

Vaccination with *Salmonella* Typhi recombinant outer membrane protein 28 induces humoral but non-protective immune response in rabbit

Anjani Saxena¹, Rajesh Kumar² and Mumtesh Kumar Saxena¹

1. Department of Veterinary Biochemistry and Physiology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India; 2. Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Corresponding author: Mumtesh Kumar Saxena, e-mail: mumtesh@rediffmail.com

Co-authors: AS: anjanimumtesh@rediffmail.com, RK: rajeshvet@rediffmail.com

Received: 16-03-2017, **Accepted:** 13-07-2017, **Published online:** 19-08-2017

doi: 10.14202/vetworld.2017.946-949 **How to cite this article:** Saxena A, Kumar R, Saxena MK (2017) Vaccination with *Salmonella* Typhi recombinant outer membrane protein 28 induces humoral but non-protective immune response in rabbit, *Veterinary World*, 10(8): 946-949.

Abstract

Aim: Typhoid is one of the most important food and water borne disease causing millions of deaths over the world. Presently, there is no cost effective vaccine available in India. The outer-membrane proteins (Omps) of *Salmonella* have been exhibited as a potential candidate for development of subunit vaccine against typhoid. The objective of the present study was to evaluate the use of recombinant Omp 28 protein for immunization of rabbit to elucidate its protection against virulent *Salmonella* Typhi.

Materials and Methods: Immune potential of recombinant Omp28 was tested in New Zealand Rabbits. Rabbits were divided into two groups, i.e., control and test group. Control group was injected with phosphate buffer saline with adjuvant while test group were injected with recombinant Omp28 along with adjuvant. Rabbits were bleed and serum was collected from each rabbit. Serum was tested by Enzyme-linked immunosorbent assay (ELISA) for humoral response. Rabbits were challenged with virulent culture to test the protective immunity.

Results: Humoral response was provoked at 15th day and maintained till 30th day. The mean ELISA titer at 15th day was 1 : 28000 (mean titer log 10 : 4.4472) and on the 30th day was 1 : 25866 (mean titer log 10 : 4.4127). Protective immune potential of Omp 28 was assessed by challenge studies in rabbits for which vaccinated and control rabbits were challenged with 10⁹ cells of virulent culture of *S. Typhi*. In control group, out of six, no rabbit could survive after 48 days while in vaccinated group, three out of six rabbit were survived.

Conclusion: Immunization of rabbit with recombinant Omp 28 induced a strong humoral response which was exhibited by high antibody titer in ELISA. Subsequently, intraperitoneal homologous challenge of the immunized New Zealand rabbit resulted in lack of significant protection. These findings indicate that Omp 28 though provoked the humoral immunity but could not provide the protective immunity in rabbit model.

Keywords: adjuvant, outer-membrane proteins, recombinant protein, *Salmonella*, vaccine.

Introduction

Typhoid fever is a public health problem with an estimated 22 million cases and 220,000 related deaths occurring worldwide each year [1]. It is a life-threatening illness caused by *Salmonella* enterica serovar Typhi. With the advancement of molecular biology polymerase chain reaction (PCR)-based techniques have been extensively used for detection and differentiation of pathogens [2-6] which had made possible to detect the disease in early stages but the development of resistance to antimicrobial agents and the possible reversal of the resistance in *S. Typhi* has become a significant issue leading to difficulties in the management of disease [7,8]. Vaccination against *S. Typhi*

is an essential tool for the effective management of typhoid fever. Presently available typhoid vaccines have several limitations such as short term immunity, and they are not cost effective [9]. Therefore, there is a need for better and improved new generation vaccine against *S. Typhi*.

The outer-membrane proteins (Omps) are conserved protein of Gram-negative bacteria and have immune reactivity. Several Omps had been isolated and targeted as an antigen and few of them had exhibited their immune potential [10,11]. However, it is very suggestive to purify a single Omp for commercial vaccine production as it is not cost effective. To overcome from this problem recombinant DNA (rDNA) technology had been used for bulk production of desired protein. Several Omps had been target as a candidate for r-DNA vaccine against typhoid [11,12]. Omp 28 is which had been purified from *Salmonella* Typhi. It was further sequenced, and few tests had been conducted to assess its immune potential in which it had exhibited promising potential [13,14]. Therefore, in our earlier study, we targeted Omp 28 as rDNA vaccine against *S. Typhi*.

