IMMUNOPROPHYLAXIS OF PSEUDOMONOSIS: ACHIEVEMENTS AND PERSPECTIVES

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According to numerous references, strains of pseudomonas, staphylococci, enterobacteria belong to the most significant conditionally pathogenic microorganisms (CPMs) - pathogens of purulent-inflammatory diseases.

The indicators of bacteriological confirmation from 14.0% to 47.0% ашк the *P. aeruginosa* etiological significance are ambiguous and fluctuate. For distant pseudomonads, high polyresistance (about 80.0%) is characteristic of 5 or more antibiotics. These data confirm the relevance of studies aimed at preventing morbidity and improving the methods for treating purulent-septic processes caused by *P. aeruginosa*.

On the one hand, the most rational direction of the fight against pseudomonases is the further improvement of antibiotic therapy, the use of highly virulent, adapted to regional, including nosocomial strains *P. aeruginosa*, specific bacteriophages, and on the other - the creation of immune preparations in order to provide specific immunity in risk groups (medical staff of hospitals, patients with appropriate diseases of the surgical, urological profile, with burn and gunshot wounds, etc.).

These can be anatoxins, lysates, polyserotypic corpuscular synaptic vaccines.

Despite the fact that purulent-septic and nosocomial infections caused by *P. aeruginosa* remain an important problem in clinical medicine and microbiology, Ukraine has a situation when there is no domestic release and the recommended use of such vaccine preparations.

The formation of a sustainable, long-term immunity, inclusion *P. aeruginosa* vaccination against calendar vaccinations has no reasonable. For the vaccination of a risk group, it is desirable to use a polyserotypic corpuscular pseudomonas vaccine.

The main requirements for immunological preparations that are useful for the prevention of *Pseudomonas aeruginosa* infection are their ability to rapidly stimulate nonspecific and specific immunity factors, ease of use (preferably an oral form administration), and the absence of toxic and sensitizing action.

These requirements most correspond to the form killed vaccine as a lysate. An important advantage of lysates is their relative cheapness, the ability to periodically change vaccine strains taking into account the biological properties of regional (including nosocomial) strains of *P. aeruginosa*, and to design complex antigenic preparations with other pathogens of purulent-inflammatory diseases (MRS strains of staphylococci, protea and E. coli etc.).

Widespread useing in clinical practice such lysate preparations as Imudon, IRS-19, Broncho-mohunal, Ribomunal, Broncho-Vaxom. These drugs include antigens from various microorganisms, which are most often the etiological factors of upper respiratory tract inflammatory diseases - *S. pneumoniae, S. pyogenes, Klebsiella pneumoniae, Haemophilus, Staphylococcus aureus, Streptococcus viridans, N. catarrhalis*.

A significant number of publications devoted to the analysis of the efficacy of such preparations demonstrates the prospects of this direction specific immunization capable of providing the induction of activation, proliferation and differentiation of specific T and B lymphocytes, immunoglobulin production (preferably Ig A, Ig and G) [1, 2 , 3].

Kaloshin A. with colleagues obtained recombinant anatoxic form of *P. aeruginosa* exotoxin for the further immunopreparations development for the prevention of *Pseudomonas aeruginosa* infection. Lipopolysaccharide of *Pseudomonas aeruginosa* is a highly immunogenic complex, in response to the administration of which agglutinating, opsonizing and precipitating antibodies are intensively produced [4,5].

High immunogenicity of endotoxin *P. aeruginosa* became a prerequisite for the preparation on its basis the specific drugs - both therapeutic and diagnostic. Over the past 40 years, there have been numerous attempts to obtain an effective *P. aeruginosa* vaccine, a number of monovalent and polyvalent corpuscular vaccines, ribosomal vaccines, chemical vaccines, which included an antigens mixture and some associated vaccines.

In the references there are reports of their high effectiveness and the inability to provide adequate immunity to heterologous strains and, often, the reactogenicity of such drugs.

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. The smooth or rough phenotypes are regulated by the presence or absence of outer O-polysaccharide chains [6]. 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 [7,8]. Another approach is to use only the nontoxic PS part of the LPS in vaccine preparations, called OPS-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

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preclinical studies [9,10]. 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. [11]. 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. 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 [12] 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 [13]. 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. Despite all these efforts, clinically relevant LPS or O-polysaccharidebased 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 multivalentconjugates.

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 [14]. 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 [15,16]. 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 [17]. 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-cellindependent to a T-cell-dependent antigen, and elicits a higher and boostable immune response in animals [18]. Alginate preparations differ principally in molecular size, the ratio of M:G residues from 10:1 to 6:4, and the level of Oacetylation 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. Campodonico et al. [19] 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.

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 [20,21]. It may bind to mucins, 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 [20]. 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 has to be bivalent and broadly protective. DNA vaccines encoding native Pseudomonas B-type (FliC) or A-type (FlaA) flagellin are strongly immunogenