C₄ عالية في الارانب المنعة بالمستضد الجسمي مقارنةً بالمجاميع الاخرى، اذ بلغت التراكيز 266.5 و 60.4 ملغم/لتر على التوالي. يعتقد ان هذه النتائج اظهرت ان المستضدات افقة الذكر قد حفزت الاستجابة المناعية الخلوية والخلطية.

Keyword: Humoral. Cellular. *E.coli*. Antigen

Introduction

The antigenicity of EPEC was complex, which composed of three types of antigens, the first is the heat-stable like which called somatic antigens or lipopolysaccharide (LPS)(7), the second type heat-labile like capsular antigen or killed antigen which found along the somatic antigen in some types of enterobacteriaceae, however, it composed of polysaccharide and proteins (32), while the third type is the flagellar antigen, in which there was more than 56 types of this antigen in EPEC strains

The immunoglobulin IgG act against somatic antigen and LPS of EPEC strains (21). When the EPEC penetrate the surface of interior epithelial cells, the mucosal immune system stimulated to secrete s-IgA against the pathogens in intestine and the production of IgG was increased systemically (36). In the first stages of life, the EPEC stimulate production of IgM that neutralized the LPS (24). The infection with EPEC primed the immune response and activated the complement in first exposure through alternative pathway starting with properdin Bb factor, and in the second exposure the classical pathway starting with C1 fractions till reaching the C3 fraction in the complete cascade (31). The present study was undertaken to determine the role of EPEC type I antigens in immune response of local rabbits.

Materials & Methods

1. Laboratory Animals

Forty mature native rabbits which have been brought from the local markets were used in this study. These animals were classified according to the information of natural history museum confirmed by opinion of (10). The rabbits were adapted to the animal house condition for two weeks before treatment and kept at *Libidum* through experimental periods (26). The weight of each animal was ranged between 2-2.5 Kg, and their ages ranged from 6-8 months.

(12). This bacterium capable of secreting many of toxins which has a higher virulence for host cells, for example LPS toxins were endotoxins that connected with bacterial cell wall, the strains of this pathogen produced many of enterotoxins which called verotoxins (8,28). The EPEC was adherent on the surface of epithelial cells which form a band of pilli lead to production of IgG which neutralized the toxin of this pathogen (25).

2. Isolation of EPEC

The EPEC was isolated from 8 months age of infant baby infected with diarrhoea, hospitalized in Hilla hospital for Pediatric and Maternity, according to (16).

3. Serological Diagnosis

The serotypes, O₁₅₈, O₁₄₂, O₁₁₄, O₄₄ and O₂₆ anticoli I (Sifin company, England), were used to diagnosis the EPEC.

4. Preparation of EPEC antigens

The killed, somatic and flagellar antigens of EPEC were prepared according to the (2)

5. Immunization protocol of rabbits

The rabbits were divided into four groups, ten replicates for each group. The first group was treated with killed antigen, while the second and third groups were treated with somatic and flagellar antigens, respectively. The control group was treated with physiological normal saline. The rabbits in the first group were injected four doses (1 ml for each) for two weeks intervals. The first dose which includes killed antigen mixed with Freund's complete adjuvant in ratio 1:1 and this dose was divided into four parts (0.25 ml each), and then each subdose was injected in the two sides

of the neck and both sides of grion region. The second dose (1 ml) was intraperitoneally injected once , and this dose contained killed antigen solution mixed with sunflower oil (SFO) as adjuvant in ratio 1:1. The third dose was similar to the first dose, but contained killed antigen and SFO. The fourth dose was similar to the second dose. The rabbits in the second and third groups were immunized in the similar procedure as in the first group (2).

6. Single Radical Immunodiffusion Assay

This test was used to determine the specificity and concentrations of immunoglobulins (IgA, IgM and IgG) and complement components C₃ and C₄ according to (18) and the informations of Biomaghreb company .

7. Cellular and Humoral Immune Response

The cell-mediated immunity (delayed type hypersensitivity) was performed according to the (3) while the humoral immune response (immediate hypersensitivity) was carried out according to (2).

8. Statistical Analysis

The results of the experiments were analyzed using completely randomized design (6).

Results

The immunization experiments were revealed that the mean of diameter reaction for immediate hypersensitivity (humoral immunity) in rabbits injected with killed, somatic and flagellar antigens were 16.6, 15.4 and 19.4 mm respectively (Fig.1).

The reaction diameters of delayed hypersensitivity reaction were recorded after 24 hours of immediate hypersensitivity, in which the median of diameter reaction were

19.8, 17.8 and 26 mm for killed, somatic and flagellar antigens, respectively (Fig. 2).

Table 1 showed the concentrations of total protein of treated and control groups. It was found that the medians were 87.3, 83.9 and 80.6 g/L in sera of rabbits which primed with killed, somatic and flagellar antigens, respectively.

The median of IgG concentrations of treated and control groups were reached to 1615.9, 2203 and 1422 mg/dL of rabbits primed with killed, somatic and flagellar antigens, respectively, while it was 847 mg/dL in control group.

The median of IgM concentrations were reached to 174.4, 326.9 and 249.8 mg/dL in sera of rabbits immunized with killed, somatic and flagellar antigens, respectively. The median of IgA concentrations were 292.7, 577.5 and 423.4 mg/dL in sera of rabbits primed with killed, somatic and flagellar antigens, respectively, while it was 207.8 mg/dL in control group (Table-2).

Fig. 3 showed the concentrations of complement component C₃, which reached 200.9, 266.5 and 248.5 mg/dL in sera of rabbits immunized with killed, somatic and flagellar antigens, respectively, while the concentration in control group was 110.9 mg/dL.

The concentrations of C₄ component were 44.4, 60.4 and 49.9 mg/d in sera of rabbits primed with the above mentioned antigens, while in control group it was 30.5 mg/dL (Fig.4).

Discussion

The flagellar antigen was more stimulated the immediate hypersensitivity reaction compared with killed and somatic antigens when injected in rabbits. (22) were reported that the TI-2 antigens didn't have properties of B-cell receptor, in which these antigens formed mainly as a result of repeated, polymer antigens such as LPS in bacterial cell wall or polymer proteins that formed bacterial flagellum which activated B-cells. These antigens are able to connect

with B-cell receptor molecules which lead to formation of specific antibodies inside the cells characterized by its long lived which regard as indicator of antibody response for TI-2 antigens.

A high significant difference ($P < 0.01$) was noted between immunoprimered and control groups, in addition to the flagellar antigen which was more effective than other antigens in stimulating delayed hypersensitivity (DTH) reaction. The DTH probably was required 48 hours to occur secondary changes after stimulating with antigens for circulating of helper T-cells (memory cells) and beginning release of lymphokines, attractant of macrophages and cytotoxic T-cells, furthermore, these cells mediated DTH reaction and activation of phagocytosis process inside the tissue (14).

Since the microbial antigens are able to stimulate the immune system and the serum protein represent the small part from body proteins, therefore, it was the uniprotein that can be studied (35). The injection of *Shigella* spp. antigens in mice and rats were stimulated the immune system and increased the serum protein concentrations (5).

Many of studies showed that the complete stimulation of immune system occurs during production of hyperimmune sera against microbial antigens (30). A significant difference in concentration of immunoglobulin class IgG between sera of treated and control groups, but the somatic antigen was more stimulator. (1) found that the somatic antigen connected to LPS was triggers for B-cell development, which lead to stimulate the production of antibodies class IgG that regards a direct antibody for commensal intestinal microorganisms or antibody specific for antigen produced as a result of infection with EPEC. (11) showed that the injection of white rats with LPS of EPEC serotype O₁₈ triggers B-cells for secreting IgG antibody.

The inoculation of mice with pathogenic factors of bacterial antigens lead to trigger of lymphopoiesis, and B-cell production, which capable of secreting IgM

antibody (20). The immunoglobulin IgM of rat was played a major role against LPS of EPEC serotype O₁₈ and was increased between 100-1000 times against polysaccharide antigens of RBC surfaces compared with the IgG antibody (15,33).

The somatic antigen was potent stimulator to immune response for secreting of IgA antibody. (17) reported that the immunization of mice with antigens derived from pathogenic microorganisms mixed with immune adjuvant was of increased specificity and production of immunoglobulin IgA.

Many studies showed increase in level of IgA when the concentrations of EPEC toxin injected in rabbits was higher and homologous for Shiga toxin (19). The somatic antigen and LPS of *Salmonella* stimulate the immune system for production of immunoglobulin IgA, which play a direct role against new antigens or commensal intestine bacteria (1).

The *E.coli* somatic antigen and LPS were able to stimulate complement system through two separating pathways, microorganisms and their cell wall components stimulated antibodies, furthermore, the action of complement system for killing of microorganism. Meanwhile, microorganisms and endotoxins were direct stimulator for alternative pathway (9). (29) were reported that the major role of complement in organization of immune response, furthermore, C₃ was a major component of complement found in circulating system that play a main role in activation of complement pathways because its capability for cleavage of their molecules.