Copyright: Saxena, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

We had cloned, sequenced and mapped major epitopes (B and T epitopes) of Omp 28. Our preliminary study on the basis of bioinformatic analysis indicated that Omp 28 can provoke humoral as well as cell mediated immunity [15]. Similar type of findings was reported in the case of *S. Typhimurium* [16]. Therefore, we expressed Omp 28 and in the present study tested the immune potential of Omp 28 in rabbit model to explore the possibility of development of Omp 28 based subunit vaccine against *S. Typhi*.

Materials and Methods

Ethical approval

The ethical approval for this work was accorded by Institutional Animal Ethics Committee of G.B.P.U A&T Pantnagar.

Bacterial strains and vectors

The culture of *Salmonella* Enterica subsp. enterica serovar Typhi MTCC 733 was procured from Institute of Microbial Technology (Chandigarh, India) and grown on LB agar. *Escherichia coli* strain DH5 α used in the cloning experiments was purchased from Bangalore Genei (India) and grown in Luria broth. The *E. coli* M₁₅ cells were used for the expression of recombinant protein, and pQE 30 expression vector was procured from Qiagen (USA).

Animals

New Zealand rabbit of 6 weeks was procured from Central Drug Research Institute (CDRI), Lucknow, Uttar Pradesh, India. Animals were certified as disease and pathogen free by CDRI, Lucknow, Uttar Pradesh, India. 12 rabbits were used in vaccine trial. Animals were reared as per the ethical guidelines approved by Institutional Animal Ethics Committee of the university. Animals were housed in small animal house of the department and provided the recommended feed and water. Before vaccination, all the rabbits were bleed by ear veins under aseptic conditions. Serum was collected and tested for pre-existing antibodies against *Salmonella* by double immune diffusion test with *Salmonella* Omp antigen. Animals were also tested by cloacal swab culture for the presence of *Salmonella*.

Vaccination and trial studies in rabbit

The rabbits were divided into two equal groups. The first group of six rabbits was taken as control and injected with adjuvant in phosphate buffer saline (PBS). The other group, test group of six rabbits was used for vaccination with r-Omp 28 antigen. Test group was injected subcutaneously with 100 μ g purified recombinant protein with equal amount of adjuvant, followed by two boosters at 21st and 42nd day post vaccination. Rabbits were bleed 15th day after last booster. But no significant titer was observed. Therefore, two more boosters of 500 μ g with equal amount of adjuvant were injected at 15 day intervals. Controls were also injected with same volume of PBS with adjuvant with same schedule.

Collection of sera

Rabbits (control and vaccinated) were bled through ear veins on the 15th and 30th day after last booster and blood were collected in sterile microfuge tubes. The collected blood samples were kept at room temperature for an hour and further kept at 4°C for retraction. Finally, the serum was separated and kept at -20°C till further use.

Humoral immune response

Sera collected from rabbits at 15th and 30th day were used for assessment of humoral immune response by indirect enzyme-linked immunosorbent assay (ELISA) [17]. 96 well ELISA plates were charged with 100 ng/well protein and incubated overnight at 4°C. Plates were washed with Phosphate buffer saline with tween (PBST) 0.5% twice to remove unbound antigen and blocked with 2% bovine serum albumin for 1 h. Serum from each rabbit was diluted and used in duplicate. Plates were incubated at 37°C for 2 h followed by three washing with PBST to remove unbound primary antibodies. After three washings 100 μ l secondary antibodies (anti rabbit HRP conjugate) in dilution of 1:3000 were poured in each well. Plate was incubated at 37°C for 2 h, followed by three washing with PBST. Substrate ortho phenyl diamine was added, and after the development of color OD was taken at 450 nm (substrate buffer: 0.1 M citric acid solution, 0.2 M dibasic sodium phosphate solution, and ortho phenyl diamine 40 mg, H₂O₂ 40 μ l). Dilution at which OD of vaccinated group was more than twice of control group was considered the titer of ELISA. Mean optical density at the dilution of 1:25600 of test and control group was compared to determine the efficacy of humoral response. To assess the protective immune response of vaccine 4 weeks after the last booster dose rabbits were challenged with 10⁹ CFU (I/P) of virulent strain of *S. Typhi* [18]. Mortality as observed till 48 h of challenge.

