

Intranasal Mice Model to Study the role of *Bordetella pertussis* antigens in Immunity

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Pertussis known as whooping cough is a highly contagious disease. Whole cell pertussis vaccine is the most economical and effective strategy for preventing and controlling pertussis. The efficacy of whole cell vaccine is ascertained most commonly by intracerebral challenge assay, but it does not reflect the true efficacy of vaccine as Pertussis essentially is a respiratory disease. Therefore, in order to mimic the natural infection, intranasal challenge model in mice was developed. In intranasal challenge assay mice were immunized with vaccine and challenged through intranasal route. Mice lungs were dissected and examined for bacterial count. The degree of count was related to efficacy of vaccine, higher count indicated low efficacy and low count pointed to better efficacy.

Keywords: *Bordetella pertussis*, Agglutinin (agg), Intranasal Challenge Assay (INCA), Vaccine, Immunization, Protection

Introduction

Bordetella Pertussis is the causative agent of disease pertussis, also known as whooping cough or cough of 100 days¹. In the last two decades, epidemiology of pertussis has noticeably changed, with a shift of cases to adolescents, adults and children too young to have completed their infant immunization series². This resulted in higher infant mortality³. Vaccination is the most economical and effective strategy for preventing and controlling pertussis⁴. The whole cell pertussis vaccines should contain agglutinin 2 and 3 to produce vaccines with very high efficacy. Agglutinin 1 (agg 1) antigen which is present on fimbriae and cell surface of pertussis vaccine strains is not well studied for its protective role. Hence, in the present study an attempt has been made to investigate the role of agg 1 in intranasal challenge assay and intracerebral challenge assay.

Materials and methods

Bordetella pertussis 353 vaccine preparation:

B.pertussis 353 strain was obtained from Department of Molecular Genetics, University of Cincinnati, USA and vaccine was produced at Serum Institute of India Pvt. Ltd., Pune. A freeze dried

ampoule was reconstituted and used to inoculate growth medium. The bacterial suspension was centrifuged at 7000 rpm for 30 minutes in a cold centrifuge. Cell mass thus obtained was harvested in 200 ml 0.02 % thiomersal saline and inactivated at 56 °C for 15 minutes in a shaker water bath. Inactivated cell suspension was checked for opacity by measuring OD, purity by growth on nutrient agar plates, viability by growth on BG agar plates and presence of agglutinin antigens by slide agglutination method. Safety of this vaccine was established in mouse weight gain test and test for absence of dermonecrotic toxin (DNT)⁵. Vaccine was stored at 2-8 °C for further use in the study.

Points considered while developing Intranasal Challenge Assay (INCA)

- Mice strain selection,
- Selection of dilution for immunization,
- Selection of method for Challenge preparation.

Mice strain selection

NIH, Balb/c and Swiss albino female mice, 6-8 weeks of age were bred and maintained at Serum Institute of India Pvt. Ltd. (Siip), Pune, India, animal house. Animals were kept in well ventilated cages and with free access to food palettes and water. Unimmunized mice of each strain were taken in a group and were challenged with 25 µl virulent *Bordetella pertussis* 18323 strain intranasally. A challenge dose of 2×10^8 organism/ ml was used in

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the study. Six mice of each strain were dissected for lungs after 2 hrs of challenge and remaining six after 7 days of challenge. Dissected lungs were then homogenised and plated on BG agar plates. Plates were incubated at 37 °C for 4 days and CFU (Colony Forming Unit) count was done.

Selection of method for Challenge preparation

Bordetella pertussis 18323 challenge preparation was prepared by spectrophotometer method and liquid nitrogen method. In spectrophotometer method, lyophilized tube of challenge organism was opened and revived on BG agar slant. After second passage the growth was harvested and diluted to contain 10 IOU as measured spectrophotometrically. A dilution which contained 10 IOU is further diluted to contain 2×10^8 organisms. Liquid nitrogen challenge preparation at 10 IOU was prepared as described previously⁶ and further diluted to contain 2×10^8 organisms.

Selection of dilution for immunization

Bordetella pertussis RWRS vaccine with assigned unitage of 63 IU, which contain agg 1, 2 & 3 was used for immunizing a group of six mice intraperitoneally at 4 IU/ml and 0.2 IU/ml concentration. But no bacterial count was observed at 4 IU/ml in lung homogenates, so a low dose range of 0.2 IU/ml and 0.04 IU/ml was used in following experiments. *Bordetella pertussis* 353 strain was first diluted to 32 IOU and then immunized at 1:2 and 1:40 dilution. Very low bacterial count was observed at 1:2 dilution of immunization. Therefore a higher dilution range of 1:8 and 1:40 was used in further experiments. A comparison of *Bordetella pertussis* 353 vaccine efficacy in terms of lung CFU count was also made in intranasal challenge assay with Siipl pertussis vaccine production strains e.g. Bp1, Bp2 and Bp3 at 1:8 and 1:40 dilution of immunization.

