Pathological And Immunological Study On Infection With Escherichia Coli In ale BALB/c mice

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Abstract. Escherichia coli bacteria consider as one of the common responsible for the frequency and severity of infections that it causes hospitalized patients. E. coli simultaneously carries a harmful side in which only a slight genetic recombination can bring about highly pathogenic strain that most frequently causes the scourge of bacterial infections worldwide including sepsis, neonatal meningitis, pneumonia, bacteremia, and traveler’s diarrhea. This study was carried out to assessed Escherichia coli infection induced different Pathological and immunological. Following Escherichia coli isolation, identification and coupling, the lethal dose (LD-50) was determined before infection. Twenty-two mice were used in this study for 21 days infection, the animals were sacrificed at 3, 6, 9, 12, 15, 18 and 21 days, and tissues of different tissue were collected, examined for bacterial infection. Bacteria and mice Immunization and ELISA were used to detect immunoglobulin G level in serum as well. For histological study, different infected organs were used. The results indicated that the LH50 was 1×10⁹ cell; and all organs were infected after 3 days followed by decreased in infection level shown in brain at day 12, lung, kidney and intestine at day 15 and in liver, spleen and heart at day 21. Moreover, ELISA results revealed that concentration 1:200 of serum in positive and negative state and optimum concentration of Ag 1:40 dilution and compact dilution is 1:1000. In addition, diversity of histopathological alteration occurs in tissue on time-depended manner. This study concluded that the ability of activated E.coli to stimulate the intestinal secretory immune system of germ might result from a retardation of immunological maturity.

Keyword: Escherichia coli infection, histopathological in mice, immunological maturity

Introduction

Escherichia coli are a gram negative bacterium which is responsible for the frequency and severity of infections that it causes hospitalized patients. E. coli simultaneously carries a harmful side in which only a slight genetic recombination can bring about highly pathogenic strain that most frequently causes the scourge of bacterial infections worldwide including sepsis, neonatal meningitis, pneumonia, bacteremia, and traveler’s diarrhea [1, 2].

There are many physiological changes accrue during infection with EPEC such as transport of iron [3], inflammatory responses initiation, and increase in the cellular permeability [4]. These physiological events are similar to the changes accrue by EPEC of host signaling pathways, such as inositol phosphate fluxes [5], protein kinase C, mitogen-activated protein kinases [6], tyrosine kinase enzyme, myosin light-chain kinase [7], and NF-KB [8].

The motivation of some these lanes have been proven to the biological changes induction of EPEC. Though of achievement over the last 25 year for understand the EPEC illness, there is still needed more studies to comprehend the EPEC activation of biological changes. Earlier studies with different animal models have been reported to investigate host immune reactions such as modulated rabbits and mice [9, 10].
The diseases which are induced by REPEC in animal is comparable to EPEC causes infections [9] but, there are confines to the same model, like genetic and immunological difficulties. However, the present study was done to examine of *Escherichia coli* for BALB/c mice was used for animal immunological and histological studies.

**Material and methods**

**Isolation and identification of Bacteria**

In the present study, the samples of bacteria were collected aseptically in sterilized labeled containers from Al-Yarmook Hospital, Baghdad, Iraq, and were brought to the Microbiology Laboratory, Division of Biotechnology, Department of Applied Science, University of Technology. The *E. coli* were isolated by following standard protocol using sterile bacteriological media, including Nutrient agar and MacConky agar. The specimen were aseptically inoculated on the plates and incubated aerobically at 37 °C for 24 hours. The morphological investigation of Bacterial strains have been done using Gram's staining. In the other hand biochemical tests were also included such as, catalase, (MR-VP), gelatin liquefaction test, starch hydrolysis, nitrate reduction test, indole, motility, coagulase, urease, oxidase, TS.I sugar fermentation and bacterial counting as described by [11].

**Laboratory Animals**

Male BALB/ mice were the tested animals, which were 8 week old at the beginning of experiments, and their weight was 22 ± 0.5 gram. They had free excess to water and food during experiments. The mice were divided into 2 groups.

