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Research Article

THE POSTCHALLENGE PARTIAL IMMUNE PROTECTION OF SMOOTH AEROMONAS HYDROPHILA LIPOPOLYSACCHARIDE IN A MOUSE MODEL

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ABSTRACT

The smooth lipopolysaccharide preparations of Aeromonas hydrophila, the human and animal gut pathogen were previously extracted and partially purified as fraction 1(F1) and fraction 2 (F2). F1 and F2 were assessed for: Mitogenicity, immunogenicity and immune protectivity. F1 and F2 were mitogenic in mouse food pad model giving skin induration and bone marrow lymphocyte blastogenicity, rise up of humoral antibody response specific for A.hydrophila, cytokine network activator IL4,IL6 and TNF alpha, and partially immune protective through death percentages. Thus, the smooth F1, F2, LPS of A. hydrophila were mitogenic, immunogenic and partially immune protective in mouse model.

Key Words: Cytokine network, humoral response, mitogenicity, protection lipopolysacchride.

INTRODUTION

The immune response to an immunogen can be as an immunogenic but non immune protective and be immunogenic, immune protective as well[1].The smooth lipopolysaccharide as an immunogen derived from Aeromonas hydophila ,the human and animal gut pathogen[2,3], in small mammalian model to which class immunogen mentioned above can we put them. Knowing that gram negative smooth LPS were; Mitogenic, polyclonal B lymphocyte activator cytokine network activator[4] and what remains to be mentioned if crude smooth LPS purified and fractionated, could different fractions give different immune potentials. The objective of the present work was to report on the nature of the immune protectivity mediated by two fractions of the smooth A.hydrophila LPS in postchallenge mouse model.

MATERIALS AND METHODS

Bacterial strains:- *A.hydrophila* was obtained from Advance genetic engineering lab. Department of Biology Faculty of Science, University of Babylon. The isolate identification was confirmed biochemical tests.

Method of LPS isolation:- LPS Extraction according to Westphal et al.[5] **partial purification in accordance with** Boyer RF[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 - (*A.hydrophila* F1) Included 5 mice which inoculated with LPS as 2.5mcg/gm of mouse in pad at dose 0.2 ml / mouse.

Group II - (*A.hydrophila* 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 respectively.

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 tremed 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- (*A.hydrophila* 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- (*A.hydrophila* 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 IIIandIV: - were positive and negative group respectively.

Post immunization challenge: - Twenty mice, their weight (20-25gm) divided into six groups each one of groups included 5 mice:
Group I- (*A.hydrophila* 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 *A.hydrophila* 5×10^8 at 27days.
Group II- (*A.hydrophila* 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 *A.hydrophila* 5×10^8 at 27days .
Group III and IV: - were positive and negative group respectively.

Antibody assessment[8]:-
Bacterin preparation: Heat killed bacterin from *A.hydrophila* fresh cultures were made as suspensions and subjected to heat treatment at 100C° for 1 hour then bacterin densities were adjusted 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* heat killed bacterins with decimal double dilution of the sera of immunized mice.

Cytokine assessment:-Interleukin-4, Interleukin-6 and TNFα Assay Procedure according to manufacturer's instructions (Boster´s –Korea).

RESULTS

Mitogenicity:- The mitogenicity of *A.hydrophila* LPS in mice was assessed through skin induration and blastogenicity for both fraction 1and fraction 2.The skin indurations were 1.95±0.4 ,1.87±0.4 for *A.hydrophila* fraction 1 and fraction 2 LPS respectively .While blastogenicities were 3.975±0.8 , 2.55±0.6 for *A.hydrophila* fraction 1 and fraction 2 LPS accordingly. Table (1).

Table 1
The mitogenicity of F1 , F2 LPS *A. hydrophila* in mice through skin induration and blastogenicity percentages.

Test modulant	Skin induration	blastogenicity
A.hydrophila F1 LPS	1.95± 0.4	3.975± 0.8
A.hydrophila F2 LPS	1.87 ± 0.4	2.55± 0.6
Control tuberculin 0.05 IU Size 0.1	4	3.50

Immunogenicity
Humoral immune response
Direct microtitration test:- Immunized mice groups (5mice from each group) were rising humoral antibody response , The antibody titers were upto (35 ± 7.8) group immunized with *A. hydrophila* LPS (table 2).

Table 2
The antibody titers specific for *A. hydrophila* in *A.hydrophila*Lps immunized mice.

