Current Status of *Pseudomonas aeruginosa* Vaccine

Lessandra Michelim¹*, Gregory Saraiva Medeiros¹ and Alexandre P. Zavascki²

¹Department of Infectious Diseases, Hospital Geral de Caxias do Sul, Caxias do Sul, Brazil; ²Infectious Diseases Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil.

Abstract: *Pseudomonas aeruginosa* is one of the major pathogens responsible for a wide variety of severe nosocomial and community acquired infections. Numerous vaccine candidates and several monoclonal antibodies have been developed over the past 40 years but only a few have reached clinical trials and none of these vaccine candidates has obtained market authorization. The understanding of *P. aeruginosa* pathogenesis and its virulence factors is essential in the identification of immunogens that can be used for a *P. aeruginosa* vaccine. This review summarizes the present status of vaccine development for this important pathogen.

Keywords: Cystic fibrosis, Clinical trials, Hospital infections, Immunity, *Pseudomonas aeruginosa*, Vaccines.

1. INTRODUCTION

*Pseudomonas aeruginosa* is a frequent cause of severe nosocomial and community acquired infections at various body sites including the urinary tract, surgical or burn wounds, the cornea, and lower respiratory tract [1]. These opportunistic Gram-negative bacteria have a non-fastidious behavior with low nutritional requirements and are found mostly in water reservoirs and soil. Infections caused by these bacteria have been associated with higher rates of morbidity and mortality [2].

These organisms are particularly problematic among two distinct populations of patients: cystic fibrosis (CF) and hospitalized patients. *P. aeruginosa* chronically infects patients with CF and is associated with further impairment in lung function resulting in increased morbidity and mortality [3]. This pathogen is a leading cause of nosocomial infections, especially, pneumonia and ventilator-associated pneumonia [4], and these infections are usually extremely difficult to treat, a fact that contributes to several adverse outcomes among such patients [5, 6].

Despite advances in therapy, effective treatment and control of *P. aeruginosa* infections remains a constant problem, mainly because its ability to acquire resistance to multiple antimicrobial agents by diverse mechanisms [2]. Extensively-drug resistant isolates and even, pan-drug resistant isolates have been a common cause of both infections in CF patients and nosocomial infections, representing a major public health concern [7]. The discovery and development of new antibiotics against Gram-negative pathogens, especially *P. aeruginosa*, has been a challenge since several years. Thus, adequate immunotherapies or vaccines have long been desired as an alternative method to prevent infections in susceptible populations. Several vaccine candidates which include capsule components, sub-cellular fractions, purified and recombinant proteins have been evaluated in animal models and human clinical trials [8]. This review summarizes the present state of the development of vaccines and immunotherapies against *P. aeruginosa*.

1.1. Host Defense

Human immune responses and the bacterial defense mechanisms are important factors for adequate vaccination. Innate and adaptive immune responses work in synergy against *P. aeruginosa* infections [8]. Humoral, mucosal or systemic opsonizing immunity is most efficient to prevent colonization and infection being an extracellular pathogen. Nonetheless, T-cell responses can mediate protective immunity in patients with *P. aeruginosa* infections. The vaccine would be ideal for prevention of acute infections in immunocompromised hosts, particularly in patients with organ transplantation, HIV infection, using cytotoxic drugs or with hindered localized phagocytosis due to severe burns with vascular damage [9-11]. Moreover, chronic infections of the respiratory tract are the primary cause of morbidity and mortality for individuals with CF [1], and a vaccine to prevent lung infection is essential for these patients [12]. In CF patients, the defect in cyclic adenosine monophosphate-regulated chloride ion channels in the epithelial lining of the respiratory system favours the colonization of *P. aeruginosa*, resulting in inflammation. Upon the entry of *P. aeruginosa* in lungs of patients with CF, penetration of mucus surface and colonization is followed by a genetic transformation of non-mucoid to mucoid (alginate-producing) phenotype. Non-mucoid bacteria are more virulent and are better recognized by the immune response while the mucoid phenotype increases bacterial adherence and resistance to phagocytosis.
As the infection progresses, bacteria release virulence factors through quorum sensing, inducing an inflammatory response. Unlike normal airway secretions, the inflammatory response increases concentrations of neutrophil-derived DNA and filamentous actin in the mucus of CF patients that allow for bacterial adherence and biofilm production [13]. Vaccine-based immunity should ideally be induced against multiple antigenic components with diverse host-induced immunologic effectors for the induction of a strong protection against clinically diverse \( P. \) aeruginosa isolates. However, we have an incomplete understanding of the range of effectors of acquired immunity that contribute to protection against \( P. \) aeruginosa infections in humans. Additionally, a limited array of effectors may not be sufficient to protect against the range of clinically relevant strains at all sites of infection [14].

