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Evaluation of Produced Vaccines against *Pseudomonas aeruginosa*

Hassan Roozbahani[1]*, Sasan Rahbar Farzami Hagh[2], Mahsa Siahmansouri[1], Sadaf Alsadat Seyedi[1] and Ali Majdi[2]

[1]Department of Microbiology, Faculty of Basic Science, Lahijan Branch, Islamic AzadUniversity, Lahijan, Iran.
[2]Young Researchers Elite Club, Tonekabon Branch, Islamic AzadUniversity, Tonekabon, Iran.

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Pseudomonas aeruginosa **is the leader among gram-negative bacteria in causing burn wound infections. Exotoxin A (ETA) is a major virulence factor that produced by this organism. Specifically infecting strains tend to elaborate a smooth lipopolysaccharide (LPS) and they are resistant to the bactericidal effects of serum. It is important that antitoxin A antibody and anti-LPS antibody may provide protection through an independent and additive mechanisms.Howeverappears that optimal protection against*** ***P. aeruginosa*** **would be obtained by use of a vaccine capable of engendering both anti-LPS and antitoxin A antibodies. *P.aeruginosa* is motile with a single polar flagellum that has played an important role in the pathogenesis. Promising results in researchs show prevention of the acquisition of *P. aeruginosa* infection in CF patients immunized with a bivalent type a and b flagellum vaccines. One of the most component of colonization is the adhesion of type IV pili to asialo-GM1 receptors on the surface of epithelial cells. The toxin-pilin protein can candidate vaccine to prevent *Pseudomonas* colonization in CF.*P. aeruginosa* has an outer membrane which contains Protein F (OprF). The outer membrane protein F gene (oprF) of *Pseudomonas aeruginosa* was recently showed protect in mice against *P. aeruginosa* chronic pulmonary infection. *P. aeruginosa* is an opportunist pathogen that causes acute life-threatening infections, including pneumonia and bacteremia, individuals with immunocompromised. The control of infectious diseases requires multiple approaches and vaccination is an extremely attractive way that can induce long-term protection.**

Key words: *P. aeruginosa*, Vaccine,Antibody, Protection, Immunocompromised.

Pseudomonas aeruginosa an opportunistic Gram-negative bacterial pathogen found in most environments and is responsible for localized infections of a variety of organ systems in susceptible individuals[1]. Despite great advances in clinical medicine and the development of new effective antibiotic substances, *Pseudomonas aeruginosa* infections remain a clinical problem in the therapeutic management of nosocomial infections[2-3]. *P. aeruginosa* exhibits innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial drugs[7-8]. Most infections with this microorganism occur in immunocompromised hosts such as disrupted physical barriers to bacterial invasion for example experienced in burn injuries and dysfunctional immune mechanisms, cystic fibrosis, AIDS, neutropenia, complement deficiency, hypogamma globulinemia and iatrogenic immuno suppression[4,5-6]. Chronic pulmonary infection with *P. aeruginosa* remains the most important cause of lung disease in patients with Cystic Fibrosis [9-10]. In order to provide a good immunological response, such as is available with other vaccines against bacterial infections, several parts of the *Pseudomonas aeruginosa* cell body have been identified as potential candidates for producing

* To whom all correspondence should be addressed.
E-mail: Roozbahani_hassan3@yahoo.com

an efficient vaccine. The elements of the outer membrane proteins, OMP, Pili, Exotoxin A, LPS(alginate), the proteic part of the locomotion apparatus, the flagellum, and recently, translocation proteins related to the secretive activity of *P. aeruginosa* are of particular interest. All the above proteins, that are potentially efficient in an antibody stimulating vaccination, are still the objects of preliminary studies[11-12].