Intracerebral challenge assay

Intracerebral challenge assay was performed in Swiss albino mice as per method described in WHO TRS 941⁵. Potency of *B. pertussis* 353 vaccine preparation was estimated against working standard and compared with Bp1, Bp2 and Bp3.

Results and Discussion

Bordetella pertussis 353 vaccine preparation:

Opacity of 205 IOU was assigned to *B. pertussis* 353 vaccine preparation. This vaccine was shown to contain only agglutininogen 1 as it showed positive

reaction with NIBSC reference anti agglutininogen 1 serum and not with anti agglutininogen 2 and anti agglutininogen 3 serum. The vaccine was found safe in mouse weight gain test as 90.18% weight gain against a passing criterion of 60% was observed. The vaccine was also checked for absence of DNT and no dermonecrosis in nuchal region of suckling mice was seen after administering the vaccine.

Mice strain selection

Unimmunized NIH, Balb/c and Swiss albino mice were dissected after 2 hrs of challenge and the CFU count obtained after plating their lung homogenates indicated that initial infection had been established (Figure 1). An increased count was observed when remaining mice in each strain group were dissected after 7 days of challenge. The highest increase was recorded for NIH mice strain and amounting to 2.1 log₁₀. For Balb/c mice strain, the difference between CFU count after 2 hrs and 7 days of challenge was 1 log₁₀ and in case of Swiss albino this difference was 1.53 log₁₀. But in case of Swiss albino mice, the count was found least among all three mice strains in both initial as well as after 7 days of challenge (Figure. 1).

Selection of method for Challenge preparation

Two methods for challenge preparation viz liquid nitrogen and spectrophotometer were evaluated in INCA. Initially after 2 hrs of challenge almost similar bacterial count was observed for both the preparation whereas after 7 days of challenge a slightly higher count was observed for liquid nitrogen preparation and the difference in CFU count was 1.18 log₁₀ (Figure 2).

Selection of dilution for immunization

Freshly reconstituted *Bordetella pertussis* RWRS vaccine with an assigned unitage of 63 IU was assayed in INCA in two separate experiments. In first

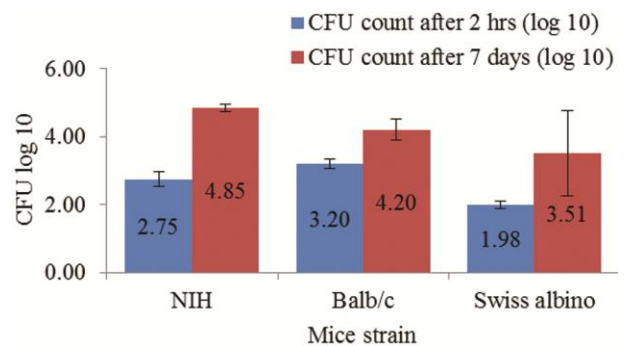


Fig. 1 — NIH, Balb/c and Swiss albino mice strain evaluation for sensitivity to *Bordetella pertussis* 18323 infection after intranasal challenge (Data represents Means ± SE).

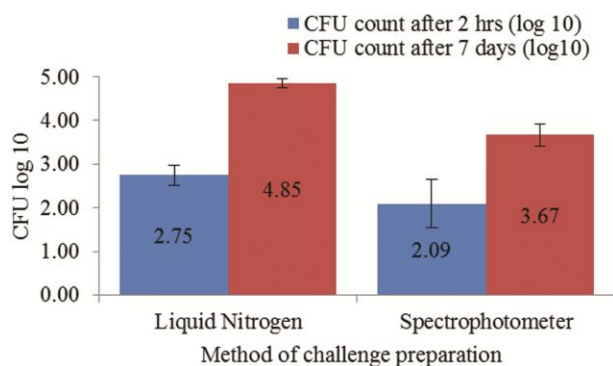


Fig. 2 — Comparison of Liquid nitrogen and Spectrophotometer method (Data represents Means \pm SE).