**Lethal Dose -50 (LD<sub>50</sub>)**

The lethal dose-50 was determined in mice before carrying infection efficacy experiments by injecting intraperitoneally (i.p.) different doses of viable *E. coli* suspended in phosphate-buffered saline (PBS). The doses were 1×10<sup>1</sup>, 1×10<sup>2</sup>, 1×10<sup>3</sup>, 1×10<sup>4</sup>, 1×10<sup>5</sup>, 1×10<sup>6</sup>, and 1×10<sup>7</sup> CFU per mouse. Five mice were utilized for each dose group. Another group of five mice were used as control and administered with PBS only. The living and dead mice were detected after 28 days for LD-50 and calculated using the formula as below:

\[
\text{Proportional distance} = \frac{50\% - (\text{Mortality at dilution next below})}{(\text{Mortality next above}) - (\text{Mortality next below})}
\]

**Infection of Mice**

Approximately 1 × 10<sup>7</sup> of *E. coli* cells in 250 μL of PBS were injected interperitonial into 33 mice. Control animals of 11 mice were received 250 μL of PBS. During the experimental time of treatment, animals were detected every 24 h for observing action and aquatic intake, and mass was measured as well. Following infection, the animals were sacrificed at 3, 6, 9, 12, 15, 18 and 21 days, and tissues of liver, spleen, kidney, lung, brain intestines and intraperiton were collected, examined for bacterial infection and processed for further analysis.

**Preparation of *E. coli* Ag for Immunization**

*E. coli* cells were inoculated into nutrient broth for 24 h at 37°C and then washed with PBS by centrifugation at 3000 rpm for 5 min at 4°C; and then re-suspended to the appropriate density in PBS. Bacterial count were done to verify the number of bacteria at 1×10<sup>7</sup> CFU/ml. Bacterial strain were killed by heating 60°C for 60 min. The sterility of antigens was tested before use according to [12]. Protein concentration has been measured by biuret protein assay according to standard protocol.
**Immunization of Mice**

The mice were divided equally into two groups, 10 mice of each. The group one was injected subcutaneously 2 times for two weeks with 250 μL containing 1x10^7 CFU/ml. Second group (control group) was injected subcutaneously with 250 μL of PBS. Serum were collected at 3, 6, 9, 12, 15, 18 and 21 days. Serum samples were stored at -20 °C until use for analysis by ELISA.

**Enzyme-Linked Immunosorbent Assay**

ELISA technique are used to measured the level of immunoglobulin G (IgG) in the serum as described techniques by [13]. 200 μl prepared as described above. Diluted 1.5 μg mL⁻¹ in carbonate bicarbonate buffer as coating antigen which kept overnight in refrigerator. Plates were washed three times with washing solution (PBS containing 0.1% Tween 20). Blocking solution 200 μl well⁻¹ was added (1% BSA in PBS), plates were incubated at 37 °C for 60 min. Then washed three times with washing solution. Diluted 100 μl of each serum samples 1:100 in 1% BSA was distributed into appreciate well, positive and negative control sera were added and then incubated at 37 °C for 60 minutes. Washing the plates three times, 100 μl of anti-IgG peroxidase conjugate diluted 1:1000 in diluents buffer containing 1% Bovine serum albumin were added to each well and plates were incubated at 37 °C for 60 minutes. Washing 3 times again and then add 100 ml of diluted OPD (1 tablet plus 75 ml deionized distilled water and immediately H₂O₂ 30% for each diluted OPD) incubated 20 minutes in dark place at room temperature. Absorbance values at 490 nm were measured using ELISA Reader.

**Histological study**

The histological examination selected organs of treated mice were washed with PBS, fixed in 10% formalin, followed by use of paraffin dispensing module EG 1150H (Leica, Germany), and embedded in paraffin. Sections were prepared by use of a microtome RM2255 (Leica, Germany) and followed by hematoxylin and eosin (H&E) staining. The processing and staining of sections were performed according to a standard procedure used in histopathological laboratories [14].

**Results**

The biochemical evaluation for *E. coli* showed that colonies growing in MacConkey agar have a ping color due to the presence of fermented lactose sugar and give a positive test in catalase and negative test in urease as shown in Table (1):

| Test                  | Result |
|-----------------------|--------|
| Gram stain            | Negative |
| Oxidase               | Negative |
| Catalase              | Positive |
| Urease                | Negative |
| Coagulase             | Positive |
| Hemolysin             | Positive |
| Fermentation mannitol | Negative |

**Lethal Dose-50**

The results of LD50 for bacteria *E. coli* in mice after injection subcutaneously with bacteria have proved that the LD50 is (1×10⁹ cells) as shown in table 2.

| Group | No. of cells | loose | Live | Accumulation of loose effect | Accumulation of living effect | Accumulation percentage | Percentage |
|-------|--------------|-------|------|------------------------------|------------------------------|-------------------------|------------|
| 1     | 10⁵²         | 4     | 0    | 12                           | 0                            | 12/12                   | 100        |
| 2     | 10⁴¹         | 3     | 1    | 8                            | 1                            | 8/9                     | 88.88      |
Infect ed organs with E. coli.  
After 21 days of infection with E. coli. It is noted that all organs of mouse were infected and reached to maximum levels at 3 days of infection. After that the bacterial infection was started to decrease and less infection was recorded in the brain at 12 days of infection as compared to other organs. Low level infection in lung, kidney and intestine were recorded at 15 days while, in liver, spleen and heart was at 21 days as given in Table (3):

| Day after infection | Liver     | spleen    | heart    | lung     | Kidney    | intestine | Intraperitoneal space | brain     |
|---------------------|-----------|-----------|----------|----------|-----------|-----------|-----------------------|-----------|
| 3                   | ++ +      | ++ +      | + + +    | ++ +     | ++ +      | ++ +      | ++ +                  | ++ +      |
| 6                   | ++ +      | ++ +      | + + +    | ++ +     | ++ +      | ++ +      | ++ +                  | ++ +      |
| 9                   | ++ -      | + - +     | + - +    | + - +    | + - +     | + - +     | + - +                 | + - +     |
| 12                  | + - -     | + - -     | + - -    | + - -    | + - -     | + - -     | + - -                 | + - -     |
| 15                  | 0/12      | 0/12      | 0/12     | 0/12     | 0/12      | 0/12      | 0/12                  | 0/12      |
| 18                  | 0/16      | 0/16      | 0/16     | 0/16     | 0/16      | 0/16      | 0/16                  | 0/16      |
| 21                  | 0/16      | 0/16      | 0/16     | 0/16     | 0/16      | 0/16      | 0/16                  | 0/16      |

Histopathological changes

Sections of infected organs were stained with haematoxylin and eosin and then evaluated for histological changes. The tested organs were liver, lung, spleen and kidney.

In the liver, infection with E. coli induces focus suspension and poly focus of neutrophiles, congestion of veins with micro abscess in tissue of liver especially at first days of infection. At second week, a severity infection was characterized by presence of inflammatory cells such as neutrophiles, lymphocyte and macrophage while at third week a signs of prominent central area of necrosis was seen in the infected sections but not in control sections 'Figure (1)'.

![Figure (1): Photograph of liver section showing micro abscess and necrosis in liver cells of infected mice with E. coli. (H&E stain; x 400).](image)
In spleen, increase in growth of white core of spleen tissue and contact between with other core and congestion in red core of spleen tissue and eccentric artery, in third week amyloidosis type sago spleen was seen in the infected sections. No abnormal changes were observed in control sections ‘Figure (2)’.

Figure (2): Photograph of spleen section showing amyloid in white core and sago spleen amyloidosis in cells of infected mice with *E. coli* (H&E stain; x 400).

As shown in ‘Figure (3)’, the pancreas of infected mice with *E. coli* notes suspension around pancreatic cell, pancreatic acini and congestion in vein of pancreatic. After three week later fibroblasts, lymphocyte, macrophages were observed in the pancreatic loop. No abnormal changes were observed in control sections ‘Figure (3)’.

Figure (3): Photograph of pancreatic section showing amyloid in pancreatic ascini and clot in vein of infected mice with *E. coli*. (H&E stain; x 400).

Histopathological examination of lung showed growth in lymphocyte behind tunic and amyloid with congestion in vein and loose size in interstitial pneumonia. No abnormal changes were observed in control sections ‘Figure (4)’.

Figure (4): Photograph of lung section notes inflammation and amyloid with lymphocyte of infected mice with *E. coli*. (H&E stain; x 400).
As shown in ‘Figure (5)’, the infected heart revealed amyloid with neutrophile and macrophage, lymphocyte. Congestion in vein with focal epicarditis and focal myocarditis was observed at infection in later state. Bacterial contamination was also recorded in lipid tissue surrounding the heart.

**Figure (5):** section in heart tissue notes myocarditis and amyloid in epicarditis X 400 (H& E).

The kidney: infection tissue of kidney effect interstitial nephritis and amyloid with macrophage and lymphocyte ‘Figure (6)’.

**Figure (6):** section in kidney tissue notes macrophage, amyloid and lymphocyte X 400 (H& E).

The intestine: infection accuse present of neutrophile in mucus of intestine that lead to congestion of vein in wall of intestine, advance infection macrophage, plasma cell and lymphocyte in mucus and secretion mucin ‘Figure (7)’.

**Figure (7):** section in kidney notes chronic catarrhal enteritis and mucin in intestine X 400 (H& E).
The brain: congestion in vein and loss white blood cell around perivascular cuffing lymphocyte in all tissue of brain, with focal gliosis and different change 'Figure (8)'.

![Figure (8): section in brain tissue notes congestion in vein and perivascular cuffing lymphocyte X 400 (H&E).](image1)

The peritoneum: notes congestion in vein after infection and present macrophage and lymphocyte 'Figure(9)'.advance infection notes growth in fibroblast and mononuclear cells that lead to chronic peritonitis.