Immune response

Alveolar macrophages have roles in both the innate and adaptive immune responses to infections. Macrophages become activated when microbial products bind to cell-surface receptors, Including TLRs[13-14]. The recruitment of neutrophils is a major component of the protective host response to *P. aeruginosa* and the depletion of neutrophils results in excessive mortality after *P. aeruginosa* infection[15-16]. *P. aeruginosa* pyocyanin has been shown to induce apoptosis of neutrophils and may be a mechanism by which the bacteria resist host defenses, leading to persistent infection[17-18]. The role of lymphocytes in the immune response against *P. aeruginosa* is not well characterized[18], But in a study of 14 patients with CF and *P. aeruginosa* infection, a Th1-type immune response was associated with better lung function compared with those patients that had a predominant Th2 response. Thus, the type of T-cell response in the lung may contribute to the level of resistance to *P. aeruginosa*[19-20]. Recent data also indicate that ExoS of *P. aeruginosa* may activate monocytes by binding to both TLR2 and TLR4[21]. The role of proinflammatory cytokines is intriguing, As most data suggest that TNF- α is critical for activating phagocytic cells to clear the bacteria[22-23]. However, IL-1 and IL-18 seem to have deleterious effects in host defense against *P. aeruginosa*. Experimental work has also demonstrated a beneficial effect of IL-4 and IL-10 in host defense against *P. aeruginosa*[23]. Studies showed that several *P. aeruginosa* virulence factors are potent inducers of neutrophil chemotactic chemokines(CXC), particularly human IL-8. Bacterial products that induce chemokine production include pili, flagella, peptidoglycan, and the homoserine lactone autoinducer[24]. These products may initially interact with discrete receptors, But downstream activation of TNF- α B and/or MAP kinases is necessary toregulate CXC chemokine expression [25]. The administration of exogenous IFNγ via adenoviral vectors before bacterial challenge with *P. aeruginosa* has been shown to enhance bacterial clearance[26]. IL-4 is produced primarily by Th2 lymphocytes and by mastcells[27]. The protective effects of IL-4 may be related to its ability to modulate leukocyte function. IL-4 enhances expression of complement receptors CR1, CR3, and CR4 and increases complement-dependent phagocytosis[28]. The complement system has been shown to be important in host defense against *P. aeruginosa* and the lack of complement or its receptor did not affect the nature of the inflammatory response[29-30]. Syndecan shedding is a protective response activated during tissue injury. Thus, by enhancing the shedding of syndecan, *P. aeruginosa* takes advantage of a host protective mechanism to promote its survival. The shed ectodomains also inhibit the activity of antimicrobial peptides released by the host[31-32]. Surfactant proteins enhance the phagocytosis and killing of microbes. Both *in vitro* and *in vivo* studies provide evidence that SP-A and SP-D have important roles in the innate immune response to *P. aeruginosa*[33-34].

Flagella and Flagellin

P.aeruginosa is motile with a single polar flagellum that has the added structural feature of being glycosylated [35], and is a major virulence factor in this microorganisms [41-42]. Flagellin is the primary protein component of the flagellar filament, and it can be classified into two serotypes, types a and b[36-37]. Flagella carry out many functions, such as motility and attachment of bacteria to host cells and can also elicit the activation of the host inflammatory response with Toll-like receptor 5 (TLR5)[38-39]. Promising results in terms of prevention of the acquisition of *P. aeruginosa* infection in CF patients immunized with a bivalent type a and b flagellum vaccine have been showed[40]. A DNA vaccine encoding recombinant type a or type b *P. aeruginosa* flagellin can induced protective immunity against lethal *P.aeruginosa* lung infection[43]. A fusion protein of outer membrane proteinF (OprF), mature OprI and flagellins a and b generated significant immune responses show in mice and promote enhanced clearance of strain PA01 in a pulmonary challengemodel[44]. In addition to being highly immunogenic, the flagellin component of flagella serves as a pathogen-

associated molecular pattern (PAMP), activating TLR5 and inducing innate immunity in the lung, stimulating a protective inflammatory response[37,39,43-45]. Since *P. aeruginosa* serotype a and b flagella are conserved, contribute to virulence, stimulate innate immunity, and have induced protective efficacy in both animal[46-47] and human[40] vaccine studies, it is clear that the flagellum or the flagellin monomer may be a useful target as a vaccine component, particularly as a carrier protein to link to protective carbohydrate antigens such as lipopolysaccharide (LPS) O-side chainsor the alginate capsule[48,49-50]. In astudy compared immunization with flagella and that with flagellin for *in vitro* effects on motility, opsonic killing, and protective efficacy using a mouse pneumonia model. Observations suggest that, Antibodies to flagella were superior to antibodies to flagellin at inhibiting motility, promoting opsonic killing, and mediating protection against *P. aeruginosa* pneumonia in mice. Antibody to type a but not type b flagella or flagellin inhibit TLR5 activation by whole bacterial cells[51]. Recently recombinant exoA-flagellin (fliC) fusion protein with based on Western-blotting results, show a good antigenic interaction with sera from patients with various *P. aeruginosa* infections[52].

