IMMUNOGENICITY OF BACTERIAL PROTOPLASMIC SONICATE PROTEINS

Abstract

Bacterial Protoplasmic Sonicate Proteins BPSP were separated,identified,quantified and recovered from human skin burn infections.These proteins,the BPSPs were of Pseudomonas aeruginosa PAPSP and Klebsiella oxytoca KOPSP .They were considered to be as ,the test immunogens.The test immune system was that of rabbits.The immunization protocols were multisite multi-injection type.Mucosal and systemic ,humoral and cellular immune arms were matched.The PAPSP induces an increase in; NBT % neutorphil phagocytosis,significant leukocyte migration inhibition cytokines, IL6 cytokine elevationand rise up of humoral agglutinins responses both at mucosal and systemic compartments.While,that for KOPSP it induces increase of; humoral agglutinins and IL6 cytokine elevation both at mucosal and systemic responses.As well as non-signifcant increase in leukocyte inhibitory cytokines.The specific immune primed rabbits in both of PSPs were of higher levels than control animals.The PAPSP functional epitope mapping suggest that they are of both T cell denpendent [TH1] and T cell independent B cell dependent epitopes[TH2].While,the functional epitope mapping of KOPSP suggested to be direct or indirect B cell dependent epitopes[TH2].Such findings areholding the position of novelity,based upon the need for an autovaccine to multi-drug resistant *P.aeruginosa* and *K.oxytoca* skin burn infections since immunogenicity is an integral part of development and evalution of bacterins.

Key Words

Antigens, Bacterin , Cellular , Epitope, Humoral, immunogen, Protein,vaccine.

Introduction

Bacterial antigens[BAGs] were being investigated to be of use in,bacterial diagnosis, bacterial infection diagnosis,vaccine development and vaccine production. Immunogenicity of BAGs holds a backbone position in vaccine development strategies[1 – 7].The objective of the present work was the investigation of immunogenicity of protoplasmic sonicate protein antigens of skin burn infections with *P.aeruginosa* and *K.oxytoca*.

Materials and Methods

Protoplasmic Sonicate Protein Antigens P SPA;

The PSPA separation was in direct way for P.aeruginosa[8],while,by in direct way as it needs removal of the capsule [9,10] before processing for obtaining PSPs.The method for separation,identification,purification and quantitation of these PSPs were as in the method described by Bjorn et al.[8].The details of the processing method was as in the followings; Six mls of tris buffer 0.01 N and PH 8 was added to the surface of 24 hrs growth of

P.aeruginosa and K. Oxytoca(aftersuspenidg bacteria then removal ofcapsule[9,10]) on the nutrient agar plates.Growth were vortoxed in vortox test tubes for thee minutes. Suspensions were centrifuged at 5000 rpm for ten minutes.Supernatant were discarded and pellets Ps were kept.Ps suspensions were tubbed and washed three times with tris buffer 0.01N.Ps were reconstituted with 6 mls,and were tubbed in the cell disintegrator tubes. Then jacketed with cooled ice.The best sonication conditions were five times for five minutes at 20 oscillation ampiltude.The sonicated cell suspensions were centrifuged at 5000 rpm for ten minutes.Supernats were collected and ultrafiltred with 0.22 um milliporefilter.Fiterates were collected in sterile plastic tubes,then proteins were separated with PEG 6000 ,6% as inShnawa and AlSadi[11].The protein concentration determination was measured by Biurt test[12].To this end the preparations can be designated as PAPSP and KOPSP both P.aeruginosa and K.oxytoca respectively.These PSPs were distributed into alliqoutes of 0.5 mls. In anappend off plastic tubes and kept at -20C,till use.

Immune Reagents;

Specific immune priming of rabbits were donewith the PSP concentrations of 2.71 mg/l for PAPSP and1.8 md/l for KOPSP[12].The somatic antigens for both bacteria were prepared as heat killed as in [13].Complete Freund Ajuvant that from Difco,Co.Ltd. The test proposed immunogens were made as; one voliume of CFA mixed with one voliume the test proteins

Immunization Protocols

A mounthwise twice dosage of PSP-CFA in 2 mls amounts were dosaged in the first and second months to therabbits .The specific immune priming was that of multisite injection protocol[14].

Rabbits:

A local breed rabbits brought from the local market were checked for ecto and endoparasites .As well as for pyrogens and found to be free off.They were acclimatized for housing conditions two weeks befor experimentation at an ad libitum conditions.Among which nine were elected and subdivided into two test and one control group each of three rabbits.

Samplings And Immune Function Tests

At the temination of the specific immune priming protocols,blood with and with out heparin were collected from the test and control rabbits by cardiac puncture for humoral and cellular immune tests.Sera were saved for serology and cytokine studies.Heparinized blood

were used for leukocyte inhibitory factor[15] and for NBT phagocytosis[16].Appendix for test and control rabbits were collected and open up,washed from digesta and processed for separation of mucosal globulins[17].Mucosal leukocytes were separated by dextran 2% as in [18].IL 6 determinations were made as in the recommendation of the instruction of the manufacturer.Standared tube agglutination test were made as in [19].