![Figure (9): section in peritoneum notes inflammatory in peritoneum and macrophage ,lymphocyte X 400 (H&E).](image2)

**ELISA result**

To detect level of immune response against *E.coli* in detect time table to differential optimum dilution between positive and negative control and detect optimum quantity of Ag we found that concentration 1:200 of serum in positive and negative state and optimum concentration of Ag 1:40 dilution and compact dilution is 1:1000 Table (4).

**Table (4) : ELISA results for IgG level.**

| Groupe | Day | Maximum | Minimum | Average |
|-------|-----|---------|---------|---------|
| 1     | 3   | 0.313   | 0.201   | 0.257   |
| 2     | 6   | 0.379   | 0.302   | 0.340   |
| 3     | 9   | 0.743   | 0.624   | 0.683   |
| 4     | 12  | 1.021   | 0.942   | 0.981   |
| 5     | 15  | 1.034   | 1.023   | 1.028   |
| 6     | 18  | 0.808   | 0.979   | 0.893   |
| 7     | 21  | 0.706   | 0.892   | 0.799   |
Discussion:

The results of present study improved that immunization of male mice by *E. coli* results in activation of immunity in treated group compared with control. These results is in contract with a study of [15] which reported mice immunity stimulation with two doses of the same bacteria vaccines containing O157 or O157:H7; K99 to antibodies in serum. Other study has been reported different immune response parameters such as lymphocyte numbers and spleen weight where the infection with *E. coli* increase the spleen weight and number of lymphocytes after infected with 36x 106 CFU/ml [16]. Other studies have been demonstrated that continuous injection of *E. coli* increases number of immune cells such as intestinal monocytes when we compare the number produced through infection with a live *E. coli* [17].

Several studies have been reported the LD50 dose in mice; for example, Tzong et al. [18] informed that dose of 250µg subcutaneous administrated was sub-toxic to mice, while control PE was toxic at 0.5 and 1µg. Moreover, Armstrong and Merrill [19] have reported the inhibition of protein synthesis by activation of ADP on factor 2 and the lethal dose was at 1µg in *in vivo* and *in vitro* study. Susan et al., [20] documented that toxin had median lethal in mice at i.p injection dose of 0.15µg /22 g. However, Mohawk et al., [21] reported that there is no one reviews all *E. coli* O157:H7 infection features, and many respected mouse prototypes have been established to authorization examination of *E. coli* O157:H7 pathogenesis and disease can be measured.

Histopathological investigation in administrated mice in this study reveal that toxin had a marked effects on different tissues characterize by the presence of inflammatory cells such as neutrophils, lymphocyte and macrophage. Vulcano et al., [22] investigated the histopathological and immunological alteration in mice infected with *E. coli* and suggested that EPEC activates immune action and intestinal histological changes with instruction to use mice as an appropriate model for the proposal and experimental of immuno-biological products for active or passive immunization. Not many *in vivo* researches have studied the differences between *E. coli* improved from patients with renal clinical signs and patients with signs of pyelonephritis. One of the mechanism is that *E. coli* has LPS in its wall, which is consider as a possible elements that generate an inflammatory reaction by motivating cytokine producing inflammatory mediators such as interleukins, TNF-α and CAM.[23]. While LPS stimulation through Toll-Like Receptor-4 (TLR-4) causing cytokines production [24]. Thus in view of the B-cell mitogenicity of bacterial lipopolysaccharide it may well be the Gram-negative component of the intestinal flora which most significantly effects expansion of immunocytes and thereby plays a more fundamental role in the activation, differentiation and maturation of the local immune system [25]. Furthermore, Ogawa et al. [26] have reported that STEC infected infant rabbit model shown intragastric epithelial changes including; vacuolation in epithelial cells, detached and necrosis due to increase growth of STEC; cecum and colon epithelial changes were exfoliation of epithelial cells, infiltration of immune cell and mitotic activity. Other studies have been demonstrated that continuous injection of *E. coli* increases number of immune cells such as intestinal monocytes when we compare the number produced through infection with a live *E. coli* [17]. Additionally, higher IgG level in mucosa in cattle has been observed by Martorelli et al., [27] in response to *E. coli* O157 where the level of IgA was low in serum. Hoffman et al. [28] explanation was that production of Stx2 by *E. coli*O157:H7 throughout infection time might diminish lympho-proliferative responses in intra-gastric epithelial cells and peripheral lymphocytes. It would be interesting if further examination of other protein response in serum and mucosa study.

This finding suggests a relationship between immunization, immune response and histology. Nevertheless, further studies are necessary to investigate the role of antibodies and cellular immunity in the *E. coli* infection. In addition, immune responses improvement can be proposed to control *E. coli* affects.

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