



Comparative immune response of purified native OmpH protein derived from *Pasteurella multocida* P52 and oil adjuvant vaccine against hemorrhagic septicemia in mice

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ABSTRACT

Hemorrhagic septicemia (HS), caused by *Pasteurella multocida* serotype B:2, is an economically important disease of cattle and buffalo; and causes serious economic losses to cattle industry in Asian subcontinent. In the present study Native OmpH protein of *Pasteurella multocida* (serotype B: 2) strain P52 was purified and used for immunization of mice. Humoral and cell mediated immune response were assessed by ELISA and MTT respectively. Protective ability of vaccine was assessed by direct challenge test. The immune response of native OmpH protein was compared with standard oil adjuvant vaccine (OAV). High titers of specific antibody and strong T cell proliferative response were observed in mice, vaccinated with native OmpH protein as well as OAV, as compared to PBS control group. Furthermore, both the vaccines induced balanced Th1 and Th2 response as determined by IgG isotype analysis. Despite strong humoral and cell mediated immune response native OmpH vaccine could only provide partial protection to mice against direct challenge with live *P. multocida* P 52.

Key words: Haemorrhagic septicemia, Native OmpH protein, Oil adjuvant vaccine, *Pasteurella multocida*

Hemorrhagic septicemia (HS), caused by *Pasteurella multocida*, is an economically important infectious disease of cattle and buffalo. This disease has to be controlled as it is still a severe problem in the cattle industry in India. Several vaccines, viz. alum-precipitated vaccine, aluminum hydroxide gel vaccine and oil adjuvant vaccine (OAV) are used to control the HS in India.

OAV is considered as the most potent vaccine for the control of HS with 1 year protection (Muneer *et al.* 2005, OIE 2008). The inactivated vaccines (bacterins) induce only serotype-specific immunity and oil adjuvant vaccine suffers from inherent disadvantages of poor syringeability due to higher viscosity, thus the search for better and effective vaccine against HS is still going on.

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Outer membrane proteins (OMPs) are potent immunogens of *P. multocida* and confer protection to poultry (Zhang *et al.* 1994), rabbits (Lu *et al.* 1991), mice (Srivastava 1998) and buffaloes (Pati *et al.* 1996). Major *P. multocida* OMPs include OmpH, P6-like protein (Omp16), PlpB, OmpA and Omp 87 among which the heat-modifiable or OmpA and the porin or OmpH proteins are important immunogens (Hatfaludia *et al.* 2010). The role of OmpH in protective immune response has been studied in recent past. A monoclonal antibody (mAb) against this protein passively protect mice against *P. multocida* infection (Vasfi Marandi and Mittal 1997). The purified native OmpH induces protective immunity in chickens against homologous strain challenge (Luo *et al.* 1997). Synthetic peptide vaccine mimicking the conformational epitopes of native protein OmpH, provided protection in chickens against bacterial infection (Luo *et al.* 1999). Lee *et al.* (2007) cloned and expressed OmpH from a pathogenic strain of *P. multocida* causing atrophic rhinitis. Recombinant OmpH proteins were found to be antigenic and provided strong protection against *P. multocida* infection. Recombinant OmpH elicited protective immunity in chickens against homologous challenge.

Though some work has been done on whole OMPs of *P. multocida* B:2 (Pati *et al.* 1996, Srivastava 1998) but no comparable report is yet available regarding immune

response and protective ability of OmpH protein of *P. multocida* B:2. In this report, we isolated and purified the native ompH porin protein of *P. multocida* B:2 strain P52 and evaluated its immune response as well as protective ability in mice model and compared with existing OAV.

MATERIALS AND METHODS

Mice: White albino mouse of either sex, 4 to 6-week-old obtained from Laboratory Animal Resource Division (LAR) of the Institute were used for studying immune response and protection ability of native OmpH protein. The experimental protocols used to perform various studies were approved by Institutional Animal Ethics Committee (IAEC).

Bacterial strain: The reference strain *P. multocida* Serotype B:2, strain P52, obtained from Division of Biological Standardization, IVRI, Izatnagar, was used for purification of native OmpH protein and challenge studies.

Purification of native OmpH protein: The native OmpH protein was purified by zinc reverse staining and electro-elution method (Walker *et al.* 2002). The proteins were obtained by acetone precipitation and stored at -20°C / -80°C till further use under freeze-dried condition.

Immunization of mice: Six week old 39 mice, were randomly divided in 3 groups of 13 mice each. The first group of mice received 2 injections of native OmpH protein-mixed with Freund's complete adjuvant (FCA) @ $50\mu\text{g}$ into each tibialis anterior muscle, for a total of $100\mu\text{g}$ per animal and were boosted with same protein on day 14th. Second group was injected with OAV and third group by only PBS. The second and third groups served as control. Sera samples were collected at weekly interval starting from day 0 to day 28 for evaluation of humoral immune response.

Humoral immune response studies: The indirect ELISA was performed following standard methods (Engvall and Pearlmann 1971) to evaluate humoral immune response.

