

Immune characterization of human pneumotropic local serotype 1 and 6 *Staphylococcus pneumoniae* isolates as local vaccine strains

Abstract

The serotypes C1 and C 6 of human pneumotropic *S .pneumoniae* were immune characterized as vaccine strains.Capsules were separated, identified , quantified and dispensed as 10mg deried amounts in ampoules .On reaching specific immune priming of rabbits the ampoules dissolved in 10 mls sterile saline and each ml admixed with 1ml lanoline and injected intramuscularly per each rabbit's thigh muscles. The immune primed rabbits left for 28 days then test bleed to check for an evident immune conversion then,at the day 35 after immunization the immune rabbits were challenged with live *S .pneumoniae* 1×10 to six CFU/ml., in a rate of 2 mls.The carbohydrate based capsule prototype monovalent vaccines were found as;Pure,safe,antigenic,immunogenic and immune protective to the rat of 80% for C1 and 60% for C6 serotype.They induced systemic humoral immune precipitins responses higher than mucosal pricipitin responses.Proinflammatory cytokine responses TNF alpha,TNF beta and IL6 and anti-inflammatory cytokine IL10 were mounted as mucosal responses higher than the systemic responses.The proved immune characteristics of these capsule serotypes were suggestive for use of these strain as vaccine strains along with other serotypes in development of the local prototype multiserotype vaccines of this pathogen.

Key Words

Antigenicity ,purity,safety,sterlity, protectivity,precipitins,vaccine strain

Introduction

In human clinical practice of bacterial infectious diseases,there were few bacterial specific carbohydrate based vaccines that are approved for mass vaccination use for those at risk human beings by the vaccine approval authorities[1].Such as that of multiple carbohydrate based vaccine of *S.pneumoniae* [2-10].Though at times human pneumococcal pneumonia reported every now and then at diferent part of the developing world countries[10].So the local human pneumotropic isolates gained prevelence may express pathogenic and/or immunogenic potentials that are somewhat different from that present in the current 23 *S.pneumoniae* approved vaccine although of same serotype[10,11].The present paper was aims at tempts to immune characterize human pneumotropic local serotype 1 and 6 *S.pneumoniae* isolates as local vaccine strains.

Materials And Methods

1-Seed Strains:

A collection of eight serotypes of *S .pneumoniae* that were recovered from clinical human cases of pneumonia.The most dominat of which were serotype 1 and 6 [10].

2-Laboratory Development

The 24 hr freshly revived original isolates of each serotype 1 and 6 [author collection[10] of the *S.pneumoniae* serotype 1 and 6 growth were made onto blood agar plates. Sterile heavy swab inocula were made onto 20 trypticase soy agar plates for each serotype. The inoculated plates were incubated at 5-10% CO₂ tension at 37C for 24 hrs. Pure growth were harvested by 5 ml sterile saline solution per each plate. The suspensions were brought to PH 8.5 by the addition of 10% KOH. The alkalized suspensions were heated at 90C for 1hr in water bath. These heated suspensions let stand to cool and acidified with 5N acetic acid. The acidified suspensions then centrifuged at 5000 rpm for 10min. Pellets were discarded and supernatants were mixed with two volumes of 1% sodium acetate in 95% ethanol solution then kept at 4C for 24 hrs. Supernate and pellets were formed. The formed precipitates were removed by centrifugation at 2500rpm for 10 min. Pellets were discarded and supernatants were mixed with 5% sodium acetate in 0.5 N acetic acid for 24 hrs at 4C. These mixed solutions were centrifuged at 5000 for 30 min. Supernatant discarded and pellets were washed with 85%, 95% absolute ethanol. Then dried at 37C. The 20 plate yields 200 mg powder materials. These materials were cautiously dispensed in 10 mg per ampoule under aseptic precautions and kept till use. Each ampoule dissolved in 10 mls., sterile saline representing the immunogenic doses for 10 rabbits and stands as prototype monovalent capsular carbohydrate based experimental vaccine in rabbits [12].

3-Purity:

A purity checks were done by quadruple streak method onto trypticase soy agar plates in each step of the laboratory development of the prototype monovalent pneumococcal vaccine [13].

