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Immunomodulatory Activity of Lipopolysaccharide LPS Isolated From ECOLI

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ABSTRACT

Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria such as Escherichia coli, plays a significant role in modulating the host immune response. The present study investigates the immunomodulatory activity of LPS isolated from *E. coli* and its effects on immune cell activation. LPS was extracted and purified using standard isolation techniques and characterized through biochemical analysis. The immunomodulatory potential of the isolated LPS was evaluated by assessing its ability to stimulate cytokine production, macrophage activation, and lymphocyte proliferation. Experimental results demonstrated that LPS significantly enhanced the secretion of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), indicating activation of innate immune responses. Furthermore, LPS treatment promoted macrophage phagocytic activity and induced the expression of immune-related surface markers. The interaction of LPS with Toll-like receptor 4 (TLR4) triggered intracellular signaling pathways, leading to the activation of nuclear factor-kappa B (NF-κB) and subsequent immune regulation. These findings suggest that *E. coli*-derived LPS possesses potent immunostimulatory properties and can effectively modulate both innate and adaptive immune responses. The study highlights the importance of bacterial LPS as a valuable tool for understanding host-pathogen interactions and its potential application in vaccine adjuvant development and immunotherapeutic strategies.

Keywords: Lipopolysaccharide (LPS), *Escherichia coli*, Immunomodulatory Activity, Cytokines

1. INTRODUCTION

Lipopolysaccharide (LPS) is the main constituent of the outer leaflet of the outer membrane in Gram-negative bacteria (Vaara, M. (1999)), and many LPS are highly toxic (endotoxins) in mammals (Redl et al and Suffredini, A. F et al). For the bacteria the outer membrane represents the first line of defense against e.g. antibiotics or bile salts, and therefore the integrity of the outer membrane is of prime importance for the survival of bacteria and as such is a target for the developmentof new antibacterial drugs. LPS from wild-type enterobacteria is made up of an O-antigen, the core-region, and lipid A (Holst, O. (1999)). The structural features of LPS necessary for a functioning outer membrane are not well understood. However, conserved structural features of LPS have been recognized such as the lipid A moiety, the presence of heptose and Kdo in the inner core region, and the presence of conserved phosphate groups that result in a high number of negative charges at the surface of

the membrane. It can be speculated that the chemical structure of the conserved parts of the LPS molecule has evolved to support specific functions of the outer membrane.

Thus, the number and position of negative charges may be modulated by masking groups such as 2-aminoethanol or 4-amino-arabinose leading to enhanced resistance against polycationic antibiotics, e.g. polymyxin B (Vaara, M. (1999)). Experimental evidence has been obtained recently showing that certain LPS structural motifs are recognized by outer membrane proteins (Omp) and that LPS plays a role for the correct folding and activity of proteins, such as PhoE (De Cock et al), OmpT (Kramer, R. A., et al), and FhuA (Ferguson, A. D., et al and Ferguson, A. D., et al). The isolation and detailed structural characterization of all different glycoforms present in LPS from Escherichia coli J-5 aided the interpretation of results from in vitro folding experiments of outer membrane proteins (De Cock et al and Kramer, R. A., et al) and furthermore gave an insight into the biosynthesis of LPS (MüllerLoennies, et al). Whereas the presence and distribution of negative charges is undoubtedly very important, the highly conserved nature of carbohydrates that build the inner core region implies that they are particularly suited to fulfill certain functions. In order to better understand the structural requirements of LPS for a functioning outer membrane and compensatory mechanisms upon mutations, it is necessary to obtain a detailed knowledge about the molecular composition of LPS.

