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Immunogenic Outer membrane Proteins (Omps) of Salmonella: Potential Candidate for sub-unit vaccine

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Abstract

The outer membrane is a continuous structure on the surface of Gram-negative bacteria and has particular significance as one of the potential targets for protective immunity. Recent studies have focused attention on the outer membrane proteins (OMPs) suggesting the existence of protective immunogenic components in Salmonella. Outer membrane protein (OMPs) has been reported to be immunogens for eliciting active/protective immunity against Salmonella and thus, have great potential for use in vaccination. OMPs have been investigated as potential vaccine candidates, virulence factors and these surface exposed proteins play a critical role in pathogenic processes such as motility, adherence and colonisation of the host cells, injection of toxins and cellular proteases, as well as the formation of channels for the removal of antibiotics (antibiotic resistance).

Keywords: Outer membrane protein; Immunogens; Salmonella

Introduction

The outer membrane (OM) of Gram-negative enteric bacteria is a highly asymmetric lipid bilayer. Its outer leaflet is occupied by lipopolysaccharide (LPS), a unique constituent of the OM, whereas the inner leaflet is covered by phospholipids, mainly phosphatidylethanolamine [1-3]. About 50% of the outer membrane mass consists of protein, either in the form of integral membrane proteins or as lipoproteins that are anchored to the membrane by means of N-terminally attached lipids [4]. The OMPs of Gram-negative bacteria are synthesized in the cytoplasm and have to cross the inner membrane before being assembled into a correctly folded state in the outer membrane. Proteins are usually unfolded by chemical and physical treatments, which lead to alterations on their conformations [5,6]. The outer membrane proteins of bacteria function as the dynamic interface between the bacterium and its surroundings and are involved in the maintenance of cell structure, binding a variety of substances adhesion to other cells, and regulation of transport of both nutrients and bactericidal agents. [7]. The outer membrane protein (OMP) from Gram-negative bacteria (Salmonella Typhi) is a major immunogenic target to synovial fluid lymphocytes of patients with reactive arthritis (ReA)/undifferentiated spondyloarthropathy (uSpA) [8] and immunologically important because of their accessibility to the host defense system.

The outer membrane proteins (OMPs) of Salmonella have been considered possible candidates for conferring protection against typhoid. Vaccines for nontyphoidal Salmonella are urgently required especially in Africa. Besides, O antigen polysaccharide-based vaccine might be ineffective and increase susceptibility to life-threatening extracellular Salmonella growth, and an outer membrane protein-based vaccine could induce protective antibodies [9,10]. Large number of studies with bacterial outer membrane molecules as candidate vaccines has shown considerable promise [11,12]. Over the past years, several Salmonella OMPs have been investigated as potential vaccine candidates, virulence factors, and diagnostic antigens and the molecular structure and function of OMPs and their respective genes have been studied. However, only a small number of OMPs have so far been characterized [13-17]. Study of other gram-negative bacteria demonstrated that porins represent the most abundant class of OMPs that are protective and show some degree of antigenic heterogeneity among different strains [18]. The outer membrane (OM) of Salmonella and other Gram negative bacteria contains a family of pore-forming proteins called porins [19].These porins are present in mostly trimeric forms in the outer membrane [20] and are involved in the permeability of low molecular mass substances [21]. Porins generally have multiple surface epitopes which are usually 6 to 25 residues in length [22]. Several workers have successfully demonstrated the induction of high levels of antiporin antibodies and enhanced cell-mediated immunity along with protection afforded by porins against Salmonella [23]. The major porins in Salmonella are OmpC, OmpF, OmpD, out of which OmpC was found to be major surface antigen with unique exposed epitopes expressed in more amounts regardless of the growth condition [24]. Study of other gram-negative bacteria demonstrated that porins represent the most abundant class of OMPs that are protective and show some degree of antigenic heterogeneity among different strains [18]. However, these are relatively nonspecific and have interspecies cross-reactivity. The porin proteins may also have potential use in the development of oral vaccines, biosensors, and nano-reactors [25]. Antibody responses are important to achieve protection against Salmonella infection [13]. Because of the ability of the porins to elicit antibody response that has shown to be protective in nature, several groups have studied native S. Typhi porins prepared by conventional method as vaccine candidates against typhoid fever [26,27].