4-Safety:

A 0.1 ml of the capsule experimental prototype vaccines were IM injected into rabbits through thigh muscles three replicates for each serotype. The injected animals were followed for up to five days. Then eviscerated to test for gross and histology changes [13].

5-Adjuvant:

Lanolin solution [13].

6-Immunogens:

One volume of lanolin was admixed with one volume of 1mg/ml. capsular polysaccharide. The mixture stands as the test immunogens [13].

7-Immunization Protocol;

The immunogen 2 ml mixture was IM injected into the thigh muscle of rabbit and left for 15 days then test bleed [13].

8-Laboratory Animals:

A group of New Zealand rabbits were acclimatized for housing conditions for two weeks and kept ad libitum condition during specific immune priming and challenge experiments. These rabbits were categorized into the following groups;

Safety group.....6 Rabbits

Serotype 1 specific immune priming...10 Rabbits

Serotype 6 specific immune priming...10 Rabbits

Sham Control10 rabbits

9- Challenge Models;

The specific immune primed rabbit groups at the day 22 postpriming were challenged with live *S.pneumoniae* in strength of 1×10^6 to 6×10^6 /ml, survivors were scored 21 days post challenge [14]

10- Blood Sampling:

Test and control rabbit groups were subjected to blood collection by cardiac puncture. Sera were saved, dispensed at 0.5 ml aliquots in microcentrifuge tubes and kept at -20°C till test for antibody and cytokine levels [15]

11- Tracheal Mucosal Globulins:

Parts of the challenged and control rabbit trachea were incised and opened up into sterile petri plates. Tracheal mucus was scraped into the plates then 5 ml formal normal 0.5% saline were added to the scraps and mixed thoroughly and tubed into centrifuge tubes. Scrap saline tubes were centrifuged at 5000 rpm for 10 min. Supernatants were kept for processing of mucosal globulin separations in accordance with the method [16]. Pellets were discarded.

12- Immune Investigations:

Determinations of antibody response levels were done by precipitation tests as in [17]. Cytokine response measurements were done as the manufacturer instructions.

Results;

1-Purity

All of the laboratory development purity checks onto trypticase soy agar plates were found of negative growths.

2- Safety;

Neither gross nor histologic changes were noted in the inoculated safety check rabbits.

3- Antigenicity

One from each of the vaccine lot ampoules were used for check of antigenicity test by precipitation tests.

4- Immunogenicity

4-1: Humoral Antibody Responses

The mean of serum and mucosal antibody titres for the challenged rabbits were 400 and 20 for serotype 1 and 213.33 and 13.33 for serotype 6. Systemic responses were higher than mucosal responses, Table – 1.

4-2; Cytokine Responses;

Mucosal cytokine responses were higher than systemic cytokine responses both for the serotypes 1 and 6., Table 2.

4-3; Cytokine imbalance:

Cytokine imbalance were noted in serotype 1 vaccine at mucosal response only, Table- 3.

4-4 ; Immune Efficacy :

The immune efficacy of serotype 1 was 80% and for serotype 6 was 60%, Table-4.

4-5: Prototype Carbohydrate Based Vaccine Features

S.pneumoniae serotype 1 prototype vaccine was found; Pure, safe, antigenic, weak immunogenic needs exogenous adjuvant, induce humoral systemic precipitin responses higher than mucosal responses. Mucosal Cytokine responses were higher than systemic responses. Induce cytokine imbalance at mucosal surfaces but not at systemic compartment. It was immune efficacious to 80 % in a lapin challenge model. Serotype 6 prototype vaccine was found; Pure, safe, antigenic, weak immunogenic need exogenous adjuvant. Induce systemic humoral immune responses higher than mucosal responses. Mucosal cytokine responses were higher than the systemic responses. It neither induce cytokine imbalance at mucosa nor at systemic compartment. It was immune efficacious to 60% in lapin challenge model, Table-5.

Table -1: Specific immune precipitins responses of the immune challenged rabbits.

