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Preclinical Immunogenicity of Capsule and Outermembrane Proteins of a Local Neurotropic Isolate of Hemophilus Infleunzae B in a Lapin Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The preclinical immunogenicity of capsule C and outer membrane protein OMP of human neurotropic isolate of Hemophilus influenzae b HIB in a lapin model is being reported. Hemophilus influenzae b is gram negative, short rods, encapsulated bacterium, microaerophilic, fastidious, need

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X and V factor for growth with six capsular serotypes. Among which serotype b was the dominant. Itis associated with human pyogenic respiratory and meningeal infections. The C and OMP were separated, purified, and characterized. Then, quantfied to the rate of 3 mg/m L and 2.27 mg/mL accordingly. Both of the preparations were used 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. Rabbits were were preconditioned with CFA adjuvant for two weeks then specific immune primed using multisite multi-injection protocols. Capsule immunogen induce leukocyte stimulatory factor cytokines. Four to six folds rise both titres and concentrations of anti capsular precipitins and anti OMP hemagglutinins were noted. Both C and AMP are proved to be lapin immunogens stimulate humoral immune responses at both 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.

Keywords: Antigen; cellular; humoral; immunogens; vaccines.

1. INTRODUCTION

Hemophilus influenzae b is short gram negative rods, encapsulated bacterium. Microaerophilic, fastidious need X and V factor for growth with six capsular serotypes. Among which serotype b was the dominant. Hib is associated with human pyogenic respiratory and meningeal infections (Levenison et al., 2018). Meningitis is a life threatining disease affects millions of people of all ages allover the world and is considered a major global public health issue (International Society of Infectious Diseases 2023). The nature of the immunity induced by H. Infleunzae b in rabbit, rat and mice is humoral by a T cell independent epitopes (Levenison et al., 2018; Wisplwey et al., 1989). Circulating anti capsular 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 (International Society of Infectious Diseases 2023; Wisplwey et al., 1989). Hemophilus infleunzae b OMP have been proved to be immunogens in infant rat (Hansen et al., 1981). The noncapsulated atypical H. infleunzae suppress and modulate cellular and humoral immune responses to atypical H. Infleunzae vaccine due to interference phenomena (Mawas et al., 2006; Lee et al., 2010; Griffiths et al., 2012; Latez et al., 2004; Santosham, 1993). OMP of atypical H. infleunzae is affected by the function of Treg on B cells specific for OMP in a cell culture system (Hirano et al., 2019). The objective of the present work was to map immunogenicity of capsule and outer membrane proteins of H. infleunzae b local isolate.

2. MATERIALS AND METHODS

2.1 Reviewer Inquiry

It is a reviva revista study that has been a part of AL Thahab Ph. D. Thesis at 2006 on HIB meningitis; pathogenesis and immunology in man and laboratory animal. Prof. Shnawa and Ass. Prof. Thewaini had been supervisor and coadvisor. Whereby had been faculty membership at Babylon University.

2.2 Neuropathic H. Infleunzae

Local H. infleunzae b isolate from a clinically proven human meningitis cases The isolate was, culturally and biochemically characterized through classical and API20E system. Serotyped using Difco, Co, BDTM kit (Gonzalez & Ledeboer 2023).

2.3 Preparation of Immune Reagents

The capsule was saparated, purified, identified and quantified to the concentration of 3 mg/ml (Kwapnski, 1972). OMP was separated, purified, identified and quantified to the concentration of 2.27 mg/ml (Catlin et al., 1972). Sensitin was prepared as cell free culture filtrates as in (Shnawa & Thewaini 2002).

2.4 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.

2.5 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, 3 mg/L. and the other with OMP 2.27 mg/mL. in a two weeks apart multisite multi-injection protocol followed by one weeek leave then test bleed (AlShahery & Shnawa 1989).

