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www.commprac.com
ISSN 1462 2815

MYCOBACTERIUM TUBERCULOSIS H37Rv GENE EXPRESSION OF THE OmpA FAMILY PROTEINS

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DOI: 10.5281/zenodo.12272526

Abstract

With 1.5 million fatalities worldwide a year from tuberculosis (TB), this disease continues to be a serious global public health issue. Extrapulmonary TB (EPTB), which affects one out of every five cases of TB, presents significant diagnostic and treatment problems. In addition to adapting to a physiological state of quiescence, Mycobacterium TB is renowned for its intricate relationship with the host, resulting in a variety of poorly understood disease states, from latent infection to active clinical disease. To quickly diagnose TB, track TB therapies, and learn more about pathogenesis, new diagnostic techniques in the diagnostic arsenal are desperately needed. Gram-negative bacteria frequently create outer-membrane proteins (OMPs), which are spherical buds of the outer membrane that are filled with periplasmic material. Bacteria can interact with their environment thanks to the synthesis of OMPs, which have been identified to mediate a variety of tasks, including increasing pathogenicity, facilitating bacterial survival under stressful conditions, and controlling microbial relationships within bacterial communities. Researchers have also started to investigate OMPs as a platform for bioengineering applications due to their functional diversity. Present research is addressing the current developments in the research of OMPs, emphasizing fresh understandings of the biogenesis mechanisms and the purposes of these proteins.

Keywords: *Mycobacterium Tuberculosis,* OmpA Proteins, Protein Expression, Standard Strain etc.

INTRODUCTION

Mycobacterium *tuberculosis* is the organism which causes theTuberculosis. The outer membrane of the *Mycobacterium tuberculosis*(MTB)contains mycolic acid which is a thick cell wall complex structure. The majority of the bacterium contains many cell wall proteins which plays a vital role in protecting the bacteria against the drugs. Over the period of time Mycobacterium *tuberculosis* is becoming more and more drug resistant to primary and secondary lanes of drugs. This is mainly because of the specific virulence genes of MTB becoming more resistant to drug lanes. Among them, the outer membrane proteins play a major role in the bacterial resistance [1, 2, 3].

Coming with the Outer membrane protein of the MTB there are many proteins present in MTB cell wall. By passively and deliberately controlling the influx and efflux of essential solutes like peptides or proteins, nucleic acids, and other organic compounds like lipids and polysaccharides, gram-negative bacteria are able to adapt to a variety of external environments. Functions of outer membrane proteins (OMPs) and lipoproteins include membrane structure and stability, active and passive ion and solute transport, signal transduction, defense, and catalysis [4]. The OMP's are mainly exposed to the surface of the bacteria and hence they are targeted in the current study to understand the potential importance, host interaction, defenses etc., [5].

From the 17th century the Tuberculosis exists, after the following several decades of continuous decline, the MTB is increasing and re-emerging the country.There is a drastic increase rate of the TB in the United States and it mainly acquired with the immunodeficiency syndrome epidemic. Now-a-days the MTB is becoming more drug resistant and also the outline of the disease is changed.

This is due to change in the bacterial host and it is leading to the commonly disseminated Extra Pulmonary Tuberculosis (EPTB) that is drastically increasing and found with some variations in MTB because of its cell wall.Lymph nodes, pleura, Abdomen, Skin, Kidney, Central nervous system (CNS) and osteoarticular regions are prominent extrapulmonary infection sources, however any organ will be affected.

The challenging task is treatment of EPTB, which can be elusively done by requiring a high level of suspicion. Physicians and clinicians are trying hard to identify the EPTB at the primary stage and after identification they are prescribing the primary-lane of drugs for about3-6months [5,6]. If it is not cured then secondary-lanes of drugs are used.

Hence, physicians should take a detailed medical history, paying special attention to risk factors for HIV infection and tuberculosis. There are many TB drugs, even though the mortality and morbidity of the TB in the country is high due the bacteria cell wall which is becoming drug resistant. A negative acid-fast bacillus smear, the absence of granulomas on histology, and the failure to culture Mycobacterium tuberculosis do not rule out the diagnosis.