Groups	Titres
Serotype 1 specific immune primed rabbits	
Mucosal response	20
Systemic response	400

Serotype 6 specific immune primed rabbits	
Mucosal response	13.33
Systemic response	213.33
Control rabbits	
Mucosal response	3.33
Systemic response	6.3

Table – 2 :Cytokine responses of immune primed challenged rabbits

Rabbit groups	TNF alpha	TNF beta	IL6	IL10
Serotype 1 specific immune primed challenged rabbits				
Mucosa	473.11-+29	387.21 -+1.39	360.59-+4.3	253.36-+23.5
Systemic	250-+30.7	250.11-+7.6	223.5-+20.2	202.6 -+14.5
Serotype 6 specific immune primed challenged rabbits				
Mucosa	233.55-+1.4	225.25-+7.3	238.8-+4.9	199.68-+13.5
Systemic	245.2-+35	236.32-+27.8	219.51-+59.6	200.76-+8.1
Control				
Mucosa	125.12-+55	110.35 -+11.5	93.54 -+2.5	110.25 -+ 11.5
Systemic	115.29-+11.7	101 -+11.5	100.62-+0.75	101.15 -+11.5

Table -3 ; S.pneumoniae capsular 1 and 6 primed challenged rabbits cytokine imbalance.

Rabbit groups	TNF alpha	TNF B	IL6	IL10
Sertype 1 specific immune primed				

challenged rabbits				
Mucosa	4:1*	3:1	3:1	2:1**
Systemic	2:1	2:1	2:1	2:1
Serotype 6 specific immune primed challenged rabbits				
Mucosa	1.5:1	2:1	2:1	2:1
systemic	1:1	1:1	2:1	1:1
Control				
Mucosa/ systemic	1:1	1:1	1:1	1:1

- The number of folds of cytokine concentrations for the test to control rabbits

** Imbalance is the number of concentration folds for the proinflammatory to the anti-inflammatory cytokines.

Table -4 : The immune efficacy of the developed vaccines

Prototype vaccine	Infective dose	Number of rabbits	Servive to total	Percentages
Type 1	1x10 to 6	10	8:10	80%
Type 6	1x10 to 6	10	6:10	60%
Control	1x10 to 6	10	0:10	0%

Table-5 : Features of the Carbohydrate based S.pneumoniae serotype 1 and 6 monotypic prototype vaccines in rabbit challenge model..

Features	Protype C1	Prototype C6	Approved pneumococcal Vaccine
Understanding Disease	Pneumonia	Pneumonia	Pneumonia
Understaning causal	S.pneumoniae	S.pneumoniae	S.pneumoniae
Virulence factor	Serotype 1	Serotype 6	23 serotypes
Purity	Pure	Pure	Pure
Safety	Safe	Safe	Safe
Antigenicity	Antigenic	Antigenic	Antigenic
Immunogenicity	Immungen*	Immunogen*	Immunogen*
Immune efficacy in rabbit	80%	60%	60%
Immune efficacy in man	?	?	

- Amplified on combination with lanlin

Discussion;

The subunit bacterins of the bacterial pathogens when introduced to the body of a small mammal laboratory animals like rabbits, Tables 1 – 5, may triggers TH1, TH2 and /or B cells to

grow, proliferate, expand and activated as an effector and/ or memory cells. As a consequence, humoral mucosal and systemic antibody responses as well as cytokine network activations both for innate and adaptive cytokine types [18]. The systemic humoral immune responses were higher than mucosal responses [16]. While the mucosal cytokine responses were higher than the systemic responses [4]. Cytokine imbalance was noted between pro and anti-inflammatory cytokines serotype 1 at mucosal compartment but not systemic. Serotype 6 does not express imbalance at both mucosal and systemic compartments [19]. The immune efficacy of the present prototype carbohydrate based capsular bacterins were ranging between 60- 80% [11,20]. The vaccinologic features of the prepared candidates capsular bacterins were ;Antigenic, weak immunogenic need an exogenous adjuvant leading to antibody and cytokine responses. Such responses were both at mucosal and systemic compartments with an immune efficacy ranging between 60 to 80%. The proposed *S.pneumoniae* vaccine strains may be of help in development of an eight local pneumococcal vaccine with the other reported six serotypes [10] which may be more efficacious in this area than the internationally approved 23 pneumococcal vaccine [20].

Conclusion

Local pneumotropic isolates of *S.pneumoniae* serotype 1 and 6 were found valid as vaccine strains based on lapin immune challenged models.

Ethical Issue

Care, housing, handling and management done on rabbits were following the international acts regulating care, housing, handling and interventions.

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