Effect of LPS Extracted from *Campylobacter coli* on Lymphocyte Transformation

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Abstract

Lipopolysaccharide (LPS) of *Campylobacter coli* was extracted by using digestive enzyme and hot phenol method. The effect of LPS on lymphocyte transform was studies by lymphocyte transformation index for twenty blood samples were collected from apparently healthy control.
The results were showed significant differences ($P< 0.05$) between samples which treatrd with phytohemagglutnin PHA (66.1 ± 0.6) and the samples which treated with LPS of *C*. coli (74.2 ± 0.8) when compared with control, this lead to suggest that the LPS extracted from *C. coli* may play a role as a mitogen to transformed lymphocytes.

Key word: LPS, LPS and *Campylobacter*, gram negative bacteria.

تأثير عديد السكريد الشحمي المستخلص من بكتيريا *Campylobacter coli* على التحول اللمفاوي.

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الخلاصة

استخلص عديد السكريد الشحمي العائد لبكتريا Campylobacter coli بطريقة الفينول الحار والانزيمات الهاضمة ودرس تأثير مستخلص عديد السكريد الشحمي على تحول الخلايا اللمفاوية من خلال قياس معامل تحول الخلايا اللمفاوية لـ 20 عينة دم جمعت من اشخاص اصحاء .أظهرت النتائج بان هنالك فروقات معنوية في معامل التحول للخلايا اللمفاوية بين عينات الدم المعاملة بالمشطر PHA مقارنة بعينات الدم المعاملة بمستخلص عديد السكريد الشحمي والسيطرة . وهذا يدل على أن عديد السكريد الشحمي المستخلص من بكتيريا *C. coli* ربما يلعب دوراً كمشطّر للخلايا اللمفاوية.

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Introduction

Campylobacters are small, spirally curved, Gram-negative rods with a polar flagellum at one or both ends of the cell and the cells are highly motile [1]. They are catalase and oxidase positive, and urease negative. Campylobacters are microaerophilic requiring an oxygen concentration of 3±15% and carbon dioxide concentration of 3±5%. The temperature range for growth of the thermophilic *Campylobacter* species *C. jejuni* and *C. coli* is 34 ± 44°C, with an optimal temperature of 42°C, which probably reacts an adaptation to the intestines of warm-blooded birds [2]. *Campylobacter* spp. is fastidious organisms that require complex growth media and are unable to ferment carbohydrates [3], it caused gastroente-ritis in humans, and diarrhea which considerably less frequently than *C. jejuni* [4], the human pathogens *C. jejuni* and *Campylobacter coli* are causative agents of acute human enterocolitis, and are the most common cause of food-borne diarrhea in many industrialized countries [5].

Lipopolysaccharide was the major component of gram negative outer membrane and are important virulence factor involved in serum resistance indotoxicity and adhesion [6]. The LPS consisted of three distinct regions, lipid A moiety which was anchored in the outer membrane and is the endotoxic part of the LPS molecule. Other is the core, which is attached to the lipid, and at last is the O antigen attached to the outer core. The LPS molecules of Campylobacter are involved in adherence and play a role in antigenic variations, as Campylobacter has the ability to shift the LPS antigenic composition. Surprisingly N-acetyl neuraminic acid (sialic acid) is present in the core oligosaccharide, not frequently found in prokaryotes. These sialic acid residues appeared like gangliosides in structure, when attached to – D galactosidase. This molecular mimicry is involved in the neuropathological autoimmune diseases like Guillains Barre' Syndrome and Miller-Fisher Syndrome which eventually leads to the death of the patient [7]. LPS-activated macrophages secrete a variety of important cytokines such as Interlui number 1(IL-1), Tumor Necrosis Factor (TNF), and lymphotoxin, which play roles in host defense [8, 9], also increased immunoglobulin secretion by certain B cell lines [10]. LPS function has been under experimental research for several years due to its role in activating many transcription factors. LPS challenge also produces many types of mediators involved in septic shock. Humans are much more sensitive to LPS than other animals (e.g., mice). A dose of 1 µg/kg induces shock in humans, but mice will tolerate a dose up to a thousand times higher [11]. This may relate to differences in the level of circulating natural antibodies between the two species. LPS causes an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes which leads to IL-10 production by monocytes after binding of program death–1 (PD-1) by program death–L (PD-L) [12]. In other hand some study refer to effect of other bacteria LPS on lymphocyte cells which used differentiated human monocytic cell line T-lymphocyte hiper 1 (THP-1) cells to study the interaction of *C. jejuni* with human macrophages, and indicating that during infection *C. jejuni* induces apoptosis of differentiated THP-1 cells [13], for this reason our research was designed to study mitogenic effect of *C. coli* LPS on lymphocyte.

Material and Methods

1. **Preparation of bacteria solution**

   Campylobacter coli bacteria was isolated from diarrhea samples was collected from Al-Yarmok hospital and was inoculated on blood agar which containing %5 sheep blood cells and supplements, incubated plates for 48 hr. at 42C° under micro aerobic conditions by using candle jar .The isolated colonies were identified by shape of these colonies and by biochemical tests according to Garrity [14].