Pili and Pilin

The pili of *P. aeruginosa* are polarly located and these structures mediate the transfer of bacterial effector molecules to target cells through type III and type IV secretion systems[53-54]. Previous studies showed the presence of an actual binding domain for adherence to the host epithelium at the C-terminal region of the last pili monomer and that antipili antibodies inhibit the adherence of *P. aeruginosa* to human mucosal epithelia [55-56]. The potential of mucosal immunization with a killed, whole *P. aeruginosa* vaccine has also been studied[57]. Recently, it has been shown that mucosal immunization with the *P. aeruginosa* catalase can enhance bacterial clearance in rat lungs[58]. The success of mucosal immunization against *P. aeruginosa* infection has encouraged the development of a pili vaccine. Results shown the protective effect of tracheal mucosal immunization with pili protein against respiratory infection by *P.aeruginosa* in mice[59]. Pilin (encoded by the *pilA* gene) is the monomeric subunit of the type IV pilus, an immunogenic bacterial surface appendage utilized for adhesion, surface motility and biofilm formation[60-61]. *P. aeruginosa* 1244 pilin glycosylationis mediated by theoligosaccharyl transferase PilO[62-63]. This type of O-linked, PilO-mediated pilin glycosylation is common among *P. aeruginosa* strains[64,65-66]. Because the *P.aeruginosa* 1244 protein glycosylation machinery covalently links an O-antigen subunit and pilin, studies have suggested the possibility for exploitation of this system to biologically produce glycoconjugate vaccines[67,68-69]. Furthermore, expression of plasmid-borne*pilAO*1244 in non serotype O7 *P. aeruginosa* strains resulted in pilin glycosylation, in which the glycan consisted of the host's Osubunit[69]. As a variety of Osubunits could be conjugated to pilin, this indicated that the PilO glycan specificity was low, an extremely promising phenomenon in terms of exploiting the 1244 glycosylation machinery for the generation of glycoconjugate vaccines[68-69]. The current investigation shows that a bacterial protein glycosylation system is capable of biologically producing efficacious glycoconjugate vaccines. In a study demonstrate that the pilin glycan is a major immunogenic epitope, directing the production of antibodies against O antigen composed of analogous subunits and results suggest that the pilin glycosylation system of *P.aeruginosa* 1244 may be useful for the biological production ofanti-O-antigen glycoconjugate vaccines against a variety of harmful gram-negative bacterial pathogens[70]. Evaluation a dual-function vaccine that generated of antibodies against pili and exotoxin A show, Produce antibodies that reduced bacterial adherence and neutralized the cell-killing activity of exotoxin A[71].

Lipopolysaccharide and Alginate

The lipopolysaccharide (LPS) elaborated by *P. aeruginosa*is a major factor in virulence and both innate and acquired host responses to infection [72-74]. Lipopolysaccharide(LPS) plays a similar role in bacterial adhesion and The O-antigen portion of the *P. aeruginosa* LPS is responsible for conferring serogroupspecific[73-75]. A heptavalent vaccine based on extracts from 7 different strains defined as immunotypes due to their protective activity in mice[76] underwent several clinical trials[77-78] as did a vaccine based on growth in a defined medium and extraction of the culture[79,80,81-82]. In both of these cases the protective factor was found to

