PRECLINICAL IMMUNOGENICITY OF CAPSULEAND OUTERMEMBRANE PROTEINS OF A LOCAL NEUROTROPIC ISOLATE OF HEMOPHILUS INFLEUNZAE B IN A LAPIN MODEL

Abstract

Please shorten it to 200 words

Hemophilus influenzae b is gram negative ,shortrods,encapsulated, microaerophilic, fastidious,need X and V factor for growth.

Also put the goal briefly here

It is of six capsular serotypes.Among which serotype b was the dominant.It mainly associated with human pyogenic respiratory and meningeal infectionsThe capsule and the outer membrane protein antigens were separated, purified , and characterized .Then,quantfied to the rate of 3mg/ml and 2.27 mg/ml accordingly.They were recovered from a local neurotropic isolate of H.infleunzaeb.Both of the produced C and OMP preparations stands as test antigens for the invitro use and for speific immune priming of rabbits.Cell free culture filtrate of test isolate was prepared and used as a skin sensitin in DTH test.The test immune system was that of rabbit.The immunization protocol was carried out initially by preconditioning with Complete Freund Adjuvant CFA for two weeks period.Then the conditioned rabbits were specific immune primed with C in one group ,OMP with other group and saline sham control in athird group of rabbits.The test immunogens were dosaged in two weeks apart using multisite multi-injection , then one week left and test bleed.The anti-OMP was tested by hemagglutination and the anti-capsular was by capillary precipitation tests.Immunogenicity was matched as cellular, allergenic and humoral immune conversion from the baseline immune functions of the saline sham controls.Leukocyte stimulatory factor cytokines were noted among those rabbits immunized with capsular antigen.The number of folds of rise for thetitre and the concentration during immune conversion was showing four to six folds than normal baseline function .Both of the test antigens were found basically as lapin immunogens stimulating humoral immune responses both at systemic and mucosal immune compartments.These findings may forms the background information and be an integral part of preclinical and clinical development and production of local H.infleunzae b systemic and/or mucosal vaccines. Which could be valid on approval for local mass vaccination against childhood meningitis in this area.

Key Words

Keywords must not exceed 5 words.

Antigen,allergenic, baseline, cellular, function, humoral, immunogens, vaccines.

Introduction

-It is not reasonable for the number of words in the introduction to be less than the number of words in the abstract. It should be explained and its development should be briefly added at a rate of at least 70 words so that it is greater than the number of words in the abstract.

-The term must be written in full the first time, and then we use abbreviations.

Hemophilus influenzae b is short,encapsulated,gram negative rods.Microaerophilic ,fastiious need X and V factor for growth.It showed six capsularserotypes.Among which serotype b was the dominant.Hib mainly associate with human pyogenic respiratory and meningeal infections[1].The nature of the immunity induced by H.infleunzae b in rabbit,rat and mice is humoral by a T cell independent epitopes[1,2,3].Circulating anticapsular PRP antibodies promote complement dependent bactericidal power and phagocytosis found in meningitis patients and vaccinee.There is a correlation between the presence of bactericidal antibodies and resistance to H.infleunzae b infection[2,3].Hemophilusinfleunzae b OMP have been proved to be immunogens in infant rat[4].The noncapsulated atypical H.infleunzae suppress and modulate cellular and humoral immune responses to atypical H. Infleunzae vaccine due to interference phenomena[5-9].OMP of atypical H. Infleunzae is affected by the function of Treg on B cells specific for OMP in a cell culture system[10].The objective of the present work was to map immunogenicity of capsule and outermembrane proteins of H.infleunzae b local isolate.

Materials And Methods

The type and period of study must be written.

1-Neuropathic H. Infleunzae

Local H.infleunzae b isolate from a clinically proven human meningitis cases.The isolate was culturally and biochemically charcterized through classical and API20E system.Serotyped usingDifco,Co,BDTM kit[11].

2-Preparation of Immune Reagents

The capsule was saparated,purified,identified and quantified to the concentration of 3mg/ml.[12].OMP was separated,purified ,identified and quantified to the concentration of 2.27 mg/ml.[13].Sensitin was prepared as cell free culture filtrates as in [14].