Results

The NBT neutrophil phagocytosis percentages in PAPSPA(56.75 % for mucosal and36.5 for systemic) and KOPSPA(48.5% for mucosal , 41% for systemic)primed rabbits were higher than that of control rabbits(20% for mucosal,18% for the systemic).Leukocyte inhibitory cytoknine LIF % in PAPSP primed rabbits were56.6% for mucosal and 58.45 fo systemic as compared to control ,the mucosal was 90% and systemic was 86%.While for KOPSPA primed rabbits were; 88% for mucosal and 87.5% for systemic LIF as compared to normal control were 93% for mucosal and 89% for systemic responses.The IL6 concentration determinations were shownig that PAPSPA and KOPSPA primed rabbits have got higher IL6 concentration means than normal control rabbits.PAPSPA primed rabbits IL6 concentration means were 92.8 for mucosal and 72.7 for systemic responses.In KOPSPA primed rabbits the mucosal concentration means were 83.26 and for systemic were 76.79 as compared to normal control were 8.83 for mucosal and 9.25 for systemic responses.The humoral specific agglutinin titre levels for PAPSPA were 128 for mucosal and 320 for the systemic responses and KOPSP primed rabbitswere 64 for mucosal and 640 for systemic responses as compared to control rabbits were 4 for mucosal and 20 for systemic responses.Mucosal agglutinins were resistant to treatment with 2ME,Tables 1 and 2.

Table – 1 :The immunogenicity of PAPSP in primed rabbits and controls.

Rabbits groups	NBT%	LIF%	IL6 pg/ml.	Agglutinin titres
PAPSPA M S	 46.75 36.6	 56.6 58.45	 2.8 72.7	 128 320
Control M S	 20 18	 93 89	 92.8 72.7	 4 2 0

Table – 2 : The immunogenicity of KOPSP in primed and control rabbits

Rabbit groups	NBT%	LIF %	IL6 pg/ml	Agglutinin titres	

KOPSPA M S	48.5 41	88 87	48 72	64 640	
Controls M S	20 18	93 89	8.83 9.25	4 20	

Disscussion

The concept,application and continuity of the immunogenicity theme for bacterial protein antigens are still in the current mode of researchers allover the world[1-7].Immunogenicity appeared to have two main facets.First that of theoriticalimmunologisets which advocate that immunogenicity is denoted to self-nonself recognition theme[6].While the second facets was that for most of the proper immunologists which can be summerized as the ability of an antigen to initiate humoral and/or cellular conversion from the normal baseline immune functions to an optimized cellular immune reactions outcomes that are finalized by the optimest synthesis and production of cellular secretory proteins(antibodies,cytokines) concentrations and /or optimised depression of such secretory proteins[1-5,7]. Immunogenicity appeared to be essential for diagnosis and prophylaction of human infections as well as in cancer personalized prophylactic and therpeuticmedicine.Hence the presnt study for Pseudomonas and Klesiella immunogenicity may participate in developing of a porototype bacterial protein based vaccine and/ or in development for an autovaccine for multidrug resistant skin burn infection[20]

The antigenic make up of bacteria[1-5,7] like that of P.aeruginosa are fornedfrom;flagella,pili,exottoxinA,exoppolysaachride,LPS,OMP, hemolysin, elastases, proteasis, heat stable phospholipid,heat stable glycolipid,exoenzyme S and ribosome[21-23].While that of Klebsiella oxytocaare;capsule,somatic antigens and LPS[24].Hence, PAPSP and KOPSP proteins antigens are novel antigens prepared in this experimental settings.

PAPS was proved to be immunogenic in rabbits model,Table-1,promoting humoral and cellular immune responses both at mucosal andsystemi ccopartments.These findings were in contradiction with that of[24].While,KOPSPimmunogenicity,Table -2, were by promoting humoral immune responses both at mucosal and systemic level.[21].Different burn infecting bacterial protein preparations have shown different immune potential features,Table -3,[25].The functional epitope mapping for PAPSPmay be T cell depndent and T cell independent,while that of KOPSP may be of T cell independent or Th2 cell depent B cell epitopes[26,27].

Table – 3 : the immune features of the study bacterial proteins.

Features	PAPSP[25]	KOPSP[25]

1-Chemical nature	Protein	Protein
2-Origin	Bacterial	Bacterial
3-Location	Intracellular	Intracellular
4-Processing method	Ultrasonication	Ultrasonication
5-Non-specific immune function	Rise up of phagocytosis By NBT%	Rise up of phagocytosis by NBT %
6- Specific cellular immune function	Significant inhibion of LIF cytokine	Nonsignificant inhibition of LIF cytokines
7-Induction of cytokine network	Rise up of IL6 cytokine	Rise up of IL6 cytokines
8-Humoral Immune responses	Rise up of specific agglutinins	Rise up of specific agglutinins
9-Functional Epitope Mapping[26,27]	T cell dependent and T cell in-ndepedent epitopes	T cell independent and /or, TH2 dependent B cell epitope
10-Immuen system compartments	Mucosal and Systemic	Mucosal and systemic
11-Expected Immune potentials[20]	Prototype protein based vaccine and an autovaccine	Prototype protein based vaccine and an autovaccine.

Conclusions

P.aeruginosa PAPSP and K.oxytoca KOPSP protoplasmic sonicate proteins were found as lapin immunogens.PAPSP mediate humoral and cellular immune responses both at mucosal and systemic compartments.While,KOPSP mediate humoral immune responses both at mucosa and blood stream.These proteins may be of expected immune potentials as protein based vaccine candidate and as an autovaccine formultidrug resistant skin burn infections.

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