1.2. Vaccine Development

For more than a century, the use of live bacteria and bacterial virulence factors as therapeutic tools in human medicine has been considered. The production of an effective vaccine against \( P. \) aeruginosa infections, including nosocomial pneumonia, bloodstream infections, burn wound infections, chronic lung infections in CF patients and potentially sight-threatening keratitis in users of contact lenses, is a high priority. As with vaccine development for any pathogen, key information about the most effective immunologic effectors of immunity and target antigens need to be established. Major target antigens include the lipopolysaccharide O-polysaccharides, cell-surface alginate, flagella, pili, components of the Type III secretion apparatus and outer membrane proteins with a potentially additive effect achieved by including immune effectors to toxins and proteases [15]. A variety of active vaccination approaches are potentially efficacious, such as vaccination with purified or recombinant antigens incorporating multiple epitopes, conjugate vaccines incorporating proteins and carbohydrate antigens, and live attenuated vaccines, including heterologous antigen delivery systems expressing immunogenic \( P. \) aeruginosa antigens [2]. A diverse range of passive immunotherapeutic approaches are also candidates for effective immunity, with a variety of human monoclonal antibodies described over the years with promising preclinical efficacy and some early Phase I and II studies in humans [16]. Johannsen and Gotzsche had recently published an intervention review [17] selecting randomized trials (published or unpublished) comparing oral, parenteral or intranasal vaccines with control, or no intervention in CF patients. In this review, only three trials were included comprising 483, 476 and 37 patients, respectively. No data are available from one of the large trials, which is unpublished. In the other large trial and in the small trial, the risk of getting a chronic infection was not decreased. In the large trial, one patient was reported to have died in the observation period. In that trial, 227 adverse events (four severe) were registered in the vaccine group and 91 (one severe) in the control group. So far, vaccines against \( P. \) aeruginosa are not recommended for CF patients. Table 1 summarizes potential antigens for \( P. \) aeruginosa vaccines.