3-Rabbits

A Newzeland white rabbits weighing 1- 1.5 kgs were checked to be free of parasite ,bacteria, and antibodies.These rabbits were adapted to housing conditions for one week and kept ad libitum conditions.They were grouped and assigned into three groups each of three.Two test groups and one control group.

4-Immunization Protocol

The test groups were primed with complete Freund adjuvant CFA in a rate of one ml for each rabbit, control group was primed with saline.Test and control groups were left for two weeks.After preconditioning with CFA.One group was primed with capsule, 3mg/ml. and

the other with OMP 2.27/ml. in a two weeks apart multisite multi-injection protocol followed by one weeek leave then test bleed[15].

Capsule in 3mg/ml..... three rabbits

Outermemberane protein 2.27 mg/ml.....three rabbits

Saline sham control.....three rabbits

5- Sampling And Processing

Blood samples were collected from test and control rabbits by cardiac punctutre with a rate of six mls from each rabbit.Samples were divided into two parts each of three mls amounts. Tubes withoutanticoaggulant and three mls., and tube with anticoaggulant.Sera were saved from samples without anticoaggulants in a rate of 0.5 ml amounts in an appendroff tubes and kept at -18 C.Whole blood with anticoaggulantuesed for test for leukocyte inhibitory factors by capillar method[16].Appendecies were collected from primed and control rabbits.They were opened up , freed from digesta and scrapped with sterile clean scalpel to remove mucus from the mucosal surfaces. Proportional mucus-saline were mixed in a sterile petri-dish. Mixtures were tubed in centrifuge tubes and centrifuged at 5000 rpm for ten minutes.Supenates of three mlsamounts were mixed with equal amounts of PEB 6% ,6000 and left at 4 C for one hr and centrfuged for 5000 rpm for 15 minutes.Precipitates were saved and reconstituted with sterile formal normal saline[14].

6-Immune Function Tests

The anticapsular antisera and mucosal globulin were titrated with capsule antigen using capillary tube precipitation test[17].The antiOMP antisera and mucosal globulin were titrated with OMP coated tanned sheep red cells by micro hemagglutination test[18].Mucus-Saline mixture was mixed with dextran solutions in an equal amounts for separation of mucosal leukocytes as in Metcalf et al.[19]. Systemic and mucosal leukocyte inhibitory factor was done by capillary method as in Soberg [17].

Results

1-Baseline Immune Functions

Control rabbits reveals the normal baseline immune functions in this experimental settings.The serum globulin protein concentration was 6.75 gm/dl.While,the normal appendexglubulin protein concentration was 0.425 gm/dl.Natural serumbaseline titre mean was 10 both for anti-capsular and anti-OMP antibodies and normal mucosal globulin concentration was 4 both for anti-capsular and anti-OMP antibodies.While the normal leukocyte inhibitory factor cytokines were ranging between 0.95 and 0.97%.

2- Cellular Immune Conversion

Specific OMP priming to rabbits lead to non-significant leukocyte inhibitory factor cytokines and mild leukocyte stimulatory factor cytokines in one rabbit replicate both at mucosal and sysyemicresponses.Specific capsular primed rabbits have shown leukocyte stimulatory

factor cytokines as compared to LIF of normal baseline immune functions.OMP and Capsular immune primied rabbits indicate cellular immune conversion than that of basline cellular immune functions,Tables – 1.

3- Delayed Allergenic Immune Conversion

AT 72 hrs post-sensitization with the skin sensitins CFCF through intradermal injection of the primed rabbits.Neither classical DTH nor Jone-Moote reaction noted in both of the primed groups.This may indicate that there were no delayed allergenic immune conversion in these immune primed test rabbits,Table – 1

4- Humoral Immune Conversion

The mean of serum anticapsular antibody titre was 533.3 compared to the baseline humoral immune function was 10.While,the mucosal anti-capsular antibody mean titre was 42.66. As compare to normal humoral baseline immune function was 2.In other word five folds increase folds increase inthe serum titer means and four folds in the mucosa mean titres.While,themean serum anti-OMP titrewas 853.3 compared to normal baseline function mean titre was 10.The mucosal anti-OMP meant titre was 85.33 compared to normal baseline humoral immune fuction was 2.That is to say six folds increase in serum and five folds im mucosal anti-OMP antibodies.The folds increase in mean antibody titres in serum and in mucosa indicated humoral immune conversions,Table – 2.