The research focusing on the diagnosis of the MTB is currently required. In some cases of extrapulmonary tuberculosis, new diagnostic methods such as adenosine deaminase levels and polymerase chain reaction can be helpful [7]. At present, all the clinical health sectors, private hospitals are using the same treatment regime to diagnose pulmonary TB and extra pulmonary TB, it is also similar with HIV positive and negative infected patients [6, 7].

About 4.4 million base pairs and 4000 genes make up the M. tuberculosis genome in its entirety (Cole et al., 1998) [8].There are 91 (2.1%) pathogenicity genes from Mycobacterium tuberculosis in that. Current study has concentrated on the virulence genes found in cell walls and inner membranes among all these virulence factors.

1578 proteins were discovered in the M. tuberculosis H37Rv strain and 1493 in the H37Ra strain, making a total of 1771 unique protein groups. In contrast, 278 of those total proteins were determined to be exclusive to M. tuberculosis H37Rv, whereas 193 were discovered to be specific to the H37Ra strain..

The Mycobacterium tuberculosis virulence factors have been classified into a variety of classes, some of which are based on the molecular characteristics, cellular localization, and functions of the factors [9]:

1. Lipid and fatty acid metabolism, including catabolism of cholesterol
2. Lipoproteins, secretion systems, cell wall, and other proteins for the cell envelope.
3. Proteins that prevent the action of macrophage antimicrobial effectors, such as those that prevent apoptosis, arrest phagosomes, and respond to oxidative and nitrosamine stress
4. Protein Kinases
5. Metalloproteases and proteases, such as
6. Metal-transporter proteins are divided into importers and exporters
7. Sigma factors, other transcriptional regulators, regulators of two systems of components, and regulators of gene expression
8. Proteins with unidentified functions, such as families of polymorphic GC-rich sequence (PE-PGRS)
9. Some proteins produced by virulence genes.

Cell Wall Proteins in *Mycobacterium tuberculosis*:

The *Mycobacterium tuberculosis cell* envelope is complex and diverse, which is mainly composed of proteins that contains peptidoglycan, mycolic acids, carbohydrates, lipids etc., The cell wall of the MTB is composed of very thick wall and they play a major role in bacterial survive. The MTB cell wall proteomic study has been limited and only a smaller number of studies is taken out.Understanding bacterial survival and immunological regulation in the host requires characterization of resident and cell wall associated proteins [10,11].

OmpA structure and physiologic function:

Two million people die each year as a result of Mycobacterium TB infection (WHO, 2004)[10]. Aerosolized by the bacterium, which is highly contagious and persistent, it is a potent bacterial pathogen that affects more than 2 million individuals worldwide [12]. Now-a-days the MTB bacteria is becoming more and more drug resistant. This is because of the un-continues use of the drugs, not maintaining proper treatment regime etc., hence some of the strains of the MTB are showing resistance to the primary drug lines. Hence the treatment of TB necessitates focused medication [13]. The limited permeability of the mycobacterial cell membrane confers inherent resistance to most hydrophilic medicines[14]. It is known that hydrophilic substances can pass through mycobacterial cells via porins that produce water-filled pores in the cell wall [15].

The recently discovered *Mycobacterium smegmatis* structural porin, isMspA, has a homo-octameric structure that resembles a goblet with a single central channel [15]. The trimeric OmpF pore of E. coli cannot pass through the thick cell membrane because the pore is three times longer. Recent investigations have shown that MspA inactivation significantly increases the minimum inhibitory concentration (MIC) of many antibiotics, including ampicillin, in M. smegmatis, which decreases the permeability of the cell wall [16]. When MspA is overexpressed, Mycobacterium bovis BCG multiplies intracellularly more and develops more quickly, suggesting that porins may be involved