Serological and structural features of the LPS from E. coli K12 have been investigated earlier and led to the classification as a separate core type (Holst, O. (1999) et al). Characteristic features are the occurrence of heptose in the outer (Holst, O.et al) and rhamnose in the inner core (Holst, O., and Brade, H. (1990)), the latter being associated with the immunodominant antigen (Mayer, H., et al). LPS consists of several different molecular species, and early studies indicated the presence of structurally heterogeneous LPS in E. coli K12 (Mayer, H., et al). E. coli K12 strains are widely used as hosts in molecular biology and were therefore the first used to elucidate the genetic details of LPS biosynthesis (Schnaitman, C. A. et al, Klena, J. D., et al, Pradel, E., et al, Parker, C. T.,et al , Austin, E. A., etal, Mäkelä, H. P.,etal Heinrichs, D. E., et al and Raetz, C. R., et al). Although many details of LPS biosynthesis in E. coli K12 are known, there still remain open questions. During the course of this study, Frirdich et al. (2003) raised the question whether the structure of the inner core in LPS has an influence on the biosynthesis of the outer core. Overexpression of the gene product of the waaZ gene performed in their study led to increased amounts of an α-Kdo-(2→4)-α-Kdo-(2→4)-α-Kdo trisaccharide in the inner core that was accompanied by a truncation of the outer core. At this stage, it remained unanswered whether the gene waaZ encoded a Kdo transferase that specifically transferred the third Kdo residue onto the common α-(2→4)-Kdo-disaccharide inner core structure, which is known to be generated by a single bifunctional enzyme (Müller-Loennies, S., et al). It also remained an open question whether the truncation of the outer core was secondary because of the overexpression of the WaaZ gene product. The gene responsible for the transfer of rhamnose to the inner core of E. coli K12 has yet to be identified.

2. REVIEW OF LITERATURE

Koscová H et al: Polysaccharide and lipid A are responsible for the wideranging pharmacological activity of bacterial lipopolysaccharides (LPS). The alterations in LPS

structure result in various effects on different functions of the target cells. The effects of LPS substructures, the polysaccharide (P) and lipid A (L) from E. coli on the innate mechanisms of human leucocytes were examined and compared in this study. Incubation of leucocytes with LPS and L and P analogues (1 and 100 microg/ml) enhanced their biological activity in dependence on their structure. These results showed that LPS was a less active immunomodulator of leucocytes than L and P analogues isolated from E. coli strains adapted to antimicrobial agents.

R L Duncan Jr et al: Lipopolysaccharides (LPS) have been isolated from culture supernatants and from cell lysates after the in vitro phagocytosis of E. coli by murine macrophages. By using E. coli radiolabeled specifically in the LPS component with [3H] galactose, our studies have shown that the macrophage-"processed" LPS is enhanced with respect to its immunostimulatory activity in comparison with control phenol-water extracted LPS. As assessed by its ability to induce interleukin 1 production in naive macrophages or proliferation in cultures of murine splenocytes, the macrophage-processed LPS is between 10- and 100-fold greater in specific activity. Evidence is presented for both structural and chemical alterations in the LPS macromolecule.

Ramezan Ali Ataee1 et al: The purpose of this study was to isolate and purify lipopolysaccharide (LPS) from A. tumefaciens and E. coli and compare its ability to produce nitric oxide and TNF- a in peritoneal mice macrophages. We isolated and purified LPS from A. tumefaciens and E. coli. The endotoxin activity of LPS extracted from A. tumefaciens and E. coli were examined via the Limulus Ambocyte Lysate Test (LALT). The effects of different concentrations of lipopolysaccharides were assayed on mice macrophages as stimuli to produce nitric oxide and TNF- a production. Then, they were measured by Griess and Enzyme Linked Immunosorbent Assay respectively. Data were analysed by SPSS version 19.0. Using the Westphal method LPS can be isolated from both aforementioned Gram-negative bacteria. The results suggest that the quantity of extraction and purification of LPS from A. tumefaciens and E. coli was dependent on culture volumes; 5 to 10 mg of LPS can be obtained from 1 liter of 24-hour culture respectively. The results indicate that the stimulating effects of 500 ng/ml LPS concentration extracted from E. coli has the same effect as 1000 ng/ml concentration of LPS A. tumefaciens. E. coli LPS was more effective in stimulating production of TNF-a and to produce nitric oxide. The findings of this study suggest that the effect of 1000 ng LPS from A. tumefaciens was equal to 500 ng LPS from E. coli in stimulating macrophages to produce nitric oxide. This demonstrated that the immunomodulatory effect with less toxicity.

Opdahl H et al: Polymorphonuclear neutrophil granulocytes (PMN) may contribute to the lung injury induced by no pulmonary infections with gram-negative bacteria. The direct effect of E. coli lipopolysaccharide (LPS) on isolated human PMN or mixed leukocytes (ML), as well as the priming effect of preincubating cells with LPS, was examined in assays measuring the maximal rate of oxygen consumption (OC), cell chemiluminescence (CHML), and aggregation (AGG). LPS, 1-10 micrograms/ml, caused no acute response in PMN or ML suspended in Fisher's-HEPES medium with BSA (FHA), but increased both CHML and AGG of cells suspended in autologous plasma. Preincubation in FHA with LPS, 1 microgram/ml, for more than 15 min increased the OC of PMN activated with zymosan-activated plasma