Table – 1:Cellular Immune functions as leukocyte Inhibitory factor cytokines[A] and Skin DTH[B] for H.infleunzae b.

A	Specific Immune Primed Rabbit	Systemic Leukocyte Inhibitory Factor	Mucosal Leukocyte Inhibitory Factor
Capsule primed	R1 R2 R3 Rmean Control	1 1 1 1 0.98	1 1.09 1.07 1.08 0.995
OMP primed	R1 R2 R3 Rmean control	1 0.9 0.9 0.903 0.98	1 0.95 0.91 0.95 0.97
B/Skin DTH/hours	erythema	Induration	Necrosis
Capsule primed 6 48 72	 - - -	 - - -	 - - -

OMP primed 6 48 72	 - - -	 - - -	 - - -

Table-2:Humoral Immune Function for capsular [A] and OMP [B] primed rabbits as precipitins A and hemagglutinins B.For the neurogenic H.infleunzae b.

Animal groups	Capsule Primed rabbits	Serum antibodytitre means	Serum concentration means g/dl	Mucus titre means	Mucus concentration means g/dl
Test Group A	R1	320	26.3	64	1
	R2	640	31.42	32	2.7
	R3	640	32.1	32	2.3
	Rmean	533.3	29.94	42.6	2.06
	Control	10	6.75	2	0.45
Test group B	OMP Primed				
	R1	1280	33.1	64	2.7
	R2	640	21.2	128	1.0
	R3	640	22.31	64	1.0
	Rmean	853.3	25.4	85.33	1.56
	Control	10	6.35	2	0.4

Discussions

The OMP and capsular polysaccharide of H.influenzae b are; pathogenicity determnant ,virulence associated antigens and virulence factor.So both in conjugate state or in separate state they are standing as targets for vaccine candidates and human approved vaccines in more thanone vaccine qualifying boards all over the world[20].In the present communication tempts to investigate immunogenicity of subcellular fractions of local neurotropic neurogenic H.infleunzaeb.Thesesubfractionsare;Capsule and OMP in a lapin model,Tables - 1 &2.The specific immune priming protocols tempts to precondition rabbits with Freund Complete Adjuvant CFA for a period of two weeks that provok non-specific immune stimulation to both of innate and adaptive immune cells[15].As a pre-immunization adjuvant followed by two weeks apart b capsular antigen in separate and OMP antigen in separate rabbit groups.This based on the theme that immuoadjuvant can be of use either pre,mix with or post-antigen priming to an immune animal model[21].Both of the prepared antigens bear forigeness characters from the organism producing them that are encoded by the genetic system of that organism[22].Parallel to this the immune system cells of test animal model are encoded by gene sets that on their expression,these cells recognize that the introduced material facing them are foreign[23].Immunogenicity in theoritical immunology sense is a matter of self/non-self recogntion theme[24].Both of capsule b and OMP were found to be as lapin immunogens inducing humoral immune responses both at systemic[blood sera] and mucosal[appendex globulin] levels.the immune

conversion were estimated by the folds increase of antibody titre and concentrations than the baseline titre and concentrations from the baseline immune function in normal control rabbits.Both of systemic anticapsular and anti-OMP were express five to six folds increase. While for mucosal antibody titresrise was four to five folds increase than normal immune function was five folds.Capsular b antigen induce leukocyte stimulatory factor up to 1.08% as compared to saline control was 0.95%, which is a kind of cytokine that promote leukocyte migration in capillary tube approach[25,26]

Both of theCapsule b and OMP antigens does not express delaye allergenic immune conversions than the baseline control rabbits .Since they neither produce typical skin DTH reaction nor Jone-Moote reactions[27],during the observation period 6 up to 72 hrs. Post to sensitinintrademal injection in primed test animals groups.