2. VACCINE ANTIGENS TYPES

2.1. Bacteria Component

2.1.1. Lipopolysaccharide, Polysaccharide and Polysaccharide-conjugate Vaccines

Lipopolysaccharide (LPS) is the main constituent of the outer leaflet of Gram negative bacteria and is composed of three distinct regions: lipid A, a relatively conserved inner and outer core oligosaccharide, and O antigen (mutable peripheral long chain polysaccharides). \( P. \) aeruginosa produces two forms of O antigen, which are the homopolymeric A band and the heteropolymeric B band [18]. The smooth or rough phenotypes are regulated by the presence or absence of outer O-polysaccharide chains [19]. Since it is toxic when administered in a purified state, pure LPS, or vaccines containing LPS, are generally considered too dangerous to humans. The toxicity is specifically linked to the lipid A and it is absent when this structure is removed from the core and O-PS regions. In order to circumvent the problem of toxicity, LPS can be introduced into liposomes to mask the toxic lipid A moieties [20, 21]. Another approach is to use only the non-toxic PS part of the LPS in vaccine preparations, called O-PS-based vaccines that have to be multivalent due to the specificity of LPS to \( P. \) aeruginosa serotypes [20]. Several vaccines based on LPS showed promising results in animal preclinical studies [22, 23]. These were tested not only in animal models but also in patients, especially burned, with cancer and lung disease, where the incidence of lethal \( P. \) aeruginosa infection is very high. To increase immunogenicity, O-polysaccharides were conjugated to carrier proteins such as exotoxin A, keyhole limpet hemocyanin (KLH) or tetanus toxoid, and some data on animal protection have been published with good results. Clinical trials for multivalent LPS-based vaccines have been performed in immunocompromised individuals with variable efficacies and toxic side effects. [24]. Using purified \( P. \) aeruginosa O-PS molecules, an octavalent conjugate vaccine was developed, later called Aerugen™, which conferred significant protection after intramuscular and intranasal administration in mice [8]. A study in CF patients showed the presence of high-affinity antibodies associated with lower rate of infection over the observation period and the follow-up of 10 years, with an important reduction of chronic infection with \( P. \) aeruginosa, as well as improved quality of life. In another cohort of 25 CF patients followed by 10 years, yearly immunization with an octavalent conjugate OPS-toxin A vaccine was safe, clinically effective and immunogenic [25] and qualitative analyses revealed that the protective capacity of specific serum IgG antibodies was linked to high affinity and to specificity for OPS serotypes rather than for LPS core epitopes [26]. Nevertheless, a consecutive double blind, randomized, placebo-controlled phase III study involving 476 patients with CF failed to prove positive results of the other trials and the production of this vaccine was suspended [8]. Despite all these efforts, clinically relevant LPS or O-polysaccharide-based vaccines continue to be a challenge. The main obstacles for the progress of this kind of vaccine are extensive serological heterogeneity, LPS-associated toxicity, cost and complexity of development of lipid free multivalent-conjugates.
### Table 1. Summary and main characteristics of *Pseudomonas aeruginosa* vaccines.

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Antigenic fragment</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Phase</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine target</td>
<td></td>
<td>Long-lasting protective systemic or localized antibodies.</td>
<td>Need of including additional flagellar types. Variants without flagella</td>
<td>III</td>
<td>Campodonico et al. [43], Doring et al. [37], Honko et al. [42], Rosok et al. [35]</td>
</tr>
<tr>
<td>Flagella</td>
<td></td>
<td>Clinically effective and immunogenic (high level of opsonic antibodies)</td>
<td>Serological heterogeneity, LPS-associated toxicity, cost and complexity of development of lipid free multivalent-conjugates</td>
<td>III</td>
<td>Kashef et al. [24], Lang et al. [26], Zuercher et al. [25]</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td>Safe and well tolerated</td>
<td>Only for Cystic Fibrosis.</td>
<td>I</td>
<td>Campodonico et al. [32], Theilacker et al. [31]</td>
</tr>
<tr>
<td>MEP</td>
<td>One of the most promising vaccine antigens</td>
<td>Controlled clinical trials are required</td>
<td></td>
<td>II</td>
<td>Baumann et al. [66, 98], Ding et al. [69], Gocke et al. [67], Krause et al. [28], Mansouri et al. [65]</td>
</tr>
<tr>
<td>Opr</td>
<td>High immunogenicity</td>
<td>Variable efficacies to reduce bacterial adherence mainly because of the difficulty of generating specific RBD antibodies</td>
<td></td>
<td>Pre clinical</td>
<td>Audette et al. [46], Horzemka et al. [51], Kao et al. [47, 49]</td>
</tr>
<tr>
<td>Pilin</td>
<td>Safety</td>
<td>Eliciting long term immunity</td>
<td></td>
<td>I</td>
<td>Cripps et al. [75, 78]</td>
</tr>
<tr>
<td>Killed</td>
<td>Safety</td>
<td>Eliciting long term immunity</td>
<td></td>
<td>I</td>
<td>Cripps et al. [75, 78]</td>
</tr>
<tr>
<td>Live-attenuated</td>
<td>Highly immunogenic</td>
<td>Residual virulence</td>
<td></td>
<td>Pre clinical</td>
<td>Priebe et al. [81], Zaidi et al. [82]</td>
</tr>
<tr>
<td>Vector based</td>
<td>Safe, flexible, effective and widely characterized vaccine</td>
<td>Pre-existing anti-Ad immunity promotes difficulty of achieving booster effect on repeated administration. This problem appears to be effectively circumvented by the incorporation on antigenic epitopes in the fiber protein. Need of clinical trials</td>
<td></td>
<td>Pre clinical</td>
<td>Lanzi et al. [87], Krause et al. [88], Sharma et al. [86], Worgall et al. [72]</td>
</tr>
</tbody>
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LPS = Lipopolysaccharide; MEP = Mucoid Exopolysaccharide; Opr = Outer Membrane Proteins.