in how mycobacteria interact with their hosts [17]. These findings all point to the necessity of porins for mycobacterial cell wall construction. Because there are so few proteins in M. TB and M. bovis, porin-like proteins have been identified there but have not yet been properly characterized [18, 19]. The *M. tuberculosis* H37Rv genome was subjected to a BLAST search investigation, and a protein that resembles the *E. coli* OmpA porin was found [18] and was named OmpATb. For their C-terminal portion, there was a 44% commonality. This 33,574 Da protein containing 326 residues was produced and purified using recombinant *E*. *coli*. After integrating into lipid bilayer membranes, the native and recombinant forms both produced single pathways [19]. The deletion of OmpATb has little impact on development under typical circumstances, according to physiological experiments employing an *M*. *tuberculosis* mutant without ompATb. However, in slightly acidic conditions, the mutant's growth was considerably hindered [20].The fact that the membrane's permeability for a variety of tiny hydrophilic molecules was dramatically decreased at this pH level suggested that OmpATb may be the only functioning porin at pH 5.5.

The innate and adaptive immunological responses are stimulated by *S. flexneri 2a*OmpA:

The host's defense against tissue invasion is led by the macrophage, a crucial modulator of innate immunity. Activated macrophages release a vast array of microbicidal effectors and immunoregulatory cytokines that are crucial for both innate immunity and priming the acquired immune response [21]. Specifically, T- or B-cell activation OmpA of S. flexneri 2a promotes the release of a variety of macrophage cytokines, including IL-1, IL-6, TNF-, IFN-, and IL-12p70, which are known to be essential in type 1 adaptive immune coordination and antibacterial host defense [22]. It is believed that IL-12 and IFN- induce naive T cells to polarise toward Th1 cells [23]. In the fight against intracellular bacterial and viral infections, Th1 cells are crucial. Additionally, *S. flexneri* 2a OmpA stimulates macrophages' production of type-1 chemokines such MIP-1, MIP-1, and RANTES [24]. Chemokines' ability to attract and activate T cells is a crucial function. Chemokines have been discovered to exert direct antibacterial activities against a wide spectrum of gram-positive and gram-negative bacteria in addition to their function in cellular recruitment[25]. More and more, it is understood that the immune response to bacterial and viral infection depends on chemokines of the C-C or -subfamily, particularly RANTES, macrophage inflammatory protein MIP-1, and MIP-1 [26]. The cytokine and chemokine profile support the polarization of macrophages toward M1 in response to OmpA. flexneri 2a, S. Nitric oxide (NO), which is secreted in small amounts by macrophages, is likewise triggered by OmpA [27]. The defense against intracellular pathogens, including as viruses, bacteria, and parasites, relies heavily on the effector molecules [28].

Furthermore, the activation and differentiation of Th1 cells are specifically enhanced by low concentrations of NO [29]. IL-12 generated by antigen-presenting cells and NO's direct action on T cells work in concert (APCs). OmpA-stimulated macrophages also increase the expression of MHCII, CD80, and CD40 [30]. The start of the type 1 adaptive immune response requires certain chemicals. This is supported by the finding that splenic CD4+ T cells from OmpA-immunized mice express more of the RANTES/MIP-1/MIP-1 receptor CCR5 on their surface [31]. IFN- and IL-2 are secreted by OmpA-activated CD4+ T cells, and their surface expression of IL-12R-2 is also enhanced [32]. Th1 cells have been shown to express CCR5 and IL-12R-2 [33]. By committing to Th1 differentiation, CD4+ Th1-type cells generate IFN- and IL-2 to

regulate cell-mediated immunity (CMI). Th1-produced While directly suppressing the growth of Th2 cells, IFN- stimulates the formation of Th1 cells through causing the synthesis of IL-12 by activated macrophages and the expression of the IL-12 receptor on antigen-stimulated naive CD4+ T helper precursor (Thp) cells [34]. Th1 cells are in charge of orchestrating cell-mediated immune responses that eliminate intracellular pathogens through the production of interferon-, which is essential for their function. Consequently, *S. flexneri* 2a OmpA's ability to coordinate innate and adaptive immunity is a beneficial trait. However, additional research is required to see whether it can elicit long-term B cell memory responses. This suggests that it might be the best subunit vaccination option against shigellosis.