The possible epitope nature of capsular b antigen may be T independent epitope triggering anti-PRP antibodies.While that of OMP antibodies may trigger TH2 cells and Th2 cells in turn activate B cells to produce anti-OMP antibodies.Apparently, it does not contain TH1 dependent epitopes but there is a possibility for shiffting from TH2 to TH1 dependence due to co-existing epitopes mixed with idependent ones[28-32].Thus type b capsule and OMP of H.infleunzae induced local and systemic humoral immune responses in rabbits primed with them separately.Systemic humoral antibody responses were higher than that mucosal responses.This was inline with Shnawa[30],working on immunogenicity of C.fetus in rabbits.The ratio of systemic to mucosal were 10;1 in term of titre whilein term of concentration for OMP was 13:1 and for capsule was 14:1[30].

Conclusions

Human neurotropic neurogenic local H.infleunzae b OMP and Capsule b are found as lapin immunogen.Adult rabbits proved to be valid immune model for testing immunogenicity of these antigens.Capsular b antigen induce leukocyte stimulatory factor in capillary method.Capsular and OMP induces humoral hemagglutinin and precipitin responses both at systemic and mucosal compartments.Such findings be essential for preclinical and development of systemic and/or mucosal vaccine ,on approvial will be valid for local mass vaccination of childhood meningitis[7-9,33].

References do not work in the conclusion, just rephrase.

References

1-Levenison W , Chin-Hong P , JoycEA,Nussbaum J ,Schwartz B.2018.Review of Medical Microbiology and Immunology 15th ed .McGraw-Hill Lange.Ney York,164-166.

2-Caroll KC , Morse SA , MietznerT,Miller S.2015.Jawetz ,Milnick&Adelbergs Medical Microbiology 27th ed ,McGraw-Hill,Lange,New York,363-264.

3-Wisplwey B , Hansen E , Scheld M,1989.Hemphilus infleunzae OMP induced BBB permeability during experimentatalmeningitis.Infect.Immu. 57(18):2559-2562.

4-Hansen EJ , Fresch CF , McDade RL , Johnston KH,1981.Identification of immunogenic outermembrane protein of H.infleunzae type b in the infants rat model system.Infect. Immu.32(3):1084-1092.

5-Mawas F ,Newman G , Burns S , Carbel MJ.2006.Suppression and modulation of cellular and humoral immune responses to H.infleunzae type b conjugate vaccine in Hib-Dipheria-Tetanus toxoid-Acellular Pertussis combination vaccines;A Study in a rat model.J.Infect.Dis.191:58-64.

6-Lee H , Hahn S , Lee HJ , Kim KH 2010.Immunogenicity of Hemophilusinfleunzae Type b conjugaste vaccine in Korean infants;A meta-analysis.J.Korean Med.Sci.25(1):90-96.

7-Griffihs UK , Clark A , Gessner B , Miners A , Sanderson C , Sedyaningish ,Mulholland KE 2012.Dose-specific Hemphilusinfleunzae b conjugate vaccines: A systematic review and meta-analysis of controlled clinical trials.Edpidemiol.infect.140:!343-1355.

8-Latez E , Franko J , Golenbock DT ,Schreiber JR.2004.Hemophilusinfleunzae b outer - membrane protein glycoconjugates vaccine induces cytokine production by eb=ngagment with Tolllike receptor 2(TLR2) and requires the presence optimal immunogenicity .J.Immunol.172:2431-2438.

9-Santosham M 1993.Presentation of Hemophilusinfleunzae type bdisease,Vaccine 11(51):552-557,.

10-Hirano J , Kadowaski Y , SusuleM et al.2019.Interaction between regulatory T cells and antibody producing B cells for immune responses at upper respiratory mucosa against nontyableHemophilusinfleunzae ,Ann.Otol.Rhinol.Laryngol.128(Supp 6);45S-51S.

11-Gonzalez MD,Ledeboer NA 2023.Hemphilus, In,Patel R ed.Manual of Clinical Microbiology, 13th ed.ASM.

12- Kwapnski JB 1972.Methodology of Immunochemical And Immunological Researchs. Wiley- Intersciense,New-York:267-316.