(34) were found that the serotype O₉ of polysaccharide-O of *E.coli* play primary role in stimulating of lectin pathway which is similar for *Klebsiella* spp.

(4) were reported that the effects of specific antibodies for microorganism in immune system of mice model against peritoneal inflammation depending on complement system.

(27) were found that the activation of complement system and platelets for shock occurs by LPS of EPEC serotypes O8 and O9 and by reconnection of LPS which became more stronger than stimulating of immune system by LPS and K-12 for the same microorganisms.

(23) showed that the connection between mannose-binding lectin (MBL), MBL-mannose associated serine protease and LPS lead to activation of complement component C₄, furthermore, the LPS was capable to activate complement system, which in accordance with response of platelets for shock, in addition, there was a strong opinion that confirmed the responses of platelets for activation of complement system during the lectin pathway (13).

Thus, on conclusion, one may state:-

1. Immediate hypersensitivity, using the diameters of immediate hypersensitivity reaction to measure specific of immunoglobulin IgE level.
2. Delayed hypersensitivity, using the diameters of DTH reaction to measure specific of T-cells.
3. Levels of immunoglobulins concentrations, increase levels of IgG, IgM and IgA in primed groups compared with control group.
4. Levels of complement concentrations, increase levels of C₃ and C₄ components in primed groups compared with control group.

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Table 1 The concentrations of total protein of rabbits primed with EPEC antigens.

Antigen	Concentration (g/L) (Mean±standard error)
Control	65.5±2.29
Flagellar	80.6±1.47
Somatic	83.9±1.21
Killed	87.3±0.48

Table 2 The concentrations of IgG, IgM and IgA in sera of rabbits immunized with EPEC antigens.

Antigen	Concentration (mg/dL) (Mean±standard error)		
	IgG	IgM	IgA
Control	847±0.22	114.6±10.1	207.8±17.4
Flagellar	1422±77.5	249.8±12.5	423.4±16.2
Somatic	2203±79.7	326.9±16.0	577.5±19.4
Killed	1615.9±152.7	174.4±10.3	292.7±24.4

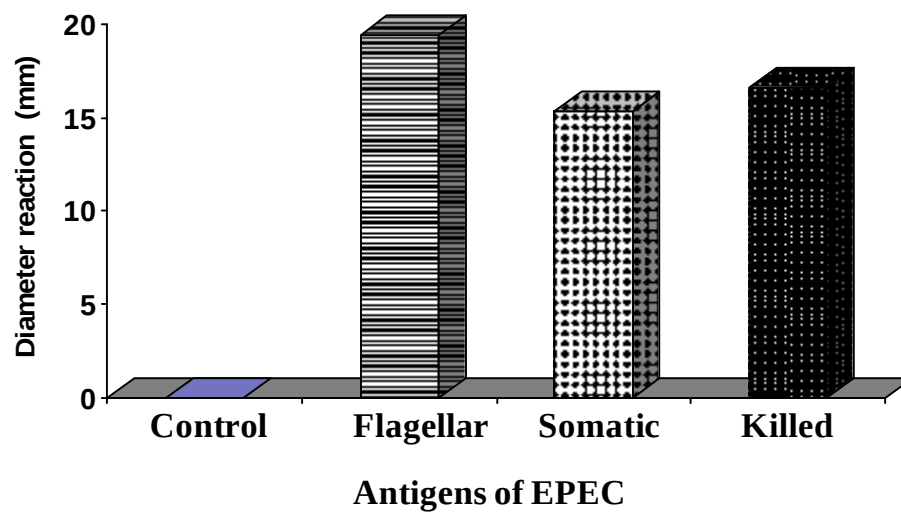


Fig. 1 The levels of specific humoral immunity of EPEC antigens which tested in skin, after 24 hours from injection of antigens.