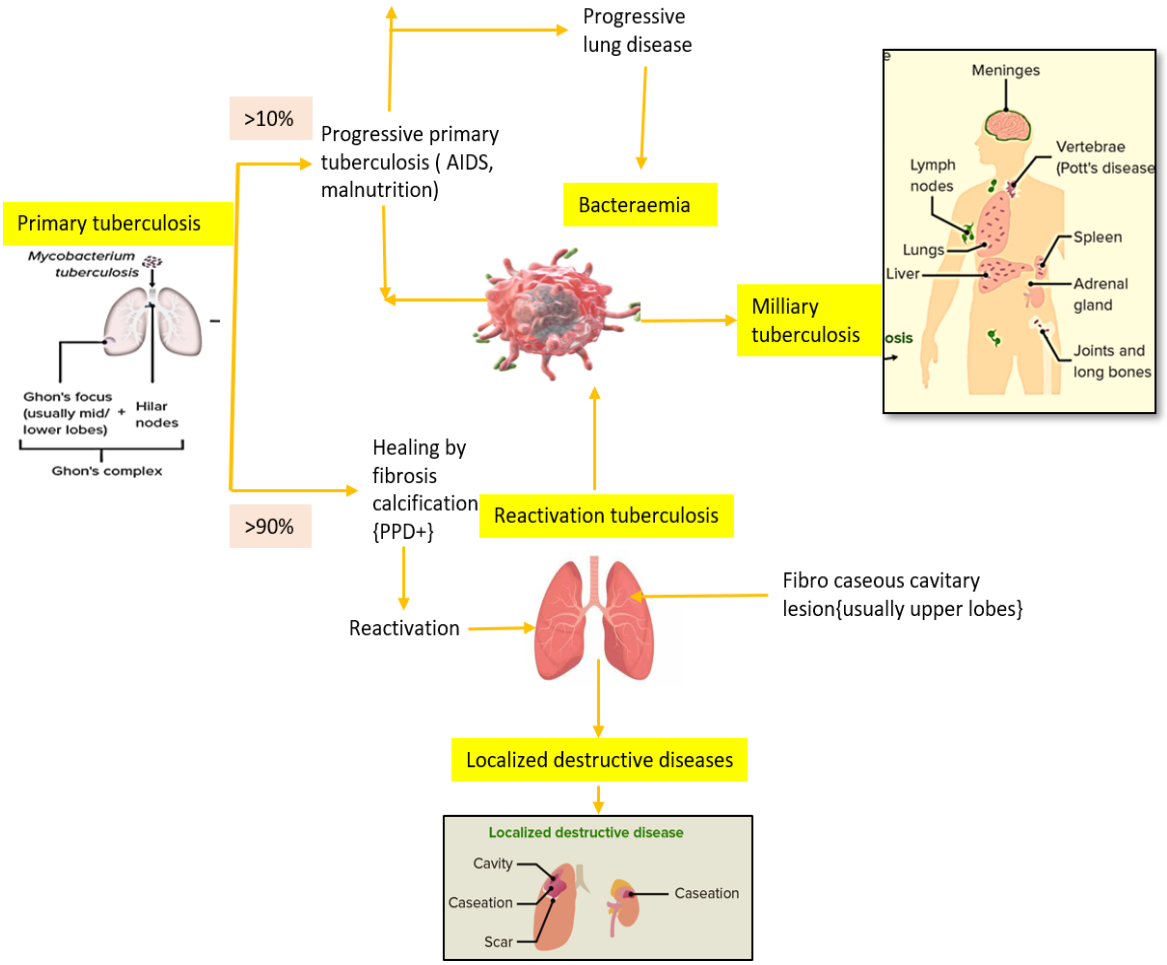

Figure 1: The overview of the *Mycobacterium tuberculosis* infection pathogenesis and is transferability

