Vol. 1, No. 2: 26-28.

WORLD JOURNAL OF EXERIMENTAL BIOSCIENCES

Research article

Lipopolysaccharide antigenic relationship between *Campylobacter jejuni* and *Vibrio cholerae* (NAG)

Ghadah Mohammed Saleh[1*], Dhuha S Salih[1]

ABSTRACT

Lipopolysaccharide was extracted from *Campylobacter jejuni* and *Vibrio cholerae* (NAG) by EDTA-heating method. Anti-sera against both LPS were raised in rabbit. Passive hemagglutination was used to check the LPS-antigenic relationship between both bacterial species. The results showed that there is no cross reaction relationship between LPS of *C. jejuni* and LPS of *V. cholerae*.

Keywords: *Campylobacter jejuni*, cross reaction, Lipopolysaccharide, *Vibrio cholerae*.

Citation: Saleh GM, Salih DS (2013). Lipopolysaccharide antigenic relationship between *Campylobacter jejuni* and *Vibrio cholerae* (NAG). *World J Exp Biosci* **1**: 26-28.

Received July 2, 2013; Accepted August 5, 2013; Published August 8, 2013.

INTRODUCTION

Campylobacter jejuni is generally considered commensals of livestock, domestic pet animals and birds. *C. jejuni* is the main cause of human bacterial intestinal disease identified in many industrial countries [1]. The infection with *C. jejuni* is associated with acute enteritis and abdominal pain lasting for 7 days or more [2]. Many species of *Vibrio* that caused diarrhea can grow in thiosulfate citrate, bile salts, sucrose agar as yellow colonies and do not agglutinate with *V. cholerae* O1 and O139 anti-sera [3]. These species are broadly defined as non-agglutinating (NAG) vibrios [4]. *V cholerae* has been responsible for several cholera outbreaks in developing countries [3].

Lipopolysaccharide (LPS) is the main outer membrane component of gram negative bacteria which constitutes about 75% of the surface [5] and 5–10% of the total dry weight of gram negative bacteria [6]. Their basic structure consists of three parts: lipid A, core oligosaccharide and repetitive polysaccharide designated as “O” antigen. Lipid A is highly conserved and exerts the endotoxic act-

*Correspondence: flagellin2013@gmail.com
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Full list of author information is available at the end of the article

ivity, while the "O" antigen carbohydrate chain is a polymer of repeating oligosaccharides, which differs between species and is responsible for the serological specificity of bacteria [7]. LPS causes pathophysiological effects such as fever, leucopenia, leucocytosis and Shwartzman reactivity [8]. It plays an important role with temperature to modulate the inflammatory immune response [9].
The present study aimed to find the LPS-antigenic relationship between two species that mainly responsible for diarrhea in our country (Iraq).

MATERIALS AND METHODS

Bacterial strains

Clinical isolates of *C. jejuni* and *V. cholerae* were procured from department of Biology, college of science university of Baghdad, Baghdad, Iraq. The isolated was stored in lyophilized tubes at -20 and re-cultured in nutrient broth before using in experiment.

LPS purification

The *C. jejuni* and *V. cholerae* LPS was extracted by EDTA method that mentioned clearly in previous study [10]. The LPS was purified by running the extract material through separose-4B gel.

Anti-LPS preparation

Clinical isolates of *C. jejuni* and *V. cholerae* were cultured into nutrient broth for overnight. The bacterial growth was harvested and washed three times with phosphate buffer saline (PBS, 0.1 M, pH 7). The bacterial count was adjusted to 10^{10} cell/ml. Rabbits were immunized with bacterial suspension according to standard previous method [11].

Preparation of sensitized sheep red blood cells

The standard method of Zgair [12] was followed to coat sheep red blood cells (SRBCs) with either *C. jejuni* LPS or *V. cholerae* LPS.

Hemagglutination technique

Serial dilutions of complement inactive anti-LPS (1/10, 1/20 up to 1/1280) were prepared in circle bottom microtiter plate (50 µl in each well). Fifty micro-liter of sensitized SRBCs (1%) was added to each well. The microtiter late was incubated for 37 ^{o}C at room temperature after mixing gently. The red clear well bottom indicates the positive results, while the dot at the well bottom was indicated for negative result. The titer of each anti-sera was the last positive dilution in each raw. Negative control (PBS and unimmunized rabbit serum) was used. The presence of cross reaction was dependent on the threshold value for agglutination reaction.

Statistical analysis

Data are expressed as mean±SD. The following equation was followed to calculate the threshold value [threshold value = mean + 2(standard deviation)].

RESULTS

The calculation of threshold value for anti-*C. jejuni* showed that the threshold value for anti-*C. jejuni* was 31.1 and 19.4. this finding depict the values of threshold was higher than the titer of hemagglutination of anti-*C. jejuni* and LPS- *V. cholerae*. That proved there was no cross reaction between anti-*C. jejuni and V. cholerae* LPS. Similar observation was found in case of hemagglutination between anti-*V. cholerae* and *C. jejuni* LPS (**Table 1**).

Table 1. The hemagglutination titers of between anti-bacteria (*C. jejuni* and anti *V. cholerae*) and SRBCs that coated with bacterial LPS (*C. jejuni* and *V. cholerae*).