Cell mediated immune response (CMI): was assessed in vaccinated as well as in control mice at 4th week post immunization as per Mossman (1983).

Direct challenge studies: Protective ability of vaccine was assessed by direct challenge test. On 29th DPV, 10 mice from each vaccinated group were challenged with 0.1 ml of 100 LD₅₀ of 18 h broth culture of *P. multocida* P52 by intraperitoneal route and observed for 7 days. Mortality pattern was carefully observed and recorded.

Statistical analysis: Data obtained in the study are expressed as mean $\pm$ standard deviation (SD) and were analyzed by Duncan's and Turkey's post hoc test using SPSS version 11.0 and was used to detect significant variance between different vaccine groups.

RESULTS AND DISCUSSION

The immune response and protective ability of whole OMP of *P. multocida* P52 was evaluated by many workers (Pati *et al.* 1996, Srivastava 1998). Native OmpH, synthetic

OmpH peptide (Luo *et al.* 1999) and recombinant OmpH (Lee *et al.* 2007) derived from *P. multocida* of avian and swine origin were found protective in fowl against fowl cholera and pigs against atrophic rhinitis. In the present study, immune response and protective ability of native ompH protein of *P. multocida* P52 was evaluated in mice model.

Indian *P. multocida* P52 strain was used in the present study. This has the ability to cause fatal septicemic infections in susceptible species including buffalo-calves and hill-bulls after experimental challenge. The strain due to its antigenic superiority is widely used for vaccine formulation in India.

Oil adjuvant vaccine (OAV) is the most potent vaccine available to control the outbreak of hemorrhagic septicemia in cattle and buffaloes (Muneer *et al.* 2005, OIE 2008). Thus the immune response of native OmpH protein was compared with OAV.

Humoral immune response of vaccine was assessed by indirect ELISA. OMP antigen specific total sera IgG levels were detected in both the vaccinated group as early as 7th DPV (day post vaccination) and antibody titre increased after booster. In the group of mice vaccinated with native OmpH, protein significantly high ($P < 0.05$) ELISA titre was observed on 14th days post immunization (DPV) and achieved peak on 21st DPV and started decreasing thereof. But the peak antibody titer in group of mice vaccinated with OAV was obtained on 28th DPV. The OAV work by forming depot of antigen and letting its slow release over long course of time, protect the antigen from rapid degradation by enzymes and recruit the antigen presenting cells (APC) at the injection site and enhances the antigen uptake by these APC (Aguilar and Rodriguez 2007).

In order to find out whether different group of vaccine induced a Th1 and Th2 response in vaccinated mice, level of IgG1 and IgG2a specific to different vaccine was also determined by indirect ELISA. The kinetics of IgG1 and IgG2a levels in serum of different group of vaccinated mice is depicted in Figs 2 and 3, respectively. Quantitative isotype analysis showed a Th₂ bias in both the vaccinated group on the basis of significantly higher ($P < 0.05$) proportion of IgG1 as compared to IgG2a in both OAV and OmpH vaccinated

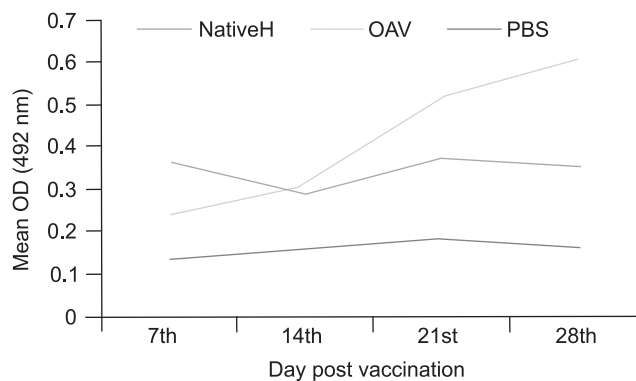


Fig. 1. Total serum IgG of different vaccinated groups.

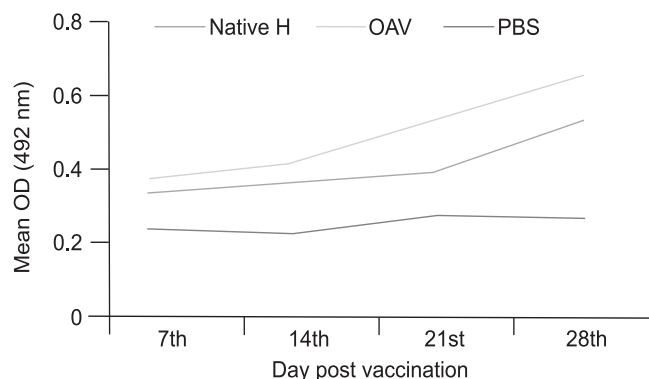


Fig. 2. Total serum IgG1 level of different vaccinated groups.