Review of Composition and Role

Escherichia coli has pre-eminent chrematistic OMP which is heat adjustable that constitutes the Outer membrane protein A (OmpA). In 1977, SDS-PAGE [35] demonstrated that OmpA has the molecular mass of 33 kDa, and this was supported by numerous studies that have reported that temperature and environment affect the molecular mass and range between 28 to 36 kDa. Bacteria has similar OmpA composition, like Pseudomonas aeruginosa consist OprF and Chlamydia trachomatis, The outer membrane of the genital pathogen is made up of MOMP, which is identified by its N-terminal domain, which is an eight-stranded, antiparallel b barrel. OmpA

confers porin activity and is noncovalently attached to peptidoglycan [36]. The molecule's outer membrane surface has three short periplasmic turns and four lengthy loops that hold its eight strands [32]. The barrel structure is not affected by the OmpA deletion[37]. Similarities of OmpA loops consisting of components have been demonstrated [38 ,39]. According to the latest report, thick polysaccharide capsule presence does not affect the surface exposure of Mannheimia (formerly Pasteurella) haemolyticaOmpA, demonstrated by immunogold-electron microscopy and immunofluorescence techniques [40]. The globoid C-terminal domain in the periplasm of OmpA and peptidoglycan probably depends on each other [41]. Hence, the Bacteria survives the osmotic stress with the ionic pore that controls the salt-influenced electrostatic gating mechanism with both the formational and ion-permeable porin roles [42]. The abundancy of OmpA estimates approximately 100,000 copies in the membrane of a cell [43].

In some Bacteria, the external conditions play a crucial role in the expression of OmpA like Protein like anaerobic condition, polyamine exposure & reduced nitrogen supply elevates the expression whereas exposure to acid & antimicrobial peptide decreases the expression [44]. Zhang et al. (2011) [45] demonstrated the disinfectant stress in OMPs of *E.coli* using Phenol & concluded that OmpA upregulates the protein expression & may increase phenol resistance. Furthermore, overexpression of OmpA in *E. coli* biofilms is specified in proteomic analysis (Orme et al., 2006). In vitro environmental conditions [46] doesn't affect the OmpA-like proteins in oral bacterium like Porphyromonasgingivalis. Through hydrophilic loops and for a number of bacteriocins, including colicin U of Shigella boydii and colicin L, OmpA also plays a significant role in a wide variety of bacteriophages [47], apart from the structural & porin roles. Type 1 fimbriae expression is enhanced with the expression of OmpA, deletion of OmpA suppresses the Type 1 fimbriae expression (Teng et al., 2006). Adding on to this, OmpA of Klebsiella pneumoniae, human respiratory pathogen has the ability to operate as PAMP (Pathogen-Associated Molecular Pattern), that can alter the activation of dendritic cells, natural killer cells, and macrophages through TLR 2 (Toll-Like Receptor) [48]. Ultimately, OmpA actively communicates with OMP TraN, which is crucial for conjugation [49].

External Membrane Intending

The available minimal knowledge on the basic principles of ompA like biochemical & biophysical aren't enough to understand the insertion & folding of external membranes. A positively charged chaperone transports a stable Skp complex with OmpA to the negatively charged membrane, and many membrane systems support this process [50,51]. An OmpA–Skp–LPS complex is formed during the transfer of OmpA from the cytoplasmic membrane into the periplasmic membrane, where the negatively charged LPS combines with the OmpA–Skp complex. Additionally, the protein self-aggregation is restricted by the communication between chaperone & OmpA, to reduce the clash with b-barrel folding [52]. Eventually, OmpA facilitates the b-barrel folding and rearrangement, which is slightly separated from the chaperone–protein complex. As a result, electrostatic interaction between the OmpA complex (positively charged) and negatively charged membranes facilitates OmpA to be inserted into the outer membrane and folder [53]. Through a noncovalent interaction between the OmpA amino acids Asp271 and Arg286 and the peptidoglycan amino acid diaminopimelate, the pathogen Acinetobacter baumannii's OmpA is joined to the peptidoglycan of the cell wall [54]. Additionally, OmpA in K. pneumoniae displays a

pathway for the transmembrane molecule's reversible unfolding in response to mechanical stress [55]. The polypeptide connecting the peptidoglycan layer to the outside membrane unfolds, absorbing the mechanical energy of stress. [56].