Capsule in 3 mg/L..... three rabbits
Outermemberane protein 2.27 mg/L.....three rabbits
Saline sham control.....three rabbits

2.6 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 without anticoaggulant and three mls., and tube with anticoaggulant. Sera were saved from samples without anticoaggulants in a rate of 0.5 ml amounts in an append roff tubes and kept at -18 C. Whole blood with anticoaggulant uesed for test for leukocyte inhibitory factors by capillary method (Soberg, 1969). 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 tubbed in centrifuge tubes and centrifuged at 5000 rpm for ten minutes. Supenates of three mls amounts 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 (Shnawa & Thewaini 2002).

2.7 Immune Function Tests

The anticapsular antisera and mucosal globulin were titrated with capsule antigen using capillary tube precipitation test (Turgeon, 2020). The anti OMP antisera and mucosal globulin were titrated with OMP coated tanned sheep red cells by micro hemagglutination test (Joshi & Chauhan 2022). Mucus-Saline mixture was mixed with dextran solutions in an equal amounts for separation of mucosal leukocytes as in Metcalf et al. (1986). Systemic and mucosal leukocyte inhibitory factor was done by capillary method as in Soberg (1969).

3. RESULTS

3.1 Baseline Immune Functions

Control rabbits reveals the normal baseline immune functions in this experimental settings. The serum globulin protein concentration was 6.75 g/L. While, the normal appendex glubulin protein concentration was 0.425 g/L. Natural serum baseline titre mean was 10 both for anti-capsular and anti-OMP antibodies and normal mucosal globulin concentration were 0.425 and 0.4g/L. both for anti-capsular and anti-OMP antibodies. While the normal leukocyte inhibitory factor cytokines were ranging between 0.95 and 0.97%.

3.2 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 systemic responses. 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, Table 1.

3.3 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.

3.4 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, the mean serum anti-OMP titre was 853.3 compared to normal baseline function mean titre was 10. The mucosal anti-OMP mean 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.

4. DISCUSSION

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 than one vaccine qualifying boards all over the world (Shnawa, 2019). In the present communication tempts to investigate immunogenicity of subcellular fractions of local neurotropic neurogenic H. infleunzae b. These subfractions are; 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 (AlShahery & Shnawa 1989). 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 (Zhao et al., 2023). Both of the prepared antigens bear forigeness characters from the organism producing them that are encoded by the genetic system of that organism (Venter, 1998). 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 (Lagou et al., 2018). Immunogenicity in theoritical immunology sense is a matter of self/non-self recogntion theme (Pradeu & Carssella 2006). 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 titres rise 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 (Sixt & Lammermann 2011; Thorley et al. 2007).

Both of the Capsule b and OMP antigens does not express delayed allergenic immune conversions than the baseline control rabbits.

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	1	1
	R2	1	1.09
	R3	1	1.07
	Rmean	1	1.08
	Control	0.98	0.995
OMP primed	R1	1	1
	R2	0.9	0.95
	R3	0.9	0.91
	Rmean	0.903	0.95
	Control	0.98	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 antibody titre means	Serum concentration means gm/L	Mucus titre means	Mucus concentration means gm/L
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

Since they neither produce typical skin DTH reaction nor Jone-Moote reactions (Burrell, 1979), during the observation period 6 up to 72 hrs. Post to sensitin intradermal 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 independent ones (Misseneir & Lary 2002; McVernon et al., 2005; Shnawa, 2006; Haddadi et al., 2022; Garcia-Diaz et al., 2013; Jackson et al., 2024). 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 (2006), working on immunogenicity of C. fetus in rabbits. The ratio of systemic to mucosal were 10;1 in term of titre while in term of concentration for OMP was 13:1 and for capsule was 14:1 (Shnawa, 2006).

5. 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 approval will be valid for local mass vaccination of childhood meningitis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors herebydeclare that NO generative AI technologies such as Large Language Models(ChatGTP,COPILOT, etc) and text-to-image generators have been used during wrting or editing of this mnanuscript.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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