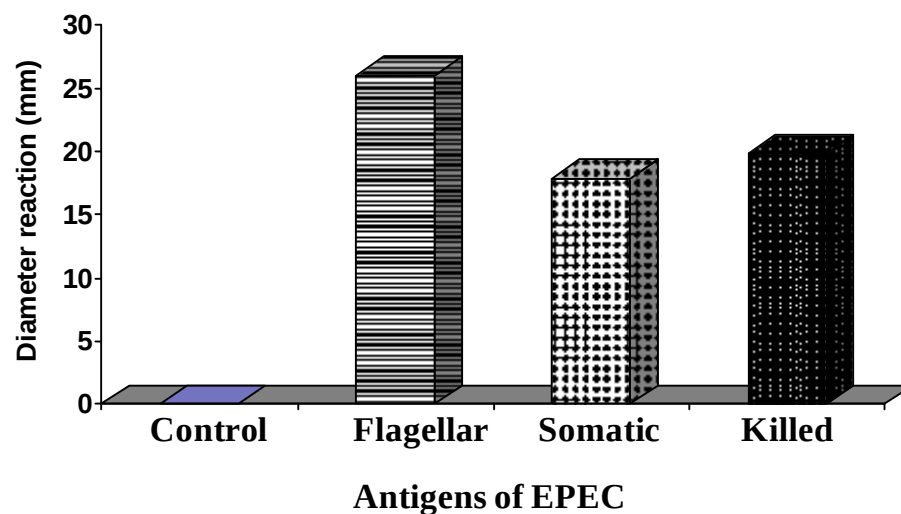


Fig. 2 The levels of cell-mediated immunity indicated with delayed hypersensitivity reaction, which tested in skin, after 48 hours from injection of antigens.

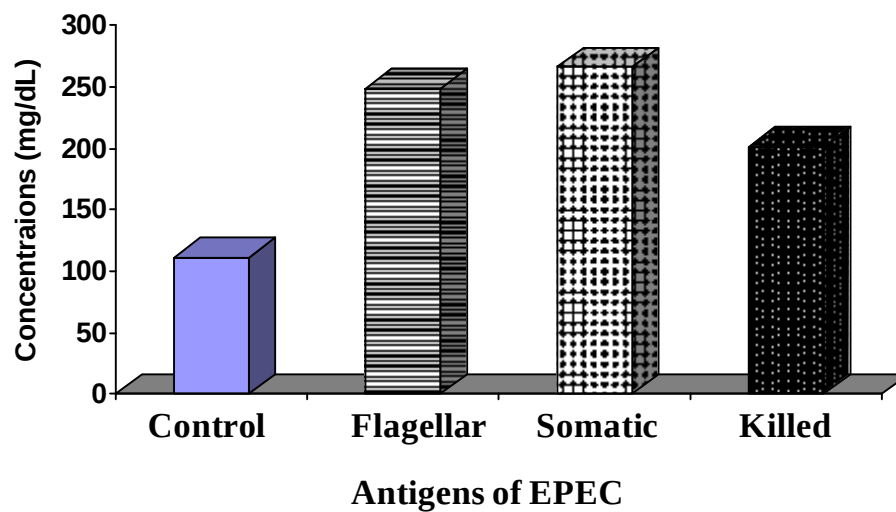


Fig. 3 The concentration of complement component C_3 in sera of rabbits immunized with EPEC antigens.

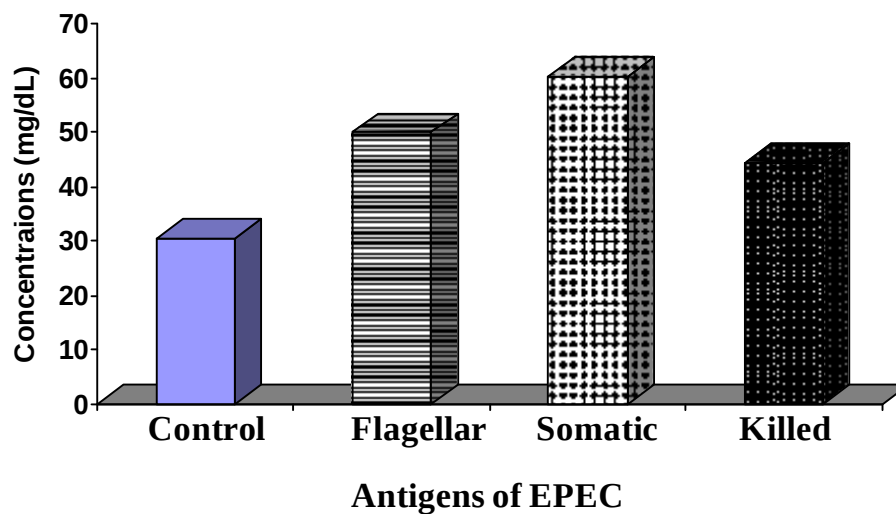


Fig. 4 The concentration of complement component C_4 in sera of rabbits immunized with EPEC antigens.