OmpA from Different Types of Bacteria

Numerous Gram-positive and numerous Gram-negative bacteria share an OmpA protein, and bacterial species are classified into families and superfamilies of proteins based on these OMPs proteins.For instance, the category includes both well- and poorly-characterized OMPs including *P. aeruginosa* OprF, the motor proteins PomB and MotY of *Vibrio alginolyticus, C. trachomatis* MOMP, and *Neisseria meningitidis* Rmp (Class 4 OMP)[49,4]. According to the proteins in the four exterior loops, bacterial species are divided into subclasses of OmpA [35]. An OMP from a specific bacteria, like *Pasteurella multocida or M. haemolytica*, is recognised and explained as the OmpA protein family, with a heat-adjustable protein in external membrane construction and a molecular weight of 30kDa, or submitted to SDS-PAGE, prior to the automated sequencing technique [50,51]. Eventually, gel extraction of protein bands demonstrated the N-terminal sequence, homologue sequence was showed in E. coli OmpA sequence, & OmpA like protein is the unknown OMP [50, 52]. Recently, C-terminal domains plays a major ro;e in identification of OMP genes sequencing & cloning with the help of beta/alpha/beta/alpha–beta structure, genomic sequence alignment & OmpA sequence homology. Additionally, N-terminus transmembrane domains demonstrates the OmpA family proteins (http://supfam.org/SUPERFAMILY/cgi-bin/scop.cgi?sunid=103088). Furthermore, numerous Gram-negative bacteria genera with similar OmpA were discovered, which are pathogenic for humans & animals. These Bacterial genera includes Actinobacillus, Aeromonas, Aggregatibacter, Bacteroides, Bibersteinia, Chlamydia, Chlamydophila, Edwardsiella, Haemophilus, Histophilus, Klebsiella, Leptospira, Mannheimia, Mycobacterium, Neisseria, Pasteurella, Riemerella, Salmonella, Shigella, Vibrio, and Yersinia. Afterall, Gram-positive Mycobacteriun tuberculosis has ArfA (also called OmpAtb and Rv0899) porin-like protein as a peptidoglycan-binding, which helps in acid stress. Eventually, pyrosequencing of C. trachomatis ompA sample genotyping offers a helpful tool for quickly identifying microorganisms [57], this was possible as they contain four variable OmpA genes.

Review of Pathogenic Mechanisms

The exposure of OmpA-like proteins in bacteria leads to pathogenicity in a number of organ systems, including the neurologic, urogenital, and respiratory systems, as a result of the diversity of bacteria [5]. Pathogenicity includes invasion, resistance to antimicrobial peptides, resistance to serum, adhesion to mucosal surfaces, and host cell activation [32]. The intrinsic immune mechanism is triggered by the surface exposed OmpA proteins that initiates the immune system. Hence, Vaccine development is eased by these OmpA like proteins which are surface exposed & are highly multiplied. Various model systems have supported the pathogenicity of OmpA importance. It was discovered that a variant of the neurovirulent, meningitis E. coli K-1 strain that lacks OmpA is bactericidal sensitive and not lethal in chick embryo or neonatal rat models. [54].Meningitis strains of E. coli has OmpA as a lethal effect & operates as adherence & invasion into the CNS capillary endothelium &astrocytes [55,5]. Other related proteins that are responsible for attachment to mucosal layers' epithelial cells, such as OmpA, include enteropathy-causing *E. coli* strains, sexually

