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Immunogenicity of Acellular Pertussis Vaccine

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Whooping cough is a severe, highly contagious respiratory disease caused by the bacterium *Bordetella pertussis* is an important cause of morbidity and mortality among children in many parts of world. Use of killed whole cell pertussis vaccine has been a major factor in control of symptomatic whooping cough. Immunization with whole cell vaccines (wcv's) has significantly reduced the incidence of pertussis. However these vaccines have drawbacks in causing side effects. An attempt was made in the present work to develop non toxic acellular vaccine that can protect against disease with reduced incidence of side effects. In the present study, recombinant acellular pertussis vaccine conferred to be 80% protection in test animals in comparison to standard vaccines.

Key words: Immunogenicity, Acellular pertussis Vaccine.

Whooping cough is an infectious respiratory disease caused by the bacterium *Bordetella pertussis*. Despite being preventable by vaccination, pertussis remains one of the top ten causes of death world wide in childhood, mainly in unvaccinated children[1]. According to the world health organization (WHO), about 17.6 million cases of pertussis occurred all over the world and about 279,000 patients died of pertussis in 2003[2]. Most of deaths occurred in the developing countries.

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Immunization with whole cell pertussis vaccines (WPVs) was started in the middle of 20 th century and was significantly reduced the incidence of pertussis in many countries[3]. However, these WPVs have drawbacks in causing side effects such as local pain, red ness, swelling, fever, including neurological effects and death[4,5]. Due to the fear of side effects and the need for booster immunizations in older age groups, acellular pertussis vaccines (APVs) were developed and they were first introduced in japan in 1981[6].

Typically current acellular vaccines are comprised of antigens directly purified from cultured B.Pertussis bacteria.They may include pertussis toxin [PT], filamentous hemagglutinin [FHA], Prn, Fim..In china, two component APVs containing PT & FHA have been developed and utilized since1990s[7].Clinical efficacy trails carried out indicated that APVs containing PT. In this report, we described extraction, purification of pertussis toxin.By using this toxin,we studied its immunogenicity and protective properties in mouse model

MATERIAL AND METHODS

Bacterial strains

B. pertussis strain, an Indian strain isolated and used for production of pertussis vaccine. Genomic DNA of this strain was used to generate recombinant proteins. *B. pertussis* strain 18323, an international reference strain, was used in the mouse intra peritoneal and intra cerebral assays.

Culture conditions

B. pertussis strains were grown at 37°c on Bordet-Gengou agar (Difco) medium supplemented with defibrinated sheep blood. E.Coli strains M_{15} (Quiagen) was used for the protein expression. They cultured in Luria Broth (LB) medium at 37°C.

Recombinant protein expression and purification

Construction of recombinant DNA fragments , protein expression and purification were performed as described[8]. Expression of recombinant protein was induced by addition of IPTG to a final concentration of 1mM. Expressed proteins were purified usingthe Histrap ™ HP column by the AKTA system (Amersham pharmacia, USA) according to the manufacturers recommendations.

Extraction

Breifly, the cells expressing recombinant protein was collected by centrifugation, and the pellet was sonicated on ice-bath. The recombinant protein was separated by centrifugation at 10,000g for 10 min at 4°C and is solubilized in a buffer solution (pH 8.0) containing 50 mM Na_2HPO_4 ,1M Nacl (Extaction buffer). Protein renature was processed by dialysis buffer containing 2M urea for 48 hrs. After dialysis the pH of dialyzed solution was adjusted to pH 6.0 with ortho phosphoric acid.

Purification

Purification of sample was performed by ion exchange chromatography method using carboxy methyl sepharose as cation exchanger. After adjusting the pH, sample was loaded on to the column equilibrated with buffer- A of pH 6.0. The column was washed with five column volumes of buffer- A. Then the column was eluted with buffer A of pH 7.4, which resulted in elution of pertussis toxin from the column. The elution fractions corresponding to the peak of the chromatogram were pooled and dialyzed against 50mM phosphate buffer saline A at pH 7.5.

Analysis by SDS-PAGE:

The purity of recombinant protein was analysed by 12 % SDS-PAGE , with 25 mM Tris,192mM glycine buffer (pH -8.3) that contained 0.1 % (w/v) SDS as the running buffer, as described by laemmli (1970), while the protein concentration was determined by the lowry method as described[8].

Western blotting

Western blotting was performed as described by Towbin *et al*[9]. In brief, recombinant pertussis protein was separated by SDS-PAGE and transferred on to nitrocellulose membrane using a semi-dry western transfer apparatus (Bio-rad,USA) at constant voltage (20v)

Non specific binding sites of the membrane was blocked by incubation with 5 % skim milk in phosphate-buffered saline (PBS) (pH - 7.4) containing 0.05 % Tween 20 for 30 min. The blot was then incubated with specific anti-PT antibodies.The blot signals were captured by using a DAB kit, following incubation with horseradish peroxidase-conjugated anti mouse secondary Ig G..

Batch	Dilution	No. of Immunized
Acellular pertussis vaccine	1X	5
	1/5X	10
	1/10X	10
Standard Cellular Vaccine	1:8	5
	1:40	10
	1:200	10
Challenge culture titrated	50 mice (11-15) grams for titrations	

RESULTS AND DISCUSSION

Immunization of Mice

Immunogenicitytest was done for the acellular pertussis vaccine by comparision with that of a standard cellular vaccine.50 swiss albino mice of 11-15gms were used for experiments. 25 mice were immunized intraperatoneal with purified pertussis vaccine of 0.5 ml at 1X , 1/5X ,1/10X concentrations.

Another 25 mice were immunized with 0.5 ml standard cellular pertussis vaccine in 1:8, 1:40, 1:200 dilutions

For intracerebral challenge assays, 50 mice were challenged with challenge strain (B. Pertussis, NIBSC strain No-18323) 14 days after immunization.The dose of challenge was 30 µl each. These challenged mice were observed for 14 days after challenge.

Batch No.	Dilution	No. of Challenged	No of Survivals	Death
Acellular Vaccine	1X(25µg)	5	4	1
	1/5X(5µg)	10	3	7
	1/10X(2.5 µg)	10	1	9
Standard Cellular Vaccine	1:8	6	5	1
	1:40	10	7	3
	1:200	9	1	8

Challenge Culture Titrations

No. of Organisms	No inoculated	Survivals	Death
100000	10	0	10
10000	10	0	10
2000	10	0	10
400	10	5	5
80	10	9	1

The observation at the end of the test was as follows..

The challenge culture titrations and the LD_{50} value obtained there of were indicative that the challenge dose was appropriate for the immunized mice in the test group and the standard group.

CONCLUSION

The purified toxin was found to be 90% pure. Three formulations with different strengths were prepared using the purified *pertussis* toxin to determine the dose response. The formulations were injected into the mice intra peritoneal and were challenged intra-cerebral 14th day post vaccination.

Formulations containing 25 µg of purified pertussis toxin conferred to be 80% protection in test animals. Control cellular vaccine has given 83% protection at 1:8 dilutions. Further animal studies have to be performed with higher dose of purified pertussis toxin to achieve better protection.

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