2. **Extraction of partial purified LPS of *C. coli*.**

   The LPS was extracted and partially purified according to Johnson and Perry [15] by digestive enzymes and hot phenol method and purification by gel filtration chromatography by using sephadex G-200 as shown in figure 1.

1. Study of lymphocyte transformation. This test was done according to Shubber et.al [16], by using RPMI-media, PHA as a mitogen.

- Blood Samples preparation: Two milliliter of twenty blood samples of apparently healthy persons were used directly to evaluate lymphocyte transformation and collected in heparinized silicon test tube (Heparin concentration was 50 IU/ml) to prevent the absorption of cells on glass tubes.
- Lymphocyte transformation test:
  In the assessment of this test, three cultures were set up for each subject, culture 1 including mixing 2.5 ml of RPMI-1640 medium, 0.1 ml of LPS (100 µg/ml), 0.2 ml of blood. Culture II was similar to culture I components which include 0.1 ml PHA crude instead of LPS, while culture III as control. The three cultures were incubated at 37 ℃ for 72 hours, and after that they were processed with a hypotonic solution 0.07 M KCl and obtained cells were fixed (3 parts absolute methanol and 1 part of glacial acetic acid), and then 4 – 5 drops of the cell suspension were dropped on clean slide, then the slide was air-dried at room temperature, then the slide was stained with Giemsa stain for 15 minutes and rinsed with distilled water. The slide was examined under oil emersion lines (100X), and at least 100 cells were examined, and the percentage of blast and dividing cell was recorded according to Shubber *etal* [16].

Statistical analysis

Data are expressed as mean ± SE, T-test was used to determine the differences between the groups by using the computer program SPSS version 13.0, statistical significant was considered at $P \leq 0.05$.

Results and discussions

In present study we found that there are significant differences ($P< 0.05$) between samples which cultured with PHA and the samples which cultured with LPS of *C. coli* (that summarized in table 1) when compared with control, this lead to suggest that the LPS extracted from *C. coli* may play a role as a mitogen to transform lymphocytes Groups as in table 2. The result of *C. coli* lipopolysaccharide purification by gel filtration was shown in figure 1.

Table 1- Biochemical test and characterization of *C. coli*.

Test	Result
Colony on blood agar	**Appear small convert grey white colony**
Gram stain	**Negative (Gram negative bacteria)**
Oxidase	**Positive**
Hiturate	**Hydrolysis negative**
Motility	**Motile**
Shape under microscope	**Spiral shape**

Table 2- Lymphocyte transformation of blood cells treated with 100 µg/ml LPS extracted from *C. coli.*

Parameter	Culture I Treated with LPS (1)	Culture II treated with PHA (2)	Culture III Control (3)	Probability
Lymph- transfer index	74.2 ±0.8	66.1 ± 0.6	48.3 ± 0.7	1 *vs* 2 (P<0.05) 1*vs* 3 (P<0.05) 2 *vs* 3 (P<0.05)

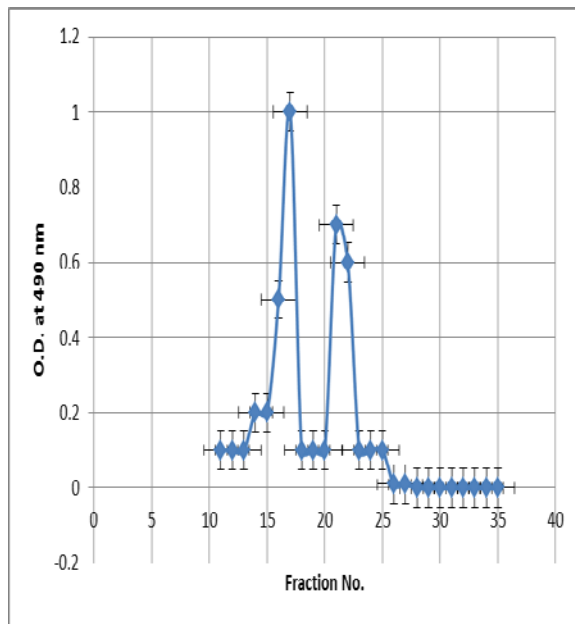

Figure 1- Purification of LPS by gel filtration (sephadex G-200)

Other studies were analyze the nature of the effect of bacteria lipopolysaccharide (LPS) on antibody production in vitro, they obtained the biological action of LPS *in vitro* may be predominantly manifested on the function of B lymphocytes or T lymphocytes depending on the conditions employed. In the absence of antigen, LPS appears to act primarily on B lymphocytes. In the presence of antigen, however, the LPS *of Campylobacter* significantly influence as helper T-cell [17]. The activation of lymphocyte by *Campylobacter jejuni* lipopolysaccharide lower than that induced by *Escherichia coli* lipopolysaccharide, which means that the LPS of gram negative bacteria had different effect on mitogensis of lymphocyte, and increased of IL-6 by stimulation with *C. jejuni*, this is probably due to its unusual acylation and phosphorylation pattern of lipid A[18]. Bacterial LPS have many biological activities and effect on cellular immune responses and immune cells activities [19]. The extraction of *Campylobacter coli* LPS showed B-cells activation and production of antibodies [20].

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