be LPS but due to the toxicity of these vaccines their final development into a useful clinical product was never realized[83-84]. A less toxic version of the LPS O-antigens was pursued as a vaccine candidate in the 1970s and 1980s, and consisted of a fraction of the O-antigens that were of a large enough molecular size to be immunogenic on their own and were referred to as high-molecular-weight polysaccharides[85-89]. Animal studies clearly showed high-level protection from infection in a number of settings with these antigens, and human studies showed the polysaccharides were immunogenic[86,89,90-91]. However, when some of the serologically related O-antigens from sero groups O2 and O5 were combined into a multivalent vaccine, it was found that antagonistic immune interactions were produced in mice and rabbits[92]. Attempts to overcome the inherent difficulties in using the LPS O-antigens as a multivalent vaccine by developing monoclonal antibodies to the major LPS O serogroups shows promise as a means for passive therapy of acute *P.aeruginosa* infection[93-94]. PS has also been shown to elicit a protective immune response but is much less immunogenic than LPS[87,95-96]. Previous studies suggest that a failure to produce opsonic antibody to the mucoid exopolysaccharide (MEP; also called alginate) capsule is associated with the maintenance of chronic bacterial infection[97-98]. The polymers of MEP with the largest molecular sizes safely elicit opsonic antibodies in a sufficiently large proportion of vaccinates to permit studies of active and passive immunization of patients against infection with mucoid *P. aeruginosa*[99]. Evaluation of antibodies specific to neutral polysaccharides, mucoid exopoly saccharide (LPS-smooth) and nonmucoid lipopolysaccharide (LPS-rough) show that protective immunity against *P. aeruginosa* directed against either nonmucoid LPS strains and mucoid LPS strains and appears that antibodies specific to neutral-polysaccharide antigens do not protect against *P. aeruginosa* infection[100]. Goldberg and colleagues have cloned the biosynthetic genes for the O11 LPS O-antigen into an attenuated *Salmonella enterica* serovar Typhimurium vector[101,102-103] and shown that oral and systemic immunization provides some protection against infection[104]. New results indicate that intranasal delivery of heterologously expressed polysaccharide antigens provides protection at distinct sites of infection[105]. Alive-attenuated *P. aeruginosa* strains as intranasal vaccines and have shown previously that an unmarked *aroA* deletion mutant of the *P. aeruginosa* laboratory strain PAO1, denoted PAO1"*aroA*, is highly attenuated and, after intranasal immunization of mice, engenders high levels of serum opsonic antibody directed against LPS-homologous strains[106-107]. Also, remarkably active intranasal immunization via PA14"*aroA* could protect against lethal pneumonia caused by LPS-heterologous strains in the absence of opsonic antibody. Further experiments show that the neutrophil-attracting, T cell-secreted cytokine IL-17 is critical for the protective efficacy of this vaccine[108].

Exotoxin A (ETA) and Polysaccharide-Toxin A Conjugate

Exotoxin A (ETA) is one of the major virulence factors produced by *P. aeruginosa*, ETA was first purified by Liu *et al.*, [109-110]. Since then, ETA has proven to be toxic for a wide variety of mammalian cells in vitro [111-112] and lethal for many animal species [113-114]. ETA is an ADP-ribosylating toxin that catalyzes the transfer of ADP-ribose from NAD to eukaryotic elongation factor 2, resulting in the inhibition of protein synthesis and ultimately cell death [115-116]. To date, several studies have been conducted in order to understand the immunochemistry of ETA and to identify the immunodominant neutralizing epitopes of this molecule [117,118-119]. Previously, reported successful induction of neutralizing antipeptide antibodies to a short amino acid sequence representing a portion of the enzymatic domain of ETA (aa 596 to 625, designated peptide 11) [120]. These antibodies provided in vitro protection to monolayers of 3T3 fibroblasts against ETA-induced inhibition of protein synthesis by specifically blocking ADP-ribosyltransferase activity [120]. In the same study, identified another synthetic peptide encompassing a region within the translocation domain of ETA (aa 289 to 333), which induced antibodies with moderate ability to neutralize the cytotoxic activity of ETA in vitro [120]. In other model examined the potential of neutralizing antipeptide antibodies to confer protection against ETA or infection with an ETA-producing strain of *P. aeruginosa* in mice, Results showed the ability of these synthetic peptides to induce a state of active immunity against ETA [121]. It is interesting that antitoxin A

antibody and anti-LPS antibody provide protection through an independent and additive mechanisms. It therefore appears that optimal protection against *P. aeruginosa* would be obtained by use of a vaccine capable of engendering both anti-LPS and antitoxin A antibodies[122-123]. In a report described the synthesis of a nontoxic *P. aeruginosa* polysaccharide-toxin A conjugate vaccine capable of engendering anti-LPS and toxin A-neutralizing antibodies[124]. In a other study, Serologically reactive O-polysaccharide from nine serotypes of *Pseudomonas aeruginosa* were covalently linked to toxin A. The conjugation condition used preserved a substantial proportion of critical epitopes on the toxin A molecule as shown by the ability of toxin A-neutralizing monoclonal antibodies to react with the various conjugates. Results suggest that all nine conjugates were capable of evoking an antitoxin A and an antilipopolysaccharide immunoglobulin G (IgG)response in mice and rabbits[11-125].