Statistical Analysis

Humoral response in vaccinated and control group at different time interval was analyzed using unpaired t-test, and the mean titer was calculated by deriving the mean of logarithmic value of reciprocal of titer with standard error. The results of challenge studies were analyzed using Chi-square test with Yate's correction.

Results and Discussion

Vaccination trial and humoral response to vaccine

The humoral response in mice was analyzed by indirect ELISA test. Humoral response was provoked at 15th day and maintained till 30th day. The mean ELISA titer at 15th day was 1 :28000 (mean titer log₁₀ : 4.4472) and on the 30th day was 1 : 25866 (mean titer log₁₀ : 4.4127). The mean absorbance value of control and vaccinated group was compared at the dilution of 1:25600 (Figure-1) it was found to be 0.061 and 0.1241 on 15th day and 0.061 and 0.1391

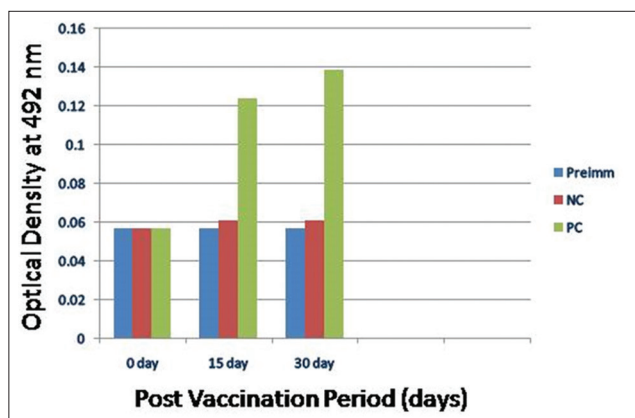


Figure-1: Mean absorbance values of the humoral immune response in rabbits evaluated at different time interval at dilution 1:25600. Preimm: Pre immunized serum, NC: Negative control, PC: Positive control.

on 30th day respectively. Humoral response in vaccinated and control group at different time interval was analyzed using unpaired t-test which was maintained till 30th day.

Protective immune potential of Omp 28 was assessed by challenge studies in rabbits for which vaccinated and control rabbits were challenged with 10⁹ cells of virulent culture of *S. Typhi* [18]. In control group, out of six, no rabbit could survive after 48 days while in vaccinated group, three out of six rabbit were survived. The findings indicated that though the mortality was reduced after the vaccination with Omp 28 but Omp 28 could not provide complete protective immunity. First report on Omp 28 indicated that the level of antibodies against Omp 28 was very high in serum of collected from patients of typhoid [13]. Further, mice antibody raised against Omp 28 in mice showed anti-salmonella activity [14] *in vitro*. These results indicated the immunogenic importance of Omp 28 isolated from *Salmonella*. Therefore, Omp 28 was selected for the study. Many other workers had also tested recombinant Omps such as Omp A, Omp C, Omp F, Omp S₁, and Omp S₂ for their immunogenic potential. Among these proteins, Omp A, Omp C, and Omp F had exhibited immunogenicity [19] but could not achieve complete protection.

Two porins Omp S₁ and Omp S₂ from *S. Typhi* could produce of long term antibody titer and complete protection against *S. Typhi* challenge [20]. Porins Omp S₁ and S₂ induced tumor necrosis factor interleukin (IL)-6 and IL-10 production. Omp S₁ and Omp S₂ despite being expressed at low levels under *in vitro* culture conditions proved as potent protective immunogen with intrinsic adjuvant properties. Similarly, Omp L had also been tested in mice model and had shown a promising protective immunity [21]. It was highly immunogenic and provoked humoral and cell mediated immune response. It conferred 100% protection to immunized mice against *S. Typhi* challenge studies. PagN an Omp of *S. Typhimurium*

as a potential vaccine candidate for salmonellosis. PagN has also been cloned, expressed and tested for its immune potential in mice and considered as a target molecule for the development of rDNA vaccine against *S. Typhi* [11].