OmpC

OmpC is a major porin protein of S.Typhi and also a major surface antigen, expressed throughout the infection period in typhoid patients. It is a good candidate to display heterologous epitopes on the cell surface [28,29]. The functional and mature OmpC is a homotrimer. OmpC monomer has 357 a without signal peptide, with a molecular mass of 39 kDa. It is a barrel protein having pore radius estimated to be 1.1 nm [19], which is significantly larger than E.coli [30]. LPSs have been shown to provide structural stability to OmpC [31]. It was reported through hybridization studies with gene segments on OmpC, that OmpC protein is highly conserved in 11 different Salmonella serovars [29]. It was reported that heterologous expression of OmpC with signal peptide is toxic to the cells [32]. It has been reported through multiple sequence alignment tools that S. Typhi OmpC consists of 8 variable regions on comparison with other porins with well-known crystal structures [33]. These variable regions have been found to be on the outer side of the membrane and therefore they have high probability to be presented for B-cell recognition and elicit immune response. These results depict that Omp C has recognized B-cell epitopes and as it shares maximum similarity with Omp C of S. Typhimurium (98%) these variable regions can be strongly predicted to act as possible B-cell epitopes capable of evoking immune response. This porin is expressed under low and high osmolarity conditions [34, 29]. Hence OmpC is expressed not only under free living conditions, but also during infection, since the osmolarity of the human serum is equivalent to high salt conditions maintained in laboratory [1]. OmpC of S.Typhi was successfully expressed with signal peptide by Verma et al. in 2009. The immunogenic nature of the recombinant porin protein was evaluated by ELISA by raising hyperimmune sera in Swiss Albino mice [35]. These reasons suggest that OmpC could be a potential candidate for development of r-DNA vaccine against Salmonella. And also can act as candidate .antigen for diagnostics and vaccination.

OmpF

Outer membrane protein F (OmpF) is a major porin in S. typhi responsible for the translocation of antibiotics. The functional unit of OmpF is a homotrimer. Each monomer of molecular weight 37 kDa forms a β-barrel structure having 16 membrane spanning β-strands. The OmpF porin forms three large water-filled channels per trimer, allowing the diffusion of small hydrophilic molecules such as nutrients, antibiotics and waste products across the outer bacterial membrane [36]. OmpF allows the passage of drugs such as quinolones, tetracyclines, and β-lactams [37,38]. The understanding of the structure function relationship of S. typhi OmpF is important for the development of new drugs against

typhoid. The mutants of OmpC and OmpF of S. Typhimurium showed attenuated virulence [39]. OmpC and OmpF of S. Typhi were shown to confer lifelong, specific bactericidal antibody response [40]. Researchers showed that denatured forms of recombinant OmpC and OmpF of S. enterica ssp Typhi are immunogenic proteins in murine models. They suggest those two OMPs as suitable vaccine candidates. However, no in vivo challenge study was performed on various types of porins. [35]. There are two variable regions in OmpC and OmpF porin proteins useful for a typhoid fever diagnostic test based on antigen - antibody interaction [41]. OmpF generates only bactericidal antibodies after boosting, suggesting that the long-lasting antiOmpF antibodies recognize mainly epitopes not exposed on the bacterial surface [40]. Marked IgG responses in mice immunized with OmpF support the immunogenic nature of recombinant OmpF suggestive of its application as a good immunogen for vaccine studies.