Outer membrane proteins(OprF, OprI)

P. aeruginosa has an outer membrane which contains Protein F (OprF). OprF functions as a porin, allowing certain molecules and ions to come into the cells, and as a structural protein, maintaining the bacterial cell shape[126-127]. Because OprF provides *P. aeruginosa* outer membrane with an exclusion limit of 500 Da, it lowers the permeability of the outer membrane, a property that is desired because it would decrease the intake of harmful substances into the cell and give *P. aeruginosa* a high resistance to antibiotics[128,129-130]. The *P. aeruginosa* major constitutive porin protein, OprF, which has previously been shown to be antigenic[131-132] and has high homology among Pseudomonas strains[133-134], was also chosen as a vaccine target[135]. Vaccination with outer membrane

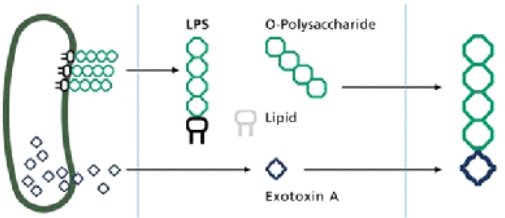

Fig. 1. A polyvalent conjugate vaccine combining 9 prevalentserotypes of *P.aeruginosa* with exotoxin A

protein antigens has been shown to be efficacious against *P. aeruginosa* infection in a number of studies using killed whole cells[136], purified outer membrane preparations[137], isolated outer membrane proteins[131-138], protein fusions, or synthetic peptides representing protective epitopes[139]. This protein has beenshown to provide protection in a mouse model of systemicinfection[131], a mouse burn infection model, androdent models of acute[140]and chronic lung infection[133].However, Evalution of protective efficacies against murine corneal infection of active and passive immunization with PAO1*"aroA* and of therapy with a rabbit antiserumraised against PAO1*"aroA* indicated that OMP antigens are the targets for the protective antibody in this Experiment[141].OPRs,can be producedby recombinant DNA technology free of contaminating*P. aeruginosa* LPS and cloned genes of OPRsapplicable for naked DNA immunization[142-143], or could betransfected into special vectors such as nonpathogenic *Salmonella*strains to induce a mucosal immune response[144-145]. In the past alsoRecombinant OprIwas expressed in *Escherichia coli* and used to vaccinate humanvolunteers,Vaccination was well tolerated[138-146]. Additionally,generated a recombinant hybrid protein consisting ofthe entire OprI molecule fused toOprF and expressed in *E. coli*. Studies showsthat Met-Ala-(His)6OprF190–342-OprI21–83 was isolatedand purified from *E. coli* to yield a clinically applicablevaccine that was successfully used without any apparent sideeffects for the vaccination of human volunteers against *P.aeruginosa*[146-147].Galloway, reported that altering theimmunization protocol to include two biolistic intradermalinoculations with the oprF DNA vaccine followed bya final intramuscular booster immunization with achimeric Pseudomonas-influenza virus displaying an insertof the peptide 10 epitope of OprF within its hemagglutinin(HA) protein,and altering thecomposition of the DNA vaccine to consist of DNA encodingthe C-terminal half of OprF fused to OprI,Are two approaches that enhance the overallprotective efficacy of DNA-based vaccination against *P. aeruginosa*[148,153]. Additionally, Evalution a capsid-modified adenovirusvector (AdOprF.RGD.Epi8) indicate that expresses thegene for OprF to induce protective immunity against *P. aeruginosa*[149].New promising perspectivesfor the development of

vaccination strategies againstvarious types of pathogens are the use of antigen-pulseddendritic cells (DCs) as biological immunizing agents[150-151]. In a study designed and tested the efficacy of OprF-pulsedDCsfor a vaccine based upon adoptive transfer in micewith *P. aeruginosa* infection, Resultsdemonstrate the ability of mouse DCspulsed with purified or recombinant OprF to protectmice against *P. aeruginosa* infection [152].

DISCUSSION

Despite great advances in clinical medicine *Pseudomonas aeruginosa* infections remain a clinical problem in the therapeutic management of nosocomial infections. The effectiveness of vaccination against *P. aeruginosa* infection in burn patients was demonstrated 30 years ago. However, currently no clinical vaccine against *P. aeruginosa* for which safety and efficacy have been shown in clinical trials with patients from one of the major risk groups for nosocomial *P. aeruginosa* infection is available for routine use. Hence, due to high prevalence of antimicrobial resistance, the immunoprophylaxis and immunotherapy might be an effective method for treatment and control of *P. aeruginosa* infections. It has been shown that neutralization of bacterial virulence factors can result in prevention and reduction of mortalities due to *P. aeruginosa* infections. A vaccine to delay or prevent initial pulmonary infection in individuals with CF would have significant impact and may be accomplished in the future.

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