An adhesion protein of *S. Typhi* named as T 2544 as potential targeted for vaccine development [22]. It had produced a high titer of IgG and IgA and strongly immunogenic and proved as potential vaccine candidate. Similarly, recombinant Omp C protein of *Salmonella* has been tested in birds for immunization of birds to elucidate its protection against virulent *S. Typhimurium* [23]. They reported r-Omp C induced a significantly high humoral immune response with a stable cell mediated immune response. Besides immune response a protective index ranged between 50% and 75% has also been observed for 3 weeks after challenge. The findings of earlier workers and our study indicate that several Omps have been targeted for development of rDNA vaccine against *Salmonella*. Some of them such as Omp S₁, Omp S₂, Omp L, and PagN have exhibited the promising potential, but some other proteins such as Omp A, Omp F, and Omp 28 could produce only partial protection. Therefore, there is a need to evaluate the effect of combination of two or more Omps along with advanced adjuvant system [24] to develop a safe and effective vaccine against *S. Typhi*.

Conclusion

Immunization of rabbit with recombinant Omp 28 induced a strong humoral response which was exhibited by high antibody titer in ELISA. Subsequently, intraperitoneal homologous challenge of the immunized New Zealand rabbit resulted in lack of significant protection. These findings indicate that Omp 28 though provoked the humoral immunity but could not provide the protective immunity in rabbit model. Therefore, a combination of more than one Omp may be targeted to achieve complete protective immunity.

Authors' Contributions

AS and RK had performed the whole experiment while MKS being the mentor of the project designed the experiment. All authors read and approved the final manuscript.

Acknowledgments

Authors are highly acknowledged to the Department of Science and Technology, for providing funds for the study under WOS-A (Women Scientist Project Grant No SR/WOS-A/LS-254/2007). Authors are also thankful to Dean, college of Veterinary and Animal Sciences and Director, Experiment Station, for providing their supports and facilities.

Competing Interests

The authors declare that they have no competing interests.

