WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 14-23. Research Article ISSN 2277 – 7105

THE POSTCHALLEMGE IMMUNE PROTECTION MEDIATED BY SMOOTH LIPOPOLYSACCHRIDE OF VIBRIO FLUVIALIS IN A MOUES MODEL

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Article Received on
06 July 2014,

Revised on 30 July 2014,
Accepted on 24 August 2014

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ABSTRACT

From V.fluvialis smooth form culture a lipopolysacchride LPS was prepared and purified into the limits of partial purity as F1 and F2.These F1 and F2 were assessed for mitogenicity, immunogenicity and immune protectivity in a mouse model.They were mitogenic through skin induration and bone marrow lymphocyte blatogenicity, rise up of V.fluvialis specific antibody titres, cytokine activator for IL4,IL6,and TNF alpha causing postchallenge cytokine imbalance between proinflammatory IL6 and anti-inflammatory IL4 and immune protective via survivors record. Thus, V. fluvialis SLPS F1&F2 were mitogenic, immunogenic and postchallenge immune protective in mouse model.

KEY WORDS; Vibrio, Smooth, culture, LPS, mitogenicity, immunogenicity Postchallenge, immune protection.

INTRODUCTION

The immune response of mice to an immunogen(Any) may be assorted into:Immnogenic non-protective, immunogenic protective, toleragenic and/or immunosuppressive(1,2,3).The smooth form of LPS, fraction 1 and fraction 2(2) that are derived from V.fluvialis, to which class of the mentioned immunogens we can assign them. Knowing that gram negative LPS preparations have been documented as Mitogenic, polyclonal B lymphocyte activators, cytokine network initiators(4, 5). What lags behind these informations. If crude S-LPS is extracted, puriefied into frctions. Could different fractions of LPS give different immune

potentials. Thus, the present work was aimed at reporting the nature of immune protectivity mediated by fraction F1 and F2 of V.fluvialis, S-LPS in a postchallenge mouse model.

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MATERIALS AND METHODS

Bacterial Strains

Vibrio fluvialis was obtained from Advance genetic engineering lab. Department of biology Faculty of Science, University of Babylon. The diagnosiswas confirmed by biochemical tests.

Method of LPS Isolation:- LPS Extraction according to [6].

Methods For In *Vivo* Mitogenicity

In mice :-Twenty mice, their weight (20-25gm)divided into four groups each one of groups included 5 mice.

Group I- (*V.fluvialis* F1) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse in pad at dose 0.2 ml / mouse .

Group II - (*V.fluvialis* F2) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse in pad at dose 0.2 ml / mouse.

Group III and IV :- were positive and negative group respectivlly.

Blastogenicity Assay In *Vivo*

the indurations were measured18 hrs post injection. To stop cell cycle, 100mg/ ml cholchicine in a rate of 0.25 ml per each animal was injected intramuscularly. One hour later, femur bone was tremmed from both ends and 5 ml of sterile saline injected for bone marrow collection. Thick bone marrow smears were made and Giemsa stained for each animal [7].

Immunization Protocol

Twenty mice, their weight (20-25gm) divided into four groups each one of groups included 5 mice.

Group I- (*V.fluvialis* F1) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group II-(*V.fluvialis* F2)Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days booster dose left for two week then blood was collected.

Group III and IV :- were positive and negative group respectivlly.

Post immunization challenge:- Twenty mice, their weight (20-25gm)divided into four groups each one of groups included 5 mice :

Group I-(*V.fluvialis* F1)Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose ,after that challenge by *V.fluvialis* 1×10^8 at 27days

Group II- (*V.fluvialis* F2)Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse s/c at dose 0.2 ml / mouse at day zero, then repeated after 15 days gave booster dose ,after that challenge by *V.fluvialis* 1×10^8 at 27days.
Group V and VI :- were positive and negative group respectively.

Antibody Assessment [8]

Bacterin Preparation: Heat killed bacterin from *V.fluvialis* fresh cultures were made as suspensions and subjected to heat treatment at 100C° for 1 hour then bacterin densities were adsjuted using 0.5 McFarland tube.

Microtiteration Direct Bacterial Agglutination Test: The anti LPS specific antibody titer were assessed through microtiteration bacterial agglutination assay between *A.hydrophila* and *V.fluvialis* heat killed bacterins with decimal double dilution of the sera of immunized mice.

Cytokine Assessment: Interleukin-4, Interleukin-6 and TNFα Assay Procedure, The following procedure is performed at room temperature according to manufacturer's instructions (Boster´s –Korea).