#### 2.1.2. Mucoid Exopolysaccharide (MEP)

The main component of the *P. aeruginosa* biofilm matrix is called alginate or MEP, a random 1-4 linked polymer of D-mannuronic acid (M) and L-guluronic acid (G) residues that is critical to the adherence in the lung. This exopolysaccharide has potential roles as a mechanism for bacterial adherence, as a barrier to phagocytosis and as a mechanism to neutralize oxygen radicals [27]. MEP also affects leukocyte functions, such as the oxidative burst and opsonization, and plays an immunomodulatory role via induction of proinflammatory cytokines and suppression of lymphocyte transformation [24, 28, 29]. Alginate is structurally less variable than LPS and has been considered as a vaccine candidate. Human and animal trials showed the role for MEP-specific opsonizing antibodies in facilitating bacterial eradication [30]. To improve immunogenicity, MEPS have been conjugated to various carrier proteins such as exotoxin A, tetanus toxoid or KLH. This converts polysaccharide from a T-cell-independent to a T-cell-dependent antigen, and elicits a higher and boostable immune response in animals [31]. Alginate preparations differ principally in molecular size, the ratio of M:G residues from 10:1 to 6:4, and the level of O-acetylation at the M residues on C-2 and C-3, and of these factors may affect the immune response, notably in the ability to generate broadly reactive, and opsonic antibody [1]. Campodonico et al. [32] showed that conjugating flagellin and alginate induced opsonic antibodies against mucoid, but not non-mucoid *P. aeruginosa*. Effective clinical product has not been yet produced despite good results in some trials. Finding a preparation of alginate that gives rise to antibodies reactive with multiple strains of mucoid *P. aeruginosa* is a challenge.