	Anti-*C. jejuni*	**Anti-*V. cholerae***
LPS-*C. jejuni*	1024-256	64-8
LPS-*V. cholerae*	16-8	512-256

Discussion

Previous study showed the immunological relationship between the LPS of *Campylobacter* and 11 members of Enterobacteriaceae, and *Vibrio* [13]. In current study, we try to find the relationship between the anti-sera against whole cells of studied bacteria (*V. cholerae* (NAG) and *C. jejuni*) and LPS prepared from *V. cholerae* (NAG) and *C. jejuni*. We found that no cross reaction between prepared LPS that coated on SRBCs and anti-sera.
Previous study on LPS that prepared from *C. jejuni* showed the structure of lipid A composed of GlcN3N that carried two groups of phosphate in location 1 and 4 [14] and some fatty acid such as octadecenoic acid and cyclopropane acid [15]. Moreover, presence a compound in core region called Neu5ac [16]. The lateral chains composed of two kinds of polysaccharide one is low molecular weight and another high molecular weight. *C. jejuni* LPS differs from *V. cholerae* LPS. The last one composes of phosphorylethanolamine in the lipid a position and high amount of fructose [17]. The LPS of *V. cholerae* characterized with low lateral chain and composed of different kinds of sugar molecules. Thus, structurally the LPS of *C. jejuni* differ from LPS of *V. cholerae* that explain there is no immunological relation ship (cross reaction)

between the both LPS and bacteria. Not only LPS can stimulate the immune system in host other antigen like flagellin can also stimulate the immune system [18]. LPS stimulates the immune system strongly, and that happens through T and B cells. The activation of both cells especially T cells may produce autoimmune phenomena [19]. We suggested used other antigens to study the immunological relationship between two genuses. This work is going on in our laboratories.

It can be concluded that according to LPS immunological relationship, there is no immunological relation ship between *C. jejuni* and *V. cholerae*.

Conflict of interest

The author declares that he has no conflict of interests.

REFERENCES

[1] **Tauxe RV.** (1992) Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In *Campylobacter jejuni: current state and future trends*, pp. 9-19. Edited by I Nachamkin, MJ Blaser, LS Tompkins. ASM Press, Washington DC, USA.

[2] **Skirrow MB, Blaser MJ.** (2000) Clinical aspects of Campylobacter infection. In *Campylobacter jejuni: current state and future trends*. Edited by Edited by I Nachamkin, MJ Blaser, LS Tompkins. ASM Press, Washington DC, USA.

[3] **Dutta D, Chowdhury G, Pazhani GP, Guin S, Dutta S, et al.** (2013) *Vibrio cholerae* Non-O1, Non-O139 Serogroups and Cholera-like Diarrhea, Kolkata, India. *Emerg Infect Diseases* **19**: 464-467.

[4] **Chowdhury G, Pazhani GP, Dutta D, Guin S, Dutta S, et al.** (2012) *Vibrio fluvialis* in Patients with Diarrhea, Kolkata, India. *Emerg Infect Diseases* **18**: 1868-1871.

[5] **Rietschel ET, Brade H.** (1992) Bacterial endotoxins. *Sci Am*. **267**:54–61.

[6] **Shnyra A, Luchi M, Morrison DC.** (2000) Preparation of endotoxin from pathogenic gram negative bacteria. In *Methods in Molecular Medicine, pp.* 13–25*.* Edited by, TJ Evans. Vol. 36: Humana press.

[7] **Erridge C, Bennett-Guerrero E, Poxton IR.** (2002) Structure and function of lipopolysaccharides. *Microbes Infect* **4**: 837–851.

[8] Luderitz O, Galanos Ch, Lehmann V, Nurminen M, Rietschel ET, et al. (1973) Lipid A: Chemical structure and biological activity. *J Infect Dis* 128(Suppl l):17–29.

[9] **Hassan SA, Zgair AK. (2013)** Thermoregulation of IL-1α production and phagocytic activity of *Escherichia coli* Lipopolysaccharide-induced mononuclear cells. *World J Exp Biosci* **1**: 14-18.

[10] **Chandan V, Fraser ADE, Brooks BW, Yamazaki H.** (1994) Simple extraction of Campylobacter lipopolysaccharide and protein antigens and production of their antibodies in egg yolk. *Int J Food Microbiol* 22: 189–200.

[11] **Bryner Jh.** (1985) Animal model for testing infectivity and immunity in Campylobacter spp. In *Campylobacter* III 39. Edited by AD Pearson et al. Public health laboratory service, London.

[12] **Zgair AK.** (2013) Involvement of (IgG and IgM)-secreting B lymphocytes in severity of autoimmune hepatitis type 1. *Med Microbiol Immunol* **202**:229–237.

[13] **Perez-perez GI, Hopkins JA, Blaser MJ.** (1986) Lipopolysaccharide Structures in Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Vibrio cholerae* Are Immunologically Related to *Campylobacter* spp. *Infect Immun* 51: 204-208.

[14] **Moran AP.** (1997) Structure and conserved characterization of *Campylobacter jejuni* Lipopolysaccharide. *J Infect Dis* **176** (Suppl. 2): S115-S121.

[15] **Blaster MJ, Moss CW, Weaver RE.** (1980) cellular fatty acid composition of *Campylobacter fetus*. *J Clin Microbiol* **11**: 448-541.

[16] **Fry BN, Korolik V.** (1998) The lipopolysaccharide biosynthesis locus of *Campylobacter jejuni* 81116. Microbiol **144** (Pt 8):2049-61.

[17] **Hisatsune K, Kondo S, Igughi T, Machida M, Asou S, et al.** (1982) Sugar Composition of Lipopolysaccharides of Family Vibrionaceae. *Microbiol Immunol* **26**: 649–664.

[18] **Zgair, AK.** (2013) Role of *Stenotrophomonas* flagella in bacterial adhesion on human epithelial cells. *World J Exp Biosci* **1:** 19-21.

[19] **Salih DS, Zgair AK, AL-khyat RMH.** (2013) T-Lymphocytes Subsets in Patients with chronic hepatitis that showed autoimmune immune phenomenon. *World J Exp Biosci* **1**: 10-13.

Author affiliation:

1. *Department of Biology, College of Science, University of Baghdad. Baghdad, Iraq*