OmpL

Outer membrane porin L (OmpL also called YshA) was predicted as a 230-residue TMBB, which contained 10 trans membrane strands and an N-terminal signal sequence, recombinant OmpL localizes to the outer membrane when expressed in Escherichia coli [42]. Studies demonstrated that OmpL an outer membrane protein of S. Typhimurium, is likely to adopt a β barrel conformation consisting of 12 antiparallel β strands connected by 6 long, flexible, surface-exposed loops and 5 short periplasmic turns. OmpL was used as a candidate protein not only because of its location on the bacterial surface but also because of its abundant expression [42,43]. Analysis of the amino acid sequence of the OmpL indicates OmpL is a widely distributed and conserved outer membrane protein among different Salmonella serovars which raises the possibility that OmpL could be used in diagnostics or as part of a subunit or conjugate vaccine. Large number of studies with bacterial outer membrane molecules as candidate vaccines has shown considerable promise [11,12]. OmpL may induce strong protective immunity in the host and remain attractive vaccine targets. Researchers suggest that OmpL immunization is protective in murine infection, reducing bacterial loads and increasing survival time after infection, which confirms and extends that OmpL-based vaccine, may be a potential vaccine candidate against multiple Salmonella Typhimurium infection. When mice immunized with rOmpL were challenged with lethal doses of S. Typhimurium, 100% of them were protected against salmonellosis. However, the task of producing and testing OmpL in subunit vaccine trials remains a challenging objective. The ELISA results indicated that the protein elicits a significant humoral response. OmpL is a potential vaccine candidate against Salmonella infections and also promises to be a potent adjuvant [43].

Omp A

Outer membrane protein A (OmpA) is a major heat-modifiable OMP in Escherichia coli and is one of the best characterized OMPs. Originally purified in 1977, the molecular mass of OmpA was demonstrated to be 33 kDa, and since then various studies have identified its molecular mass to range from 28 to 36 kDa depending on the temperature and conditions to which it is subjected prior to SDS-PAGE [44]. OmpA proteins are characterized by an N -terminal domain that forms an eight-stranded, anti-parallel β barrel, which is embedded in the outer membrane and the C-terminal domain is globular and located in the periplasmic space. Thus, the protein spans the membrane many times with the protein interacts with lipid present in outer membrane non-covalently in Chlamydia, a gram negative bacteria [45]. So, only a part of the protein, N-terminal is exposed for binding of phages and other properties while other portion, C-terminal is not essential for its localisation or for its function as phage receptor.

OmpA has both structural and ion-permeable porin roles, with its ionic pore controlled by a salt-influenced electrostatic gating mechanism that allows bacterial survival during osmotic stress [46] OmpA is abundant in the membrane with estimates of approximately 100,000 copies per cell [47]. Because OmpA homologues are highly immunogenic, in certain Gram-negative bacteria, OmpA homologues can serve as potential targets for vaccine development. Salmonella spp. are severe enteric pathogens in many animal species. Although, OmpA is not so critical to pathogenesis of enteric or systemic salmonellosis, Salmonella spp. OmpA is highly immunogenic as determined by detection of antibodies in mice infected with attenuated Salmonella and humans with confirmed cases of typhoid [48] Vaccination of BALB/c mice with formalin-killed Salmonella enhanced resistance against challenge, and survivors had high anti-OmpA antibodies [49] which suggests Salmonella OmpA may exist in more than one conformational form. Salmonella OmpA is immune-stimulatory as demonstrated by stimulation of IFN-g production and enhanced expression of MHC and co-stimulatory molecules in dendritic cells and/or T cells and may play a role in modulation of the immune response in salmonellosis [50,51]. OmpA is surface exposed and highly immunogenic stimulating bactericidal antibodies in the presence of complement [52].

Hence, studies showed that Omp A function in bacterial structure, physiology, and also adaptation to environmental stresses, whereas in disease, they can serve as virulence factors causing adhesion, invasion, and damage of host tissue or evasion of host defenses resulting in clinical disease or death. Various OmpA homologues also serve as targets for host innate and adaptive immune responses resulting in increased host resistance against infection. As per studies about pathogenic and immunogenic roles of the OmpA family of proteins, their involvement in natural and recombinant-derived vaccines will likely increase.