experiment, 4 IU/ml and 0.2 IU/ml concentrations were used to immunize mice intraperitoneally. Total bacterial clearance at 4 IU/ml concentration was obtained and at 0.2 IU/ml concentration the CFU count was found to be 2.43 log₁₀. Since there was a measurable response at only 0.2 IU/ml dose, it was decided to immunize mice at 0.2 IU/ml and 0.04 IU/ml dose level in the next experiment. An increase in bacterial count from 1.83 log₁₀ to 3.52 log₁₀ was seen as dose of vaccine was reduced from 0.2 IU/ml to 0.04 IU/ml. Similarly, *Bordetella pertussis* 353 vaccine was immunized at 1:2 and 1:40 dilution after normalizing it to 32 IOU. A count of 1.08 log₁₀ increased to 1.88 log₁₀ as vaccine dilution was increased from 1:2 to 1:40. In the next experiment, *Bordetella pertussis* 353 vaccine was immunized with a difference of 5 fold in two dilutions i.e. 1:8 and 1:40. A CFU count of 1.48 log₁₀ and 2.32 log₁₀ was observed for dilution 1:8 and 1:40 respectively. The observed CFU count was dose dependent as high dose of vaccine resulted in low CFU count and low dose in high count. The protective capacity of *Bordetella pertussis* 353 vaccine (which contain agglutinin 1 only) against infection was compared with Siipl pertussis vaccine production strains Bp1 (contain agglutinin 1 & 3), Bp2 (contain agglutinin 1 & 2) and Bp3 (contain agglutinin 1, 2 & 3). An unimmunized mice group was also included in the study. All pertussis vaccines were first normalized to 32 IOU and then used at 1:8 and 1:40 dilution to immunize allocated group of six mice. At 1:8 dilution a mean CFU count of 1.33 log₁₀ was observed for *Bordetella pertussis* 353 vaccine and this increased to 2.97 log₁₀ at 1:40 dilution. Unimmunized mice group showed a lung CFU count of 3.26 log₁₀ after 7 days of challenge. In case of Bp1, Bp2 and Bp3 strain total lung clearance was observed at 1:8 dilution for

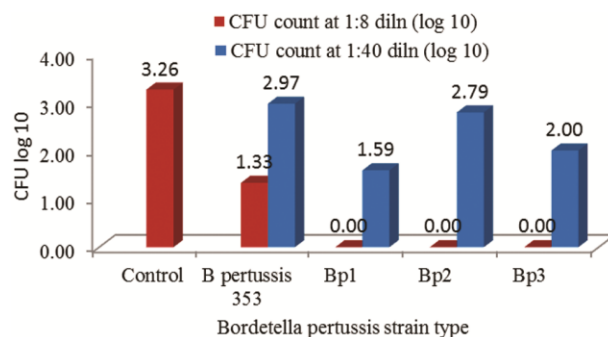


Fig. 3 — Protective capacity of *Bordetella pertussis* 353 vaccine in intranasal challenge assay in terms of CFU log₁₀ count was compared with unimmunized control group and with Siipl pertussis vaccine production strains Bp1, Bp2 and Bp3.

immunization (Figure. 3). In 1:40 dilution, a lung CFU count of 1.59 log₁₀, 2.79 log₁₀ and 2.0 log₁₀ was observed for Bp1, Bp2 and Bp3 vaccine respectively (Figure 3).

In intranasal challenge assay *Bordetella pertussis* 353 vaccine which contains agg 1 only, conferred protection as indicated by low CFU count⁷ in comparison to unimmunized control group. The protection against lung infection was dose dependent which was also reported earlier by Xing *et al.*⁸ in aerosol challenge model. But this protective efficacy was inferior to Siipl pertussis vaccine production strains Bp1, Bp2 and Bp3. This may be due to presence of agg 2 and/or agg 3 in addition to agg 1 on these strains as azgg 2 and agg 3 antigens were found to confer protection by Goringe *et al.*⁹. Potency of *Bordetella pertussis* 353 vaccine was also estimated in an intracerebral challenge assay, which is the only test that had shown a correlation with protection in children¹⁰. Results in agreement with the intranasal challenge assay were obtained where *Bordetella pertussis* 353 showed a low mean potency of 2.34 IU/dose in comparison Bp1, Bp2 and Bp3 vaccine strains, which showed a potency of 5.76 IU/dose, 7.95 IU/dose and 7.05 IU/dose respectively. Since *B. pertussis* 353 vaccine used in the study was a whole cell preparation and it contain many other antigens in addition to agglutinin 1, the protection observed in both challenge assays could be attributed to their presence.

Conclusion

In the present research work, Intranasal Challenge Assay (INCA) was developed and protective capacity of *Bordetella pertussis* 353 vaccine, which contains agg 1 only was evaluated. Three mice strains NIH,

Balb/c and Swiss albino were evaluated for use in INCA. Although initial lung infection or colonization was slightly higher for Balb/c strain as compared to NIH strain, but the extent of propagation of infection was highest for NIH strain. Swiss albino mice strain was found to be least sensitive since lung CFU count after 2 hrs and 7 days of intranasal challenge was least among three mice strains. Based on these results NIH mice strain was selected for use in INCA. Two methods liquid nitrogen and spectrophotometer method were evaluated for intranasal challenge preparation. The challenge preparation which was made using liquid nitrogen method, CFU count after 7 days of challenge was higher as compared to spectrophotometer method. Liquid nitrogen method was used in further experiments since better lung colonization was achieved in this method. Novotny *et al.*⁶ had also used single lot of frozen *Bordetella pertussis* 18323 challenge strain and this method provides additional advantage of controlling the assay variation.

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