transmitted *Neisseria gonorrhoeae*, ruminant respiratory pathogen *M. haemolytica*, multispecies pathogen *P. multocida*, and oral pathogen *Aggregatibacter (formerly Actinobacillus) actinomycetemcomitans* [56,50,57,58]. OmpA also aids in the attachment of pathogenic strains of *E. coli* and *K. pneumoniae* to leukocytes and macrophages [5]. Later, it was discovered that *Yersinia pestis* and *Yersinia pseudotuberculosis* shared OmpA similars, which clarified why mutant strains lacking OmpA were found to be recessive to the wild-type strains both in vitro and in vivo. [59]. The spirochete *Leptospira interrogans* causes zoonotic multi-organ illness, which mostly affects the liver and kidney. The protein Loa22, which is similar to *L. interrogans* OmpA and toxicates kidney cells in vitro as well as induces nitric oxide, chemoattractant proteins, and upregulates TLR2, is fatal to rats. [60]. Furthermore, Loa22-deficient *L. interrogans* was non-lethal than wild type strain in leptospirosis [61]. Concluding the studies of our laboratory explains that OmpA facilitates the adherence to epithelial cells through extracellular binding molecules like heparin & antibodies decreases the adherence in P. multocida[50]. OmpA from M. haemolytica was also found to bind to cell-surface fibronectin [62].

Respiratory Adhesion and Pathogenesis

The respiratory system uses OMPs from the OmpA family as adhesins and invasions. The airway epithelium is an essential component of lung defenses because it recognises pathogens and activates signaling pathways that cause the production of antibacterial and pro-inflammatory substances. Bacterial adherence to respiratory epithelium is the first stage of respiratory pathogenesis and the activation of host resistance systems. One of the best studied members of the OmpA family of proteins connected to respiratory epithelial adhesion is P5 from nontypeable Haemophilus influenzae, a cause of upper respiratory illnesses, bronchitis, otitis media, meningitis, and recurrent exacerbations of chronic obstructive pulmonary disease [63]. P5 has a molecular weight of 35,628 Da and shares 65% similarity with and 50% identity with *E.coli* OmpA. Human respiratory tract infections cause H. influenzae to colonise the mucosa as a biofilm, and different H. influenzae clinical isolates exhibit different OMP adhesin expression during biofilm formation, during the development of biofilms, OMP P5, P2, and P6 are expressed [64]. The surface-exposed OMP P5 epitopes of *H. influenzae* attach to human oropharyngeal cells in vitro via loops 3 and 4. [65]. When H. influenzae is incubated with respiratory epithelium, ICAM-1 is upregulated, and P5 uses ICAM-1 to mediate adhesion to the respiratory epithelium [66]. In the chinchilla infection model, OMP P5 also sticks to the middle ear and Eustachian tube mucus [67]. The P5 equivalent OMP in this pig respiratory and systemic pathogen was not linked to adhesion or invasion of porcine endothelial cells because P5-deficient *H. parasuis* displayed comparable pathogenic activity to the wild-type strain [60]. The bovine respiratory pathogen *M. haemolytica* adheres to fibronectin and bovine bronchial epithelial cells with the aid of OmpA and lipoprotein 1 [58, 68].

OmpA was found to be a virulence gene for Actinobacillus pleuropneumonia, the causative agent of severe swine pneumonia, and is likely involved in adhesion using signature-tagged mutagenesis. Similar to P.aeruginosa, the opportunistic bacteria binds to human alveolar epithelial cells in vitro using OprF, an OmpA homologue [69]. OmpA homologues for a variety of different pathogens must be linked with respiratory epithelial adhesion in order for the bacteria to be cleared from the respiratory system. Activated cells release inflammatory cytokines and chemokines through intracellular signalling mechanisms [50]. The respiratory epithelium interacts with the lung infection

in humans *Chlamydophila pneumoniae* to activate epithelial cells and trigger the production of cytokines and chemokines [70, 71]. Pre-treating C. pneumoniae cells with anti-OmpA antibodies significantly reduces the release of the neutrophil chemotactic protein Il-8. It appears to play an important role in the initiation of the inflammatory response in respiratory disease caused by *C. pneumoniae* because it activates bone marrow cells and is chemotactic for mononuclear inflammatory cells. When *C. pneumoniae* activates the human respiratory epithelium, a leukocyte growth factor called granulocyte macrophage colony-stimulating factor (GM-CSF) is secreted [70]. When C. pneumoniae is pretreated with anti-OmpA monoclonal antibodies, the production of GM-CSF from epithelial cells is reduced, proving that the C. pneumoniae OmpA homologue is a virulence factor in respiratory disease. A. baumannii is an opportunistic pathogen associated with nosocomial pneumonia. Compared to mice inoculated with wild-type germs, animals experimentally infected with OmpA-deficient *A. baumannii* showed lower blood levels of bacterial CFUs. OmpA is in charge of adherence and penetration of epithelium and macrophages through a mechanism dependent on microtubules and microfilaments, allowing *A. baumannii* persistence in biofilms [72]. OmpA location in the mitochondria or the nucleus can, respectively, cause apoptosis or cytotoxicity [71,72].