#### 2.1.3. Flagella

Flagella are conserved organelles that play a critical role in providing motility to diverse bacterial species, including *P. aeruginosa*. These organelles are effectively targeted by the host immune system, and bacteria that delete or mutate their flagella can cause severe persistent infection. Besides providing mobility and contributing to invasiveness of
**P. aeruginosa**, flagella proteins have also been found to be involved in adhesion to host cells and molecules in vitro [33, 34]. It may bind to mucus, the glycolipid asialo (GM1), as well as to Toll-Like Receptor 5 (TLR5), inducing inflammation. Furthermore, an intact flagellum structure is necessary for bacteria dissemination from the site of infection [33]. Since flagellin, the major protein element of flagella, is divided into the heterogenous type-A and the serologically uniform type-B flagellin, an effective vaccine must have to be bivalent and broadly protective. DNA vaccines encoding native *Pseudomonas* B-type (FltC) or A-type (FlaA) flagellin are strongly immunogenic and the resultant antibodies response interferes with the interaction of homologous flagellin with TLR5 [35]. This reduces the ability of the host to clear homologous, but not heterologous, flagellin-expressing *P. aeruginosa*. To circumvent this problem, Saha *et al.* [34] engineered a DNA vaccine encoding a mutant FltC R90A flagellin. The mutant antigen encoded by this vaccine was highly immunogenic, but its ability to interact with TLR5 was reduced by >100-fold. The flagellin mutant DNA vaccine provided excellent protection against both FltC- and FlaA-expressing *P. aeruginosa*. Flagella vaccine efficacy showed to be superior to that of the flagellin vaccine [34]. Besides, antibodies to flagellin monomers inhibited TLR5 activation and associated activation of innate immunity [36]. Doring *et al.* [37] conducted a randomized, double-blind, placebo controlled, multicenter phase III trial on 483 CF individuals without *P. aeruginosa* colonization to evaluate the efficacy of four intramuscular injections of a bivalent flagella vaccine. The vaccines were given over a 14 months period and the patients were followed over a 2 years period. It was well tolerated, and the patients developed high and long-lasting serum antiflagella IgG titers. Analysis of the 381 participants, exhibiting flagella subtypes included in the vaccine, were significantly less frequently isolated from vaccines than from placebo controls (*P* = 0.016; RR: 0.51; 95% CI: 0.31–0.86). In acute infections, animal studies in a burn-wound infection model and a neonatal mouse model of acute *P. aeruginosa* pneumonia have shown that immunization against the flagellum protects against the lethal effects of *P. aeruginosa* infection [38–40]. Monovalent *P. aeruginosa* flagella vaccines, prepared from purified flagella protein, have been tested in healthy human adults [41]. High and long-lasting circulating antibody titers against the flagella antigen have been noted following intramuscular immunization and adverse effects were mild. Since serotype A and B flagella are conserved, contribute to virulence, stimulate innate immunity, and have induced protective efficacy in both animal and human 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 like lipopolysaccharide (LPS) O-side chains or the alginate capsule [31, 42]. Polymeric flagella are superior to monomeric flagellin at inducing antibodies against acute lung infection [43]. Mono- or bivalent flagella vaccines have shown promise in clinical trials by inducing effective and permanent systemic or localized antibodies. Addition of other flagella types may improve the overall efficacy.

### 2.1.4. Pili

Pili are polymeric assemblies of the pilin protein that helps in bacterial adhesion, biofilm formation and twitching motility in the early stage of infection. They mediate colonization and cell invasion. Pilus antigens are serologically diversified and can be classified into five different phylogenetic groups. N-terminal region of mature pilin is highly preserved; nevertheless, it is not an ideal vaccine candidate because of its hydrophobic nature and limited accessibility [44]. Furthermore, while the pilin protein is immunogenic, few of the antibodies elicited are receptor binding domain (RBD) specific. The putative C-terminal receptor binding site is structurally preserved and is expected to interfere in *P. aeruginosa* adherence and allow cross-protection when used as a vaccine antigen [45]. Audette *et al.* [46] and Kao *et al.* [47] have developed a synthetic-peptide consensus-sequence vaccine that targets the host receptor-binding domain (RBD) of the type IV pilus of *P. aeruginosa*. Because of its structure, the type IV pilus has been suggested to mediate initial attachment of the bacteria to host surfaces before other adhesins secure the attachment. Once attached, the coordinated expression of numerous other virulence factors facilitates invasion of the surface by the bacteria. Other study with pili component have been made and immunization with intact pili, as well as synthetic peptide analogs of the RBD showing improved survival in a mouse model of *P. aeruginosa* infection [48]. There are advantages in using synthetic peptide of the RBD conjugated to keyhole limpet haemocyanin rather than native strain pilin protein for an anti-pilus vaccine [49]. Some studies in mice using purified pili protein or pilus peptides conjugated to carrier proteins has showed good results. Hertle *et al.* [50] created a dual-function chimeric exotoxin A-pilin vaccine that showed a reduction in bacterial attachment and inactivated the cytotoxic activity of exotoxin A in rabbits. Since *P. aeruginosa* strain 1244 naturally has an O-antigen repeating unit covalently linked to every pilin monomer, it seems to be a good vaccine candidate. Horzempa *et al.* [51] administered vaccines with 1244 pilin in a murine model for lung infections and in a burn model. Similarly to pilin protein vaccines, some trials with anti-pilus synthetic peptide conjugates also generated elevated antibody titers with higher affinity [49]. In summary, pilin-based vaccines have demonstrated some variability in reduce bacterial attachment mainly due to the difficulty in producing specific RBD antibodies. Heretofore, there have been no human trials with pilin vaccines.