References

1. CDC. (2014) Typhoid Fever Surveillance and Vaccine use South East Asia and Western Pacific Regions 2009-2012. Morbidity and Mortality Weekly Report. NO. 39. p855-860.
2. Shivshankara, N., Saxena, M.K. and Singh V.P. (2001) Rapid diagnosis of hemorrhagic septicemia by PCR assay. *Indian Vet. J.*, 78: 101-103.
3. Saxena, M.K., Singh, V.P., Kumar, A.A., Choudhuri, P., Singh, V.P., Shivachandra, S.B., Biswas A. and Sharma, B. (2006) REP-PCR analysis of *P. multocida* isolates from wild and domestic animals in India. *Vet. Res. Commun.*, 30: 851-861.
4. Shivachandra, S.B., Kumar, A.A., Gautam, R., Joseph, S., Chaudhuri, P., Saxena, M.K. and Srivastava, S.K. (2006) Detection of *Pasteurella multocida* in experimental infect-edembryonated chicken eggs by PCR assay. *Indian J. Exp. Biol.*, 44: 321-324.
5. Saxena, M.K., Singh, V.P., Lakhchura, B.D., Saxena, A. and Shrama, B. (2004) Detection of virulence in Indian isolates of *Salmonella* by polymerase chain reaction. *Indian J. Biotechnol.*, 3: 37-40.
6. Naggapa, K., Singh, S.P., Tamuly, S. and Saxena, M.K. (2007) Isolation of *Salmonella* Typhimurium from poultry eggs and meat of Tarai region of Uttaranchal. *Indian J. Biotechnol.*, 6: 407-408.
7. Rahman, B.A., Wasfy, M.O., Maksoud, M.A., Hanna, N., Dueger, E. and House, B. (2014) Multidrug resistance and reduced susceptibility to ciprofloxacin among *Salmonella* enterica serovar Typhi isolated from the middle east and central Asia. *New Microbes New Infect.*, 2: 88-92.
8. Kumar, Y., Sharma, B. and Mani, K.R. (2013) Anti-biogram profile of *Salmonella* enterica serovar Typhi in India - A two year study. *Trop. Life Sci. Res.*, 24: 45-54.
9. Marathe, S.A., Lahiri, A., Negi, V.D. and Chakravorty, D. (2012) Typhoid fever & vaccine development. *Indian J. Med. Res.*, 135: 161-169.
10. Hamid, N. and Jain, S.K. (2010) Immunogenic evaluation of a recombinant 49-kilodalton outer membrane protein of *Salmonella* Typhi as a candidate for a subunit vaccine against typhoid. *J. Infect. Dis. Immun.*, 2: 30-40.
11. Yang, Y., Wan, C., Xu, H., Aquilar, Z.P., Tan, Q., Xu, F., Lai, W., Xiong, Y. and Wei, H. (2013) Identification of an outer membrane protein of *Salmonella* enterica serovar Typhimurium as a potential vaccine candidate for salmonellosis in mice. *Microbes Infect.*, 15(5): 388-398.
12. Kaur, J. and Jain, S.K. (2013) High level expression of 49 kDa outer membrane protein of *Salmonella* enterica serovar Typhi. *Ann. Biol. Res.*, 4(1): 107-117.
13. Andrade, D., Ferreira, A.G., Silva, D.J.D., Nascimento, H.J. and Silva, J.G. (1998) Chemical and immunological characterization of a low molecular weight outer membrane protein of *Salmonella* Typhi. *Microbiol. Immunol.*, 42: 521-526.
14. Neves, F.A.G., Andrade, D., Vannier, S.M.A., Jonas, P., Hilton, J.N. and Silva, J.G. (2004) Complete amino acid sequence and location of Omp 28 an important immunogenic protein from *Salmonella* enterica serovar Typhi. *Protein J.*, 23: 71-77.
15. Saxena, A., Tamuly, S. and Saxena, M.K. (2012) Cloning, sequencing and *in silico* characterization of Omp 28 gene of *Salmonella* Typhi (strain MTCC 733). *J. Nat. Sci. Biol. Med.*, 3: 133-138.
16. Pandey, M. and Saxena, M.K. (2015) Cloning and immune-potential analysis of Omp 28 of *Salmonella* Typhimurium for the development of subunit vaccine for poultry salmonellosis. *Indian J. Poult. Sci.*, 50: 138-142.
17. Engvall, E. and Perlman, P. (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry*, 8: 871-874.
18. Hanes, D.E., Robr, M.G., Schneider, C.M. and Burr, D.H. (2001) New Zealand white rabbit as a nonsurgical experimental model for *Salmonella* Enteric gastroenteritis. *Infect. Immun.*, 69: 6523-6526.
19. Toobak, H., Rasooli, I., Talei, D., Jahangiri, A., Owlia, P. and Darvish, A.A.S. (2013) Immune response variation to *Salmonella* enteric serovar Typhi recombinant porinprotein in mice. *Biologicals.*, 41: 224-230.
20. Moreno, E.M.A., Tenorio, C.A., Pastelin, P.R., Perez, S.C., Gill, C.C., Lopez, S.R., Baeza, I., Fernandez, M., Bonifaz, L., Isibasi, A., Calva, E. and Lopez, M.C. (2013) *Salmonella* Typhi Omp S1 and Omp S2 porins are potent protective immunogens with adjuvant properties. *Immunology.*, 139: 459-471.
21. Yang, Y., Wan, C., Xu, H. and Wei, H. (2013) Identification and characterization of Omp L as a potential vaccine candidate for immune-protection against salmonellosis in mice. *Vaccine.*, 31(28): 2930-2936.
22. Ghosh, S., Chakraborty, K., Nagaraja, T., Basak, S., Koley, H., Dutta, S., Mitra, U. and Das, S. (2011) An adhesion protein of *Salmonella* enterica serovar Typhi is required for pathogenesis and potential target for vaccine development. *Proc. Natl. Acad. Sci.*, 108(8): 3348-3353.
23. Prejit., Agarawal, R.K., Porteen, K., Dubal, Z.B., Singh, A.K. and Biswas, R. (2013) Evaluation of recombinant outer membrane protein based vaccine against *Salmonella* Typhimurium in birds. *Biologicals.*, 41(3): 162-168.
24. Tamuly, S. and Saxena, M.K. (2012) Preparation of calcium phosphate nano-particle sand evaluation its effect on muscle of rat. *Curr. Sci.*, 102(4): 610-612.