(ZAP) or n-formyl-methionyl-leu-cyl-phenylalanine (FMLP) by more than 100%. A similar increase in the CHML of such cells was seen after FMLP, but not after ZAP. ZAP, however, primed the CHML response of the cells to subsequent activation with FMLP more than did preincubation with LPS. Previous exposure to both agents had an additive effect. Preincubation of PMN with LPS decreased the time interval from addition of phorbol myristate acetate (PMA) to peak OC response, but less so than previous activation with FMLP. Neither agent affected the maximal rate of OC after addition of PMA. LPS also increased the PMN aggregation induced by ZAP and FMLP, but not by PMA. Cells preincubated with LPS, 0.01 microgram/ml, increased their CHML in response to FMLP if suspended in Krebs-Ringer balanced salt solution, but not if suspended in FHA. Such preincubation had no effect on OC of similarly activated cells in any of the media.

3. MATERIAL AND METHODS

Instruments

1 Autoclave
2 hat air oven
3 Incubater
4 Centrifuge
5 Laminar air flow Chamber
6 Spectrophotometer
7 Weighing balance
8 Vernier calliper

Chemicals Used

1 Nutrient Broth	=	2.6 mg
(2) BPS-P^H	=	7 (6-15m)
(3) $Cacl_2$	=	3.15mm
(4)$Mgcl_2$	=	0.5mm
(5)Proteinas K	=	(100 µg /m2)
(6)R Nase	=	(40 µg /ml)
(7)D Nase	=	(20µg/ml)
(8) MgSo4	=	1 µl/ml 20%
(9) Chloroform	=	4 µl/ml
(10) Volume of not phenol	=	(65-70^{0C}) 90%
(11) polypropylene	=	1.5ml
(12) Sodium acetate	=	0.5m
(13)Ethanol	=	95%
(14) Normal saline	= 1%	
(15) Na_2CO_3	=	10mg
(16) Indian Ink		

Determination of phagocytic function: carbon clearance

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To evaluate the phagocytic activity reticulo-endothelial system in – vivo, a carbon clearance test will be performed after completion of the drug pretreatment. On day 29, the treated rats received on intravenous injection of carbon suspension (1:50 dilution of Indian ink, came) in a dose of 0.5ml/100g. body weight blood will be withdrawn from the retro- orbital venous plerus before injection of the carbon suspension 0.05ml of blood will be measured as the granulopectic index, calculated by the formula.

$$20g(odo)\ loy(odt)\ /t$$

Where od_o is the od at omins & odt is the od at time.

Indirect haemagglutination test (humoral immunity)

- The control group received as vehicle;
- Animals in the treatment groups will be given the test extracts (100 and 200 mg/kg,p.o) in 1.0% sodium methyl cellulose daily for 7 days.
- The animals will be immunized by injection 0.1 ml of 20% of fresh sheep red blood cells suspension intraperitoneally on 0 day.
- Blood samples will be collected in micro centrifuge tubes from individual animal by retro-orbital plexus on 7th day to obtain serum.
- Antibody levels will be determined by haemagglutination technique.
- Briefly, equal volumes of individual serum samples of each group will be pooled.
- Two-fold dilutions of pooled serum samples will be made in 25 ul volumes oe normal saline in microtitration plate and to it added 25ul of 1% suspension of sheep red blood cells in saline. After mixing, the plates will be incubated at room temperature for 1 hr and examined for
- Haemagglutination under microscope.
- The minimum volumes of serum required to produce haemagglutination will be noted and expressed as HA titre.

4. RESULTS

Table 1:

Treatment	Phagocytic indent
Vehicle (1mg/kg)	0.0090#0.0025
LPS(200mg/kg)	0.013,0.012.0.014
LPS(200mg/kg)	0.072,0.076,0.087
LPS(200mg/kg)	0.084,0.089

Table 2: CYCLOPHPSPHAMIDE (CYP) TEST

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Animals Group (Rats)	Weight of Animals	Entract	Dose mg/kg	Index
Group1	25.5gm	%saline	1ml/kg	0.0103
Group2	28gm	LPS	200mg/kg	0.0266
Group3	24.5gm	LPS	200mg/kg	0.0452
Group4	25.5gm	LPS	2ml/kg	0.0276

TABLE 3: carbon clearance test

Animals Group (Rats)	Weight of Animals	Entract	Dose mg/kg	Index
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5. DISCUSSION

Experimental study

Mice have been used in present study. The advantages for using mouse model are lower management efforts and lower cost than other animal.Photo (6): Mild infiltration of

neutrophils between acini (a) & simple blood vessels congestion (b) (in LPS group, 72 hrs post challenged. (H & E X 40).