### 2.1.5. T3SS

*Pseudomonas aeruginosa* uses a Type III secretion system (T3SS) analogous to that of *Vesicella pestis* and *Salmonella* spp. to deliver exoenzymes into eukaryotic cells. *P. aeruginosa* toxins, including ExoS ExoT, ExoU and ExoY, are injected directly into eukaryotic cells via a needle-like structure that pierces the plasma membrane of the target cells. ExoS and ExoT interfere with eukaryotic cell signaling pathways and host cytoskeletal; ExoU functions as a phospholipase A2; ExoY is an adenylyl cyclase that shares homology to the edema factor of the anthrax toxin [37]. The
intracellular delivery of these enzymes and their interaction with eukaryotic cofactors is highly correlated with the dissemination of bacteria from the initial sites of infection and the induction of sepsis. Other complex structures also form on the bacterial surface to assemble the delivery system, and one component of this structure is the PcrV protein. T3SS is responsible for virulence and, in the absence of exotoxins, can directly mediate macrophage and neutrophil cytotoxicity through a cell-death process called “oncosis” [52, 53]; macrophage-released factors trigger bacterial swarming and lead to direct cell-membrane perforation. PcrV protein is situated on the bacterial surface and is necessary for translocation of effector proteins [54]. Vaccine targeting PcrV showed protective response in mouse model, reduced lung inflammation and injury in a murine model and in a burn mouse model [55]. Interruption of the translocation of type III effectors by anti-PcrV was pointed as the possible mechanism for the protection [55-57]. In a recent study, a multivalent T3SS-based protein vaccine, including P. aeruginosa PcrV and needle tip proteins from other Gram-negative bacteria, proved to be immunogenic [58]. Hereafter, immunization against PcrV and extracellular toxins can be considered as part of multicomponent vaccines, since it showed efficacy in reducing the inflammatory and cytotoxic effects [59]. Monoclonal antibodies have been studied in mice model, such as KB001, an investigational PEGylated engineered human Fab’ fragment that specifically binds to a P. aeruginosa PcrV epitope and inhibits its function. A recent Phase-2a dose-finding study was conducted to determine the safety, pharmacokinetics (PK), and potential usefulness of KB001 to prevent P. aeruginosa pneumonia in intensive care patients requiring prolonged mechanical ventilation that were colonized, but not infected, with this bacterium. KB001 was safe, well tolerated and detected in endotracheal aspirates from all patients receiving it, as early as day 1 and up to 28 days and these patients developed P. aeruginosa pneumonia less frequently (33%) than placebo recipients (60%) [60]. PcrV vaccines and monoclonal antibodies have not been tested in clinical studies. The detailed understanding of structure-function relationships of T3SS needle tip proteins will be of value in further developments of new vaccines and immunotherapy.

2.1.6. Extracellular Components

Some specific exotoxins and extracellular enzymes are involved in P. aeruginosa virulence. Exotoxin A, an ADP-ribosyl transferase that suppresses host protein synthesis, is the major toxic factor. Elastase and alkaline proteases act on the host immune system by cleaving immunoglobulins, inhibiting cytokines, and interfering with the immune cell functions. Vaccines with a truncated exotoxin A subunit or with elastase and alkaline protease toxoids had an adequate response in animal models [61]. Tanomand et al. [62] describe the preparation of recombinant ExoA and FliC protein as a new vaccine candidate against P. aeruginosa infection and the results have indicated that this fusion protein may be used as a serodiagnostic antigen for rapid diagnosis of P. aeruginosa infections [62]. None of these vaccines with exotoxins or enzyme components have yet been tested in humans.