RESULTS

1-Mitogenicity

The mitogenicity of LPS in *V. fluvailis* in LPS immunized mice was detected through skin induration and blastogenicity for both fraction 1and fraction 2.The skin indurations were

2.05±0.1 ,2.4±0.06 for fraction 1 and fraction 2 LPS respectively .While blastogenicity were 5.050±0.6 , 4.375±0.7 for fraction 1 and fraction 2 LPS accordingly .Table (1).

Table (1) :- The Mitogenicity of F1 , F2 LPS *V. Fluvailis*in Mice Through Skin Induration And Blastogenicity Percent.

Test modulant	Skin induration	blastogenicity
***V.fluvialis* F1 LPS**	2.05±0.1	5.050±0.6
***V.fluvialis* F2 LPS**	2.4±0.06	4.375±0.7
Control tuberculin 0.05 IU Size 0.1	**4**	3.50

Table (2): Humoral Immune Response Immunogenicity

Direct Microtitration Test: Immunized mice groups(5mice from each group) were rising humoral antibody response , The antibody titers were upto 64 in the group immunized with *V. fluvailis* (table: 2).

Table (2): The Antibody Titers Specific For *V. Fluvailis* In *V. Fluvailis*lps Immunized Mice.

Animal group	Titer	Animal group	Titer
F1	64	F2	**64**
	64		**64**
	64		**64**
	64		**64**
	64		**64**
Mean ±SE	64±0.000	Mean ±SE	64±0.000

Table (3) Cytokine Profile: Cytokine profile of *V.fluvialis* LPS immunized mice as compared to IL_4,IL-6 and TNFα .IL-4 concentrations were 57.5996±1.097 , 29.6184±2.538 for both fraction 1 and 2 respectively. While IL_6 concentrations were 313.7008±22.413, 245.6059±8.248for both fraction 1 and 2 accordingly. TNFα concentrations were 55.1572±3.790, 106.9910±2.016 for both fraction 1 and 2 respectively. table (3).

Table (3) :- Cytokine Profile of *V. Fluvialis* Immunized Mice.

Group	IL_4	IL_6	TNF
F1 LPS	57.5996±1.097	313.7008±22.413	55.1572±3.790
F2 LPs	29.6184±2.538	245.6059±8.248	106.9910±2.016
Control	3±0.1	1.3±0.1	

Immune Protection :- Table 4 Shows The Rate Of Protection.

V.fluvialis	Live percentage	Death percentage
Fraction 1	100%	0%
Fraction 2	100%	0%

DISCUSSION

The postchallenge immune protectivity in a mouse model could be taken with opinion of being a collective multiple immune mechanisms that may covered, several immune functions like; mitogenic ablity, B lymohocyte initiating ablity, T lymphocyte helper and /or regulator functions in an up and down pathways through activation/inhibition of the cytokine network as well as the postchallenge survivors percentages[1,2,3]

The mitogenic[8,9],immunogenic[17,18] and the immune oprtective potentials of the smooth V. fluvialis LPS F1 and F2 are in agreement with workers on other gram negative pathogens. [19-29].

B lymphocytes are usually triggered by high molecular weight substances with repeating units, such as LPS [8]. The mitogenic effects of LPS are related to the lipid A moiety of the molecule. LPS exhibits mitogenic influences and induces polyclonal activation of murine B lymphocytes as it enables B lymphocytes to differentiate and multiply , expand then produce antibodies [9].

During a Gram-negative bacterial infection, bacteria, in accordance with their normal growth and Cell division, such microenvironment renders these bacteria Aminable to shed LPS together with other cell components thereby making them available for recognition by antigen presenting cells like, macrophages and neutrophils. LPS associates with the LPS binding protein (LBP) in the bloodstream or at cell membranes [10], which subsequently assist the transfer of LPS to CD14,a glycosylphosphatidylinositol (GPI) linked protein expressed on the cell surface of phagocytes [11]. Furthermore, LPS is convyed to MD-2, which associates with the extracellular portion of TLR4, followed through the formation of a homodimer and initiation of the signaling pathways by transducing the signal to the intracellular TIR domain leading to recruitment of adapter molecules [12]. There are four adaptor molecules, MyD-88 (myeloid differentiation factor protein 88), TIRAP (TIR domain-containing adaptor protein) or MAL (MyD88-adaptor-like), TRIF (TIR domain-containing adaptor inducing interferon-β) and TRAM (TRIF-related adaptor molecule)[13]. The elective usage of these adaptor molecules by distinct TLR ligands mediates different signaling pathways. The activation of