Animal group	Titer	Animal group	Titer
F1	32	F2	32
	16		32
	32		16
	64		64
	32		32
Mean ±SE	35 ± 7.8	Mean ±SE	35 ± 7.8

Cytokine profile:- *A.hydrophila* LPS immunized mice were subjected to determination of IL_4, IL-6 and TNFα. IL-4 concentrations were 39.4198 ± 2.960, 43.6993 ± 2.343 for both fraction 1 and 2 respectively. While IL_6 concentrations were 23.3462 ± 1.308, 15.5848 ± 1.537 for both fraction 1 and 2

accordingly. TNFα concentrations were 127.1946 ± 3.310, 159.6818 ± 6.697 for both fraction 1 and 2 respectively. table (3).

Table 3
Cytokine profile of *A. hydrophila* immunized mice

Group	IL_4	IL_6	TNF
F1 LPS	39.4198±2.960	23.3462±1.308	127.1946±3.310
F2 LPs	43.6993±2.343	15.5848±1.537	159.6818±6.697
Control	3± 0.1	1.3± 0.1	19.5±0.6

Immune protection:-
The protection rates were 80% for each of F1 and F2 Table4.

Table 4
The rate of protection

A.hydrophila	Live percentage	Death percentage
Fraction 1	80%	20%
Fraction 2	80%	20%

DISCUSSION

The mechanisms involved in postchallenge immune protectivity may be viewed as a collective of the immune cell functions that include, mitogenic ability, B lymphocyte polyclonal activating ability, T lymphocyte helping and /or regulatory up and down pathways through activation and/or inhibition of the cytokine network as well as survivors records[3,4]. Postchallenge immune protectivity of smooth LPS of A.hydrophila in a mouse model is based upon a sort of balance between the pathogen virulence vajor and the potency limits for the host immune defense mechanisms. Three possible cases can be expected as an outcome of the balance. The first the pathogen virulence out weight the host immune defence mechanisms which stands as no protection, the second the pathogen equate the the immune defence mechanisms for host it holds as immune protection, while the third case the pathogen abilities is slightly out weight the host immune defense mechanisms a finding indicate partial immune protection which can be assured through death percentages[1,11].

LPS was found mitogenic in mouse Table1 in accordance with workers[9,10,11].

It was good B lymphocyte mitogen as well as activated macrophage to secrete IL4 which in turn enhance Th2 lymphocyte to release IL4 andIL5 to provoke B, lymphocyte to proliferate and differentiated to plasma cells producing A.hydropila specific antibodies Table2 [4,12,13]. The events suggest a parallelism between mitogenic assessment through lectin-like LPS skin tests Table 1 and T lymphocyte potency[13]. F1,F2 LPS of A.hydrophila increase IL4,IL6 and TNF alpha as compared to the control mouse Table3[14-19] with no shift in the cytokine balance and partial immune protection Table 4 [20-31].

CONCLUSIONS

1. A.hydrophila smooth LPS F1, F2 are being lymphocyte mitogen in mouse model.
2. A.hydrophila smooth LPS F1, F2 were proved inducing humoral antibody production.
3. F1 and F2 triggers the cytokine network leading to increase in IL4, IL6 and TNF alpha as compared to normal control mouse.
4. F1 and F2 were being partially immune protective in postchallenge mouse model.

REFERENCES

1. Paul W.Fundamental of Immunolgy 6th ed. Walter Kluwer, Lippicott Williams and Wilkins, 2008 USA.
2. Levinson W,Review of Medical Microbiology and Immnuology 11th ed,Macgraw-Hill Lange,2010,New York
3. Bryant CE, Spring DR, Gangloff M, Gay NJ.The molecular basis of the host response to lipopolysaccharide Nature Review Microbiology Progress 2009. 8(1):8-14.
4. Galanos C, Endotoxin (lipopolysaccharide, LPS), Delves PJ and Roitt IM eds Encyclopedia Of Immunology, 2 nd ed Vol.2 Academic Press, 1998,806-809,London
5. Westphal O, Luderitz O and Bister F. Uberdieextracktion von bacterienmitphenole–water.Z. Naturforsch .Teil B.1952; 7: 148-155.
6. Boyer RF. Modren Experimental Biochemistry, Addison Wesley Publishing Co., 1986, Reading
7. Shnawa IMS. Tuberculin, Tetanus immunoglobulin and DPT Vaccine as an avian in vivo T-lymphocyte mitogens. World Academy of science, Engineering and technology2013; 7:2071-2073.
8. Stevens CD. Clinical Immunology And Serology: Alaboratory Perspective, 3rded, FA Davis 2010, Philadelphia.
9. Al-Hakeem AF. Study of the effect on some germs cell walls compenentsin induction of arthritis in experimental animals.Msc.Thesis .Vet.College.BaghdadUniversity 2000, Iraq.
10. Al-Amery MA.The pathological and immunological effect of Salmonella typhimurium endototxin in rabbit. Ph.D. Thesis .Vet. College. Baghdad University, 2007, Iraq.