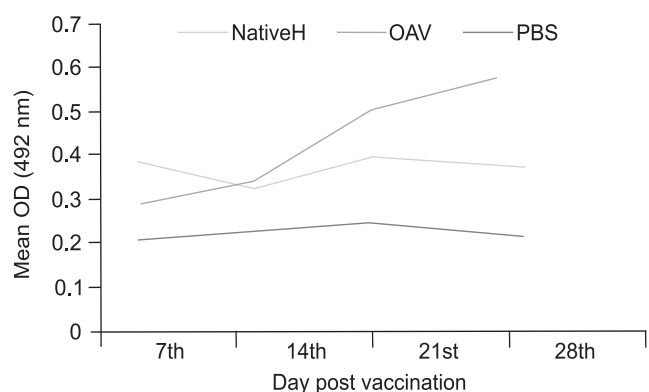


Fig. 3. Total serum IgG2a level in different vaccinated groups.

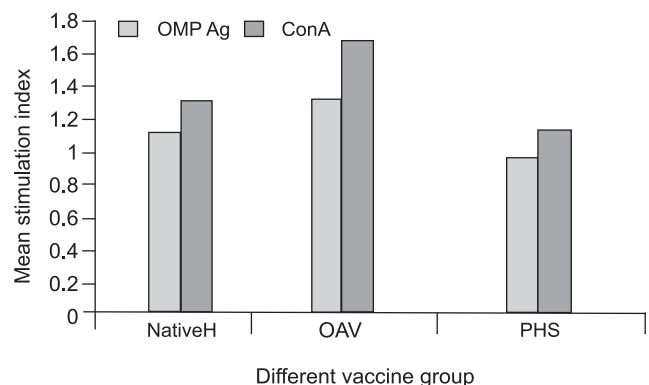


Fig. 4. *In vitro* proliferation of mice splenocyte immunized with different vaccines.

mice. Serum IgG1 level was significantly higher ($P < 0.05$) in OAV mice as compared to native OmpH vaccinated mice. Initially up to 14th DPV serum IgG2a was significantly higher in mice vaccinated with native OmpH protein, but later on IgG2a was higher in OAV group. Since, OAV act as depot and release of antigen is slow so level of IgG2a was lower till 14th DPV as compared to native OmpH group but later on it increased and persisted till 28th DPV. Thus preponderance of IgG1 response indicates the dominance of humoral response in both the vaccinated groups. This is in consonance

with (Gu *et al.* 1999) that exogenous antigen like recombinant and native protein favors humoral response.

In vitro proliferation of CD8⁺ T Lymphocyte collected from native OmpH and OAV immunized mice after their stimulation with OMP antigen suggested that both native OmpH and OAV elicited strong T- cell proliferation response. But response was significantly higher in OAV groups. Thus OAV elicited strong CMI as compared to native OmpH vaccine.

Despite of remarkable humoral and CMI, on direct challenge with *P. multocida* P 52 protection percentage (Table 1) in native ompH vaccinated group was only 40% whereas it was 80% in OAV group. The interesting observation of this study was that the group vaccinated with native OmpH protein showed comparable humoral immune response as well CMI response with OAV but protection level was lower.

The possible explanation may owe to change in conformational epitope of native protein during purification or storage which rendered protein less immunogenic. According to previous reports of immunization studies with other bacterial porins, the native conformation of porin was considered crucial for induction of protective immunity (Luo *et al.* 1999). Since purified native OmpH protein was stored for some time before immunization without protease inhibitor. It is likely that some protease contamination might have changed the conformational epitope of vaccine. Luo *et al.* (1997) found native OmpH of *P. multocida* X-73 to be 100% protective but when it was treated with protease it provided 0% protection. At the same time, it is not always necessary that vaccine eliciting strong immune response will always be 100% protective. Therrien *et al.* (2007) also reported lack of protection of mice against *Staphylococcus aureus* despite a significant immune response following immunization with a DNA vaccine encoding collagen-binding protein. The lack of protection may owe to difference in immunoglobulin isotype specificity in protection against *P. multocida*. Lower CMI response elicited by native OmpH vaccine may be another possible reason for low protection which is also required for protection against *P. multocida*. Verma and Jaiswal (1997) suggested that both humoral and cell-mediated immune responses contribute to protection in vaccinated animals against hemorrhagic septicemia.

Further study regarding effect of addition of protease inhibitor in native OmpH protein as well as effect of storage

Table 1. Protection level of different groups of mice challenged with *P. multocida* B: 2 (P52 strain)

Groups	Number of mice challenged	Number of mice survived	Protection percentage
NativeH	10	4	40
OAV	10	8	80
PBS	10	0	10

on immunogenicity of vaccine can better explain the reason for lack of protection. The possibility of native OmpH protein used with OAV in prime boost regimen can also be explored to enhance the duration of immunity of existing OAV.

The present study demonstrated that a native protein OmpH produced a strong specific antibody and cellular response in mice against *P. multocida*. This is for the first time immune response of native OmpH protein of *P. multocida* serotype B: 2 were evaluated. This native OmpH elicited comparable immune response to OAV, but protection level was lower. This protein can be used in combination with other vaccine in prime boost regimen to enhance duration of immunity of existing vaccine. Further study with the use of different adjuvant can be undertaken to maximize the protective ability of this vaccine.

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