2.1.7. Outer Membrane Protein

Outer membrane proteins (Opr) form porins and other structural and functional components of P. aeruginosa cell surface. OprF and OprI are the main Opr’s that are surface-exposed and antigenically preserved in wild-type strains [63, 64]. OprF appears to be crucial for the adaptation of the bacteria to the host defense. OprI proved to attach mucosal surfaces of the lung and intestinal tract and facilitated antigen delivery to antigen presenting cells, acting as a mucosal carrier. Immunization with OprF and OprI components may induce protective antibodies reactive to all of the known P. aeruginosa serotypes [1]. In vitro studies have demonstrated that enhanced opsonophagocytosis is the primary mechanism underlying the immunogenic protection of OprF/I. Immunization of healthy individuals with OprF - OprI vaccine was safe and elicited a long lasting systemic and lung mucosal antibody response, with higher levels of systemic IgG and mucosal IgA [65]. A phase I/II clinical trial in a population with chronic lung disease showed mucosal-specific IgA and IgG up to 6 months in more than 90% of the patients [66, 67]. Sorichter et al. [68] and Ding et al. [69] demonstrated that a single boost injection of OprF/I vaccine elicited a strong OprF/I-specific antibody response in individuals who were previously vaccinated with OprF/I in a clinical trial. The OprF/I vaccinated sera prevent P. aeruginosa binding to IFN-γ, indicating an alternative method by which the OprF/I vaccine protects against P. aeruginosa infection. Chimeric vaccines have been made composed of exotoxins and OprF and OprI, as well as formulations like peptide vaccines, DNA vaccines, dendritic cell-pulsed, viral vectors or heterologously expressed in bacterial vectors with good results in preventing infections [28, 59, 70-74]. Although Opr may be one of the most promising antigens, controlled clinical trials are needed to evaluate the real protection against P. aeruginosa.

3. LIVE-ATTENUATED OR WHOLE-CELL KILLED VACCINES

Studies of acute infection in a rodent model have demonstrated that mucosal immunization with a whole-cell killed vaccine results in effective elimination P. aeruginosa from the lung, as well as low rates of mortality [75, 76]. In preliminary study with nine bronchiectasis patients have demonstrated that oral immunization with an enteric coated whole-cell killed P. aeruginosa vaccine resulted in the detection of circulating antigen-reactive peripheral blood leukocytes, along with an important decrease in the levels of bacteria in the sputum [77]. In a Phase I clinical trial, the authors examined the safety and immunogenicity of an oral, whole-cell vaccine administered to a healthy population [78]. Thirty subjects received an oral dose of Pseudostat® in two timed, measured doses with serological follow-up to 56 days postvaccination [77]. Following vaccination, several individuals were identified as antibody responders for all three immunoglobulin isotypes tested, mainly IgA, specifically against whole-cell P. aeruginosa extract and OprF and OprI. However, more trials are needed, since inactivated whole-cell may not always induce immune response and it may not be long lived. Besides whole-cell vaccines, live attenuated P. aeruginosa strains have been created by introducing deletion mutations into aroA gene. Several trials show that intranasal
immunization of mice and rabbits with aroA mutants produce high titers of opsonic antibodies and protects against acute fatal pneumonia caused by serogroup-homologous strains. Attenuated Salmonella species that express heterologous antigens are promising vaccine vehicles, mainly for mucosal immunization [79]. An attenuated aroA mutant of S. enterica serovar Typhimurium (strain SL3261) was used to express OprF/H from P. aeruginosa. This strain was also used to express the serogroup O11-O antigen of P. aeruginosa. However, in contrast to P. aeruginosa, single aroA deletion mutants in strain SL3261 retain sufficient virulence to make them unacceptable as human vaccines [80]. A live attenuated P. aeruginosa vaccine was safe, highly immunogenic and capable, when nasally administrated in a serogroup-specific manner, of protecting immunized mice against lethal pneumonia [81]. It can also protect immunized mice against corneal infections, even that caused by different serogroups, and probably could be used to prepare therapy reagents [82]. B cell activating factor (BAFF) is a promising cytokine that can augment P. aeruginosa immune host response and can be a molecular adjuvant for a genetic vaccine [83]. Development of different methods to attenuate virulence while maintaining immunogenicity will help support such vaccines for clinical trials.