11. Rosenthal KS and Tan JS. Rapid Review of Microbiology and Immunology. Amazon company 2007.USA.
12. Melief CJ. Regulation of cytotoxic T-lymphocte response by dendritic cells: peaceful coexistence of cross priming and direct priming .Eur.J.Imm-unol.2003; 33:2645-2654.
13. Smits JE and Williams TD. Validation of immunotoxicology techniques in passerine chicks exposed to oil sand tailing water .Ecotoxicol. Envir.Saf.1999;44:105-112.
14. Kshash QH. The role of some antigens of E-coli isolated from bovine mastitis in the protection of mammary gland in mice .Ph.D.Thesis Vet. College. Baghdad University 2008, Iraq.
15. Bryn T, Yaqub S, Mahic M, Henjum, Anadahi EM and Tasken. Lipopolysaccharide activated monocyte suppress T cell immune responses with Fox pro 3 T cells through Cox3-2-PGE2 dependent mechanisms.Inter.Immunol.2008; 20:235-245.
16. Defrance T, Vandervliet B, Aubry JP, Takebe Y, Arai N, Miyajima A, Yokota T, Lee F, Arai KI, De Vries JE, Banchereau J : B cell growth-promoting activity of recombinant human interleukin 4. J Immunol, 1987; 139: 1135 .
17. Widmer MB, Acres RB, Sassenfield NM, Grabstein KH: Regulation of cytolytic cell populations from human peripheralblood by B cell stimulatory factor 1 (interleukin 4). J Exp Med 1987; 166:1447.
18. Spits H, Yssel H, Paliard X, Kastelein R, Figdor CG, De Vries JE. IL-4 inhibits interleukin-2-mediated induction of human lymphokine- activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed leukocyte cultures. J Immunol 1988; 141:29.
19. Rousset F, De Waal Malefijt R, Slierendregt B, Aubry JP, Bonnefoy J, de France T, Banchereau J, De Vries JE. 1988. Regulation of Fc receptor for IgE (CD23) and class IIMHC antigen expression on Burkitt's lymphoma cell lines by human IL-4 and IFN-y. J Immunol 1988; 140:2625,
20. Helle M, Brakenhoff JPJ, De Groot ER, Aarden LA. Interleukin 6 is involved in interleukin 1-induced activities. Eur J Immunol 1988; 18:957.
21. Brennan FM and Feldmann M. Cytokines in autoimmunity. Curr. OpinImmunol.1996; 8:872.
22. Hart PH, Vitti GF, Burgess DR, Whitty GA, Piccoli DS, and Hamilton JA. Potential anti inflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor a, interleukin 1, and prostaglandin E2. Proc. Natl. Acad. Sci. USA1989, 86:3803.
23. Mijatovic T, Kruys V, Caput D, Defrance P, and Huez G. Interleukin-4 and -13 inhibit tumor necrosis factor-a mRNA translational activation in lipopolysaccharide- induced mouse macrophages. J. Biol. Chem. 1997; 272:14394.
24. Abramson SL, and Gallin JI. IL-4 inhibits superoxide production by human mononuclear phagocytes. J. Immunol.1990; 144:625.
25. TeVelde AA, Huijbens RJ, de Vries JE, and Figdor CG. IL-4 decreases FcgR membrane expression and FcgR- mediated cytotoxic activity of human monocytes. J. Immunol.1990; 144:3046.
26. Skeen MJ, Miller MA, Shinnick TM, and Ziegler HK. Regulation of murine macrophage IL-12 production: activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines. J. Immunol.1996; 156:1196.
27. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J.A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD41 T cells. J. Immunol. 1995; 154:5071.
28. Kuperman D, Schofield B, Wills-Karp M, and Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyper responsiveness and mucus production. J. Exp. Med. 1998; 187:939.
29. Takeda K, Tsutsui H, Yoshimoto T, Adachi O, Yoshida N, Kishimoto T, Okamura H, Nakanishi K, and Akira S. Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity 19; 8:383.
30. Aste-Amezaga MXMa, Sartori A, and Trinchieri G. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. J. Immunol.1998; 160:5936.

31. Park BS, Song DH, Kim HM, ChoiB-S, Lee H and LeeJ-O. The structural basis of LPS recognition by TLR4-MD-2 complex. Nature 2009; 458(7242):1191-1195.