13-Catlin BW , Bandler III JW , Goodgal SH 1972.The type b capsule locus of hemophilusinfleunzae:map location and size.J.G.Microbiol.70:411-422.

14-Shnawa IMS ,Thewaini QNA 2002.Lapin mucosal versus systemic humoral and cellular immune responses post to intratesticular administration of heat killed C.fetus.J. Baby.Uni. 7(3):538-543.

15-AlShahery MAN ,Shnawa IMS 1989.The immunological adjuvanicity of sunflower oil.Cair. Vet.Med.J.73(2):291-298.

16-Soberg M , 1969.Interaction of human peripheral blood lymphocyte and granulocyte in the migration inhibionreactions.Acta Medica.Scand.185:221

17-Turgeon ML.2020.Immunology and Serology in laboratory Medicine,Elsevveir Health Science.

18-Joshi A , Chauhan RS 2022.Immunological Techniques;Interpretation,Validation and Safety Measures.Prakashan,Haryaon,India.

19-MetCalf JA ,Gallin JI, NauseefWM,Root RK.1986.Laboratory Manual of Neutrophil Function Raven Press.

20- Shnawa IMS 2019.Vaccine Technology At A Glance.BoffinAccess,UK.

21- Zhao T ,Cai Y ,Jaing Y et al.2023.Vaccine adjuvants,mechanisms and platforms.Signal Transduction and Target Therapy 8: 283.doi.10.1038/s41392-01557-7.

22- Venter JC .1998.Complete genome squencing of Hemophilusinfluenzae.InDootittle RF.1998.Microbial genome Open Up.Nature,392:339-342.

23-Lagou V , Garcia-Perez JE , Smet I et al.2018.Genetic architecture of adaptive immune system identifies key immune regulator.Cell Reports.25:78-810.

24- PradeuT , Car sella ED .2006.On the definition of a criterion of immunogenicity PNAS 103(47):17858-17861.

25-Sixt M ,Lammermann T 2011.Invitro analysis of chemotactic migration in 3D environment.Chapter 11 in Wells CM,Parsons M (eds): Cell Migration: Developmental Methods and Protocols.Methods In Molecular Biology Vol.769.doi.10.1007/978-1-61779-207-6-11,Springer Sciences.

26-Thorley AJ , Ford PA , Giembycz MA, GoldstrawP,Young A , Tetley TD 2007.Differential regulation of cytokine and cytokine release and leukocyte migration by lipopolysaccharide

stimulated primary human lung alveolar type II epithelial cells and macrophages. J.Immunol. 178:463-472.

27- Burrell R 1979. Experimental Immunology,Burgess publishing Co.Amazon,USA,102.

278-Misseneir H , Lary K 2002.Control of diseases attributable to Hemophilusinfleunzae b and the national immunization Program Pediatr.110(4):820- 823.

29-Mcvernon J, Johnson P , Pollard A Elack M and Moxon E 2005.Immunological memory in Hib conjugate vaccine failure.Arch.Dis. 88:379-383.

30-Shnawa IMS 2006.Lapin systemic versus mucosal humoralresponses as well as cellular immune responses following intravenous administration of C.fetus heat killed bacterin;A correlative approach.ALQadysiah Vet Med.Sci. 1(5):47-51.

31-Haddadi N-S , Mande P , Brodeur TY , Haok , Ryan GE Moses S , Sabramanian S , Picari X , AfatariK,Marshak- Rothstein A,Richmond JM 2022.TH2 to TH1 transition is required for induction of skin lesions in an inducibale and recurred murrine model of lupus like inflammation.Frot.Immunol.13:883375.doi.10.3339/fimmu/2022-883375.

32-Garcia-Diaz D ,rodriguez I ,Santisteban Y, Brown E , Iglesias E 2013.Th2- Th1 shit with the multiantigenic formulation TERAVAC-HIV-1 in Balb/c mice.Immunol.Lett.149(1-2):77-84.

33-Jackson BD ,MiernykK,Steinberg J et al.2024.H.influenaetype b vaccine immunogenicity in American indian/Alaska native infants.Pediatrics.154(4):aricle number e2024066658.