4. DNA AND VIRAL VECTOR VACCINES

DNA vaccines are relatively stable and can be easily prepared and harvested in large quantities. Additionally, naked plasmid DNA is relatively safe and can be repeatedly administered without adverse effects. Moreover, DNA is able to be maintained in cells for long-term expression of the encoded antigen; therefore, maintenance of immunologic memory is possible. Similar to DNA vaccines, viral vector system might represent an important platform for anti-pseudomonas vaccines [84]. Adenovirus (Ad) vectors are attractive delivery vehicles [85] for several vaccines due to the ability to act as immune system adjuvants and rapidly achieve robust responses against the genetic products and viral capsid proteins. The most studied serotypes of Ad virus in a vaccine model against P. aeruginosa were Human serotype 5 (Ad5) and primate serotype C7 (AdC7) [28, 72, 86]. Incorporating epitopes into the Ad capsid is an important strategy for achieving boosting with repeated vaccine administration and eliciting antigenic response while in the presence of anti-Ad5 immunity [86–88]. Adenoviral vaccine and DNA vaccines represent a safe, flexible, and widely characterized vaccine types [89]. However more P. aeruginosa clinical trials are needed since other adenoviral vaccine trials have been demonstrating safety and effectiveness [90, 91].

5. PASSIVE IMMUNOTHERAPY

Various products of P. aeruginosa-specific hyperimmune intravenous IgG (IVIG) from vaccinated donors have been used as therapies. This kind of passive treatment can potentially promote effective protection in high-risk groups of immunosuppressed patients [92]. Some trials have been conducted in burned patients with good results. A phase III trial using O-antigen-based octavalent passive immunization was stopped because it failed in demonstrating reduction of incidence and severity of P. aeruginosa infections [93]. The use of murine or human monoclonal antibodies (mAbs) as passive immunotherapy is considered superior to IVIG due to enhanced specificity, lower risk of biohazard contamination, quality in mass production and selection of highly protective epitopes from otherwise poorly immunogenic antigens. Various human and mouse mAbs with specificity for LPS-O-antigens have demonstrated protection against infection in animal models [94]. In a Phase I trial [95] there was described the generation and preclinical characterization of a fully human IgM/k MAb termed KBPA101, directed against the LPS O polysaccharide of serotype O11 of P. aeruginosa, and protection from local respiratory infections in an acute lung infection model in mice was demonstrated. Lazar et al. [95] conducted a double-blind study evaluating the safety and pharmacokinetics of KBPA-101 in 32 healthy volunteers aged 19 to 46 years, with good results. Polyreactive mAbs targeting more conserved LPS core epitopes and other virulence-associated antigens have been tested to improve immune response against P. aeruginosa [8, 96, 97]. So far, no passive immunotherapy has been successful enough in clinical trials to warrant licensure.

6. CONCLUSIONS

P. aeruginosa vaccine has been sought for 40 years; however it is still not available. The increased understanding of P. aeruginosa pathogenesis and its virulence factors supported the recognition of potential immunogens and passive immunotherapy that could be used for the development of an effective vaccine. These immunogens are situated in structural components such as lipopolysaccharides, pili, flagella, outer membrane proteins or are part of secreted products such as proteases, exotoxins and mucoid exopolysaccharides. There have been significant advances in later years; nonetheless there is clear need for additional basic research to further increase the understanding of those elements of immune response to P. aeruginosa that could potentially have a protective effect in patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

LIST OF ABBREVIATIONS AND ACRONYMS

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<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>Ad</td>
<td>Adenovirus</td>
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<tr>
<td>BAAF</td>
<td>B cell Activating Factor</td>
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<tr>
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<td>Cystic Fibrosis</td>
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<td>Confidence Interval</td>
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<td>FlaA</td>
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mAbs = Human Monoclonal Antibodies
MEP = Mucoid Exopolysaccharide
Opr = Outer Membrane Proteins
RBD = Receptor Binding Domain
RR = Relative Risk
T3SS = Type III Secretion System
TLR5 = Toll-Like Receptor 5

REFERENCES

**P. aeruginosa Vaccine**