OmpA (kpOmpA) from *K. pneumoniae* binds to human bronchial epithelial cells through TLR2 interaction, which stimulates cell activation through the NF-kb pathway, increasing ICAM-1 expression and secreting a wide range of inflammatory mediators, including IL6 and a variety of chemokines [73]. By increasing levels of ICAM-1, Il-6, and other inflammatory chemokines, purified recombinant kpOmpA delivered intravenously to mice also stimulated neutrophil influx [73]. An isogenic OmpA-deficient *K. pneumoniae* strain reduced pathogenicity in mice and caused the respiratory epithelium to generate more Il-8 and other pro-inflammatory cytokines, according to recent research by March et al. (2011) [74]. This was true even if the bacteria's adherence and internalization did not differ noticeably between the wild-type and mutant strains. Through the NF-kb and MAPK pathways, the alterations caused Il-8 secretion. kpOmpA may increase a number of inflammatory mediators that cause pulmonary inflammation or inhibit the production of the neutrophil chemotactic protein Il-8, which results in fewer bactericidal neutrophils in the lesion, in order to aid *K. pneumoniae* in evading host defenses.

CONCLUSION AND FUTURE PERSPECTIVE

Porin and OmpA are examples of effective vaccine candidates that are produced by gram-negative bacteria. Due to the fact that OmpA of *S. flexneri* 2a is a highly conserved macromolecule among Shigella species and that its epitope is expressed on the bacterial surface and induces protective type-1 cell mediated immunity, it may be an important macromolecule to research, especially in light of the need for a vaccine to combat recently emerging drug-resistant strains of *Shigella spp*. In order to start the innate immune response by secreting defense-related cytokines and chemokines, antigen-presenting cells more importantly detect the exposed epitope in a TLR2-dependent manner. The protein is attractive for the exploration of an ideal subunit vaccine antigen due to its immunoregulatory features and exposure on the bacterial surface, which makes it available to the host immune system. OmpA is presented by MHCII along with the expression of CD80 on macrophages to activate the adaptive immune response to the immunogen. In response to this interaction,

CD4+ T cells produce and express IFN-, CCR5, and IL-12R-2, respectively. OmpA additionally aids the innate response. Due to its ability to strengthen the patient's innate and adaptive immune systems, OmpA is a possible vaccination candidate.

It is yet unclear if the effectiveness and safety profiles identified in the animal model apply to genetically varied human beings with vastly variable gut microbiota compositions, nutritional statuses, and prior immune experiences. Despite the fact that our animal model and OmpA's effector immune capabilities are promising, this is the case. OmpA's efficacy as a vaccine antigen may be impacted by a number of factors. The biggest difficulty in developing an oral shigellosis vaccine for children is poor immunological responses. Therefore, the method of immunization must be taken into account. Intranasal vaccination has been found to be a successful method for promoting gut antibodies in people. Therefore, data on the confirmation of safety, immunogenicity, and initial protection will be provided by clinical investigations of S. flexneri 2a OmpA in volunteer challenge models. The high immunogenicity, ability to coordinate innate and adaptive immune responses, and protective efficacy in mice of S. flexneri 2a OmpA make it a candidate for application as a promising and effective mucosal vaccine against shigellosis in people. OmpA can be modified at multiple levels, from dose amounts to dose scheduling and whether adjuvants are necessary, to promote a protective mucosal immune response against human shigellosis.

Acknowledgement

We acknowledge the resources and financial support for the study, provided by the Department of Technology Development Transfer (TDT), Department of Science and Technology (DST), New Delhi, the Order/File No: DST/TDT/BDTD/03/2021

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