Porins are part of outer membrane proteins in Gram-negative bacteria stimulating immune system [35,53,54]. Researchers showed that denatured forms of recombinant OmpC and OmpF of S. enterica ssp Typhi are immunogenic proteins in murine models [35]. The major porins in Salmonella viz OmpC, OmpF, OmpD are widely distributed and conserved among different Salmonella serovars which raises the possibility that they could be used in diagnostics or as part of a subunit or conjugate vaccine. Various studies have shown that a crude preparation of outer membrane proteins (OMPs) of Salmonella evokes strong immune response and induces a protective immunity against infection caused by diverse Gram-negative bacteria. Therefore, from the studies on the above different Outer membrane proteins (OMPs) discussed it can be concluded that OMPs of Salmonella seem to have strong immunogenic potential and also have been implicated as possible candidates for conferring protection against typhoid Studies have also shown that some recombinant OMPs also may evoke strong immune response in animals and some of these proteins have very strong potential for the development of a subunit vaccine against Salmonella. Thus, outer membrane proteins (OMPs) could be a candidate antigen for diagnostics and vaccination and may act as promising potential vaccine candidates against Salmonella.

References

1. Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49(1): 1-32.

2. Nikaido H, Vaara M (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology Neidhardt F (ed.) American Society for Microbiology, Wash, DC. pp. 7-22,

3. Nakae T (1986) Outer-membrane permeability of bacteria. Crit Reu Microbiol 13(1): 1-62.

4. Blattner FR, Plunkett GIII, Bloch CA, Perna NT, Burland V, et al. (1997) The complete genome sequence of Escherichia coli K-12. Science 277(5331): 1453-1462.

5. Dobson CM (2003) Protein folding and misfolding. Nature 426: 884-890.

6. Fersht AR, Daggett V (2002) Protein folding and unfolding at atomic resolution. Cell 108(4): 573-582.

7. Wexler HM (2002) Outer-membrane pore-forming proteins in gram-negative anaerobic bacteria. Clin Infect Dis 35(1): 65-71.

8. Pocanschi CL, Popot JL, Kleinschmidt JH (2013) Folding and stability of outer membrane protein A (OmpA) from Escherichia coli in an amphipathic polymer, amphipol A8-35. Eur Biophys J 42(2-3): 103-118.

9. MacLennan CA, Gilchrist JJ, Gordon MA, Cunningham AF, Cobbold M, et al. (2010) Dysregulated humoral immunity to nontyphoidal Salmonella in HIV infected African adults. Science 328(5977): 508-512.

10. Feasey NA, Archer BN, Heyderman RS, Sooka A, Dennis B, et al. (2010) Typhoid fever and invasive non typhoid salmonellosis, Malawi and South Africa. Emerg Infect Dis 16(9): 1448-1451.

11. Ghosh S, Chakraborty K, Nagaraja T, Basak S, Koley H, et al. (2011) An adhesion protein of Salmonella enterica serovar Typhi is required for pathogenesis and potential target for vaccine development. Proc Natl Acad Sci USA 108(8): 3348-3353.

12. Hamid N, Jain SK (2008) Characterization of an outer membrane protein of Salmonella enterica serovar Typhimurium that confers protection against typhoid. Clin Vaccine Immunol 15(9): 1461-1471.

13. Isibasi A, Ortiz V, Vargas M, Paniagua J, González C, et al. (1988) Protection against Salmonella typhi infection in mice after immunization with outer membrane proteins isolated from Salmonella typhi 9,12,d, Vi. Infect Immun 56(11): 2953-2959.

14. Puente JL, Alvarez-Scherer V, Gosset G, Calva E (1989) Comparative analysis of the Salmonella typhi and Escherichia coli ompC gene. Gene 83: 197-206.

15. Puente JL, Verdugo-Rodríguez A, Calva E (1991) Expression of Salmonella typhi and Escherichia coli OmpC is influenced differently by medium

osmolarity; dependence on Escherichia coli OmpR. Mol Microbiol 5(5): 1205-1210.

16. Flores V, Fernandez M, Fuchs Y, Puente JL, Calva E (1987) Isolation of an ompC-like outer membrane protein gene from Salmonella Typhi. Gene 61: 75-83.

17. Lin J, Huang S, Zhang Q (2002) Outer membrane proteins: key players for bacterial adaptation in host niches. Microbes Infect 4: 325-331.

18. Munson RSJ, Shenep JL, Barenkamp SJ, Granoff DM (1983) Purification and comparison of outer membrane protein P2 from Haemophilus influenza type b isolates. J Clin Investig 72(2): 677-684.

19. Baalaji NS, Mathew MK, Krishnaswamy S (2006) Functional assay of Salmonella typhi OmpC using reconstituted large unilamellar vesicles: a general method for characterization of outer membrane proteins. Biochemie 88: 1419e24.

20. Gehring KB, Nikaido H (1989) Existence and purification of porin heterotrimers of Escherichia coli K-12 OmpC, OmpF and PhoE proteins. J Biol Chem 264: 2810-2815.

21. Reeves P (1979) in: Bacterial Outer Membranes. Biogenesis and Functions (Inouye M ed): 256-291, Wiley, New York.

22. Klebba PE, Benson SA, Bala S, Abdullah T, Reid J, et al. (1990) Determinants of OmpF porin antigenicity and structure. J Biol Chem 265: 6800-6810.

23. Akis N, Sayhan O, Karacavus A, Toreci K (2003) Immunogenicity and specificity of Salmonella Typhimurium outer membrane antigens. J Cell Mol Biol 2: 91-97.

24. Contrearas I, Muñoz L, Toro CS, Mora GC (1995) Heterologous expression of Escherichia coli porin genes in Salmonella typhi Ty2: regulation by medium osmolarity, temperature and oxygen availability. FEMS Microbiol Lett 133(1-2): 105-111.

25. Heinz C, Engelhard H, Niederweis M (2003) The core of the tetrameric mycobacterial porin MspA is an extremely stable beta-sheet domain. J Biol Chem 278: 8678-8685.

26. Salazar-González RM, Maldonado-Bernal C, Ramírez-Cruz NE, Rios-Sarabia N, Beltrán-Nava J, et al. (2004) Induction of cellular immune response and anti-Salmonella enterica serovar Typhi bactericidal antibodies in healthy volunteers by immunization with a vaccine candidate against typhoid fever. Immunol Lett 93(2-3): 115-122.

27. Singh M, Vohra H, Kumar L, Ganguly NK (1999) Induction of systemic and mucosal immune response in mice immunized with porins of Salmonella Typhi. J Med Microbiol 48: 79-88.

28. Arockiasamy A, Krishnaswamy S (2000) Homology model of surface antigen OmpC from Salmonella typhi and its functional implications. J Biomol Struct Dyn 18(2): 261-271.

29. Puente JL, Juárez D, Bobadilla M, Arias C, Calva E (1995) The Salmonella ompC gene: structure and use as a carrier for heterologous sequences. Gene 156: 1-9.

30. Nikaido H, Rosenberg EY (1983) Porin channels in Escherichia coli: studies with liposomes reconstituted from purified proteins. J Bacteriol 153(1): 241-252.

31. Arockiasamy A, Kumar PD, Baalaji NS, Rukmini MR, Krishnaswamy S (2004) Folding and structural stability of OmpC from Salmonella Typhi: Role of LPS and environment. Curr Sci 87(2): 197-202.

32. Kumar PD, Krishnaswamy S (2005) Over expression, refolding, and purification of the major immunodominant outer membrane porin OmpC from Salmonella Typhi: Characterization of refolded OmpC. Protein Expr Purif 40: 126-133.

33. Arockiasamy A, Krishnaswamy S (1995) Prediction of B-cell epitopes for Salmonella typhi OmpC. Journal of Biosciences 20(2): 235-243.

34. Muthukkaruppan VR, Nandakumar KS, Palanivel V (1992) Monoclonal antibodies against Salmonella porins: Generation and characterization. Immunol Lett 33: 201-206.

35. Verma SK, Gautam V, Balakrishna K, Kumar S (2009) Overexpression, purification and immunogenicity of recombinant porin proteins of Salmonella enterica Serovar Typhi (S.Typhi). J Microbiol Biotechnol 19(9): 1034-1040.

36. Nikaido H (1994) Porins and specific diffusion channels in bacterial outer membranes. J Biol Chem 269(6): 3905-3908.

37. Cohen SP, Mcmurry LM, Hooper DC, Wolfson JS, Levy SB (1989) Cross-resistance to fluoroquinolones in

multiple-antibiotic-resistant (Mar) Escherichia coli selected by tetracycline or chloramphenicol: Decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob Agents Chemother 33(8): 1318-1325.

38. Tavío MM, Vila J, Ruiz J, Ruiz J, Martín-Sánchez AM (1999) Mechanisms involved in the development of resistance to fluoroquinolones in Escherichia coli isolates. J Antimicrob Chemother 44(6): 735-742.

39. Chatfield SN, Dorman CJ, Hayward C, Dougan G (1991) Role of ompR-dependent genes in Salmonella Typhimurium virulence: Mutants deficient in both ompC and ompF are attenuated in vivo. Infect Immun 59(1): 449-452.

40. Secundino I, López-Macías C, Cervantes-Barragán L, Gil-Cruz C, Ríos-Sarabia N, et al. (2006) Salmonella porins induce a sustained, lifelong specific bactericidal antibody memory response. Immunology 117(1): 59-70.

41. Kumar S, Kapil S, Gautam V, Verma SK, Ray P (2010) Peptides representing the specific variable regions but not the full porin proteins can be useful for antibody based diagnosis of typhoid fever. Folia Microbiol 55(5): 520-527.

42. Freeman Jr TC, Landry SJ, Wimley WC (2011) The prediction and characterization of YshA, an unknown outer-membrane protein from Salmonella Typhimurium. Biochim Biophys Acta 1808(1): 287-297.

43. Yang Y, Wan C, Xu H, Hua, Wei H (2013) Identification and characterization of OmpL as a potential vaccine candidate for immune-protection against salmonellosis in mice. Vaccine 31: 2930-2936.

44. Chai TJ, Foulds J (1977) Purification of protein A, an outer membrane component missing in Escherichia coli K-12 ompA mutants. Biochim Biophys Acta 493(1): 210-215.

45. Findlay HE, McClafferty H, Ashley RH (2005) Surface expression, single-channel analysis and membrane topology of recombinant Chlamydia trachomatis major outer membrane protein. BMC Microbiol 5: 5.

46. Hong H, Szabo G, Tamm LK (2006) Electrostatic couplings in OmpA ion-channel gating suggest a mechanism for pore opening. Nat Chem Biol 2(11): 627-635.

47. Koebnik R, Locher KP, Van Gelder P (2000) Structure and function of bacterial outer membrane proteins: barrels in a nutshell. Mol Microbiol 37: 239-253.

48. Brown A, Hormaeche CE (1989) The antibody response to salmonella in mice and humans studied by immunoblots and ELISA. Microb Pathog 6: 445-45.

49. Chart H, Rowe B (1991) Antibodies to lipopolysaccharide and outer membrane proteins of Salmonella enteritidis PT4 are not involved in protection from experimental infection. FEMS Microbiol Lett 84(3): 345-350.

50. Jang MJ, Kim JE, Chung YH, Lee WB, Shin YK, et al. (2011) Dendritic cells stimulated with outer membrane protein A (OmpA) of Salmonella typhimurium generate effective anti-tumor immunity. Vaccine 29: 2400-2410.

51. Lee JS, Jung ID, Lee CM, Park JW, Chun SH, et al. (2010) Outer membrane protein a of Salmonella enterica serovar Typhimurium activates dendritic cells and enhances Th1 polarization. BMC Microbiol 10: 263.

52. Dumetz F, Lapatra SE, Duchaud E, Claverol S, Le Henaff M (2007) The Flavobacterium psychrophilum OmpA, an outer membrane glycoprotein, induces a humoral response in rainbow trout. J Appl Microbiol 103(5): 1461-1470.

53. Arockiasamy A, Krishnaswamy S (1999) Crystallization of the immunodominant outer membrane protein OmpC; the first protein crystals from Salmonella typhi, a human pathogen. FEBS Lett 453(3): 380-382.

54. Malickbasha M, Arunachalam R, Senthilkumar B, Rajasekarapandian M, Annadurai G (2010) Effect of ompR gene mutation in expression of ompC and ompF of Salmonella typhi. Interdiscip Sci 2(2): 157-162.