Humoral immune response

Passive haemagglutination test (PHA) was revealed higher mean of antibody titer in LPS group. A comparative reading of table (1) is showing the antibody titer in LPS groups was increased significantly than in control group, therefore we concluded that LPS has a pivotal role in enhancement of antibody producing as reported by (AlAmery MA et al). LPS was an excellent mitogen for B-lymphocyte as well as activated macrophage to secrete IL-1 which in turn enhancement Th2 to release IL-4 and IL-5 to provoke Blymphocyte to proliferation and differentiation to plasma cell and producing antibodies (Melief CJ.).

Cellular Immune response

All tests of cellular immune response were revealed that LPS as a potent antigen which stimulated immune response. In delayed type hypersensitivity skin test, Table (2) was showed the mean thickness of skin of padfoot in LPS group which increased significantly after 48 hrs than others, this result was similar to reports by (Al-Hakeem AF.) and (Al-Amery MA.). DTH test depend on ability and activity of Tdh cells to recognize antigen and secrete IL-1 which enhanced proliferation and differeniation of other T-cell into Th-cells which secrete IL-2 as a chemoatractive factor to attract macrophage around the area of activated T-cell which also secrete INF- that enhancing the cytolytic activity of accumulated macrophages leading into skin thickness (Rosenthal KS, et al). MIF indices in table (3) were showed that antigens with different concentration were revealed responsiveness reaction when compared with standard means of migration that applicated by (Bures J et. Al.), and that was in agreement with many reports which appeared that macrophage migration inhibition indices could be induced by different type of antigens as (Habasha F G,);(Yousif AA.) and (Al-Samarrae EAA.). We recorded that concentrated LPS was inducing higher migration inhibition which in turn lead to best lowest and significant MIF index than other in immunized groups and these result was in similarity to concluded reports of (Al-JobouryKHZ.) and (Al-Amery MA.) which they showed that LPS could be induced higher responsiveness of MIF index as a good cellular immune response stimulator. LPS has a particular ability to stimulate anterior pituitary gland to release migration inhibitor factor as well as activeated T-cell and macrophage which in resulting to give a significant MIF index than other antigens (Doenberg SB.). Mouse mastitis has been induced in control group by direct injection of E.coli O111:B4 into mammary gland (R4,L4) with ultrafine needle which allowed for safe penetration into gland without noticeable damage to the mammary gland of mice. The best protective status with less significant changes in parameters of challenged mice was performed by LPS which induce higher humoral and cellular immune response, thereby prophylactic dose of LPS reduced isolated E.coli count with variant degrees of clinical signs (non to mild illness)was observed within only 24hrs post challenge and the mammary weight index approximate as in negative control group with microscopic lesions were limited by mild neutrophils infiltration, blood vessels congestion and no changes were noticeable in infected mammary gland after 7 days post inoculation. All results were refered to the important role of toxic dose of LPS of E.coli to induce infection which conducted in control group. Elucidation of mechanism by which E.coli induce mastitis in mouse as that E.coli after inoclated to mice it grown. The reduction

in number of E.coli in mice of LPS group was occurred due to that CD14-LPS complexes activate epithelial cells of mice by binding to Toll like receptor –4 (Toll-LR4) on epithelial cells, lead them to secrete IL-8 (a potent chemoattractant of neutrophils) and in resultant early recruitment of neutrophils is crucial to clearance of E.coli from the mice (WangY,).

6. CONCLUSION

Lipopolysaccharide (LPS) is the major and an integral part of the outer membrane of the cell wall of gramnegative bacteria, and by weight LPS makes up about 10 % of cell wall of gram –negative bacteria and that a single E.coli cell contains approximately 3.5 ×106 LPS molecules(Marshall JC, et al). Mice were selected in this study as a model to induce E.coli mastitis and sttudying the role of purified LPS in performed the protection against induce E.coli infection of mammary glands in mice. After LD50 of purified antigen were determined (250 µg/ml of purified LPS). The study was reported that LPS was possessed high and significant efficacy and potent ability to rise the humoral and cellular immune response and reduced significantly the severity of mammary gland infection in mouse with lowest histopathological change in mammary tissue when compared with control group as a good role of LPS in reduced severity of mammary gland infection when compared with control group.

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