TLR4 triggers two distinct signaling pathways, the MyD88-dependent, leading to the production of pro-inflammatory cytokines like IL6 and the MyD88-independent, mainly involved in the production of interferons type.1 [14]. In the MyD88-dependent pathway, MyD88 associates with the cytoplasmic signaling domain of TLR4 and recruits IL-1 receptor-associated kinase 4 (IRAK4) and IRAK1 through the N-terminal death domain of the protein [15]. Phosphorylation of IRAK4 initiates the phosphorylation of IRAK1 which subsequently associates with TRAF6 (tumour necrosis factor receptor-associated factor 6) initiating further phosphorylation and ubiquitination of several cytosolic signal proteins, including TAK1 (TGFβ- activated kinase 1) and MKK6 (mitogen-activated protein kinase 6). These then modulate the activation of transcription factors e.g. NF-ƙB inducing the expression of pro-inflammatory cytokines such as TNF-α or IL-6 [13].The events suggest a parallelism between mitogenicity assessment through lictin –like LPS skin test and T-lymphocyte potency [16]. V.fluvialis F1 F2 were mitogenic in mouse model as the were mitogenic in bird and rat models[18].

Microtitration direct bacterial agglutination test was revealed higher mean of antibody titer in immunized mice with *V.fluvialis* LPS which showed higher antibody titer, Table (3) was showing the antibody titer in immunized mice with *V.fluvialis* LPS are increased , therefore we concluded that LPS has a pivotal role in enhancement of antibody producing as reported by[19].

All cytokine profile of *V.fluvialis* in immunized mice were revealed that LPS as a potent immunogenic antigen which stimulated immune response with an intrinsic immunologic adjuvanicity which can lead to enhancement of immune response and at the same time can immunosuppresed the cells which affecting their function might be through the action of T reg. subset cells [20] . IL-4 has been shown to act as a growth factor for activated T lymphocytes, thymocytes, natural killer lymphocytes, and B lymphoctes.[21] IL-4 can enhance specific cytotoxic T lymphocyte (CTL) activity [22] but suppresses IL-2-induced lymphokine activated killer (LAK) cell ac t i v i t y [23] . IL. -4 induces the expression of class II major histocompatibility complex (MHC) antigens on normal and malignant B cells [24],[25]. For a guinewine understanding of the molecular mechanisms through which cytokines such as IL-4 inhibit the production of TNF-a and IL-12 may lead to new ways to treat diseases like rheumatoid arthritis[26]. During an infection, LPS stimulates the production of multiple cytokines including TNF-a, IL-12, and IL-18. This production of IL-12 and IL-18 in turn

leads to the production of IFN-g by NK cells [27]. The regulation of TNF-a production is complex and occurs at the level of transcription and message stability and is suppressed by IL-4, IL-10, or IL-13 [29]. As is the case for TNF-a, the production of IL-12 is greatly enhanced by IFN-g and is suppressed by IL-4, IL-10, or IL-13 [29].Thus ,V.fluvialis triggers humoral and cellular immune responses.

V.fluvialis S-LPS fractions F1 and F2 were proved to be immunoprotective Tables 1-4 ,in contrast to the partial immune protection mediated by A.hydrophila S-LPS F1 and F2 (28). Studies have been done on the gram negative LPS of Rhizobium and V,cholerae have shown that there were structural heterogeneity and microheterogeniety in the polysaccharide chains of the LPS[30,31].Following the theme of, these heterogeneities the current study proved that there were functional heterogeneity between V. fluvialis and A hydrophila LPS fractions in concern with cytokine imbalance that were evident in the former and lacked in the later[17,28]. The V.fluvialis LPS postchallenge immune protectivity in mouse model Tables 1-4 is layed upon a sort of balance between the potency of the host immune defence mechanisms and the pathogen virulence vajor.From this balance three cases may be identified ;First the host holds the position of out /or,equate the weight of the pathogen ablity a position of complete survivor record,hence, immune protectivity is assigned .Second ,the host defence mechanisms were slightely deficient so that there were percent deaths ,thus partial immune protection is identified.While the third case showed down weight of the host defence mechanisms against out weight of the pathogen potentials a state of nonprotective condition is delinated. V .fluvialis LPS offers complete immune protectivity with an evident cytokine im balance state between IL6 and IL4[32].

CONCLUSIONS

1-The F1 and F2 S-LPS recovered from V.fluvialis were,mitogenic ,immunogenic ,and immunoprotective in mice.

2-F1 and F2 induces cytokine imbalance between IL6 and IL4.

3-The functional heterogeneity were noted in higher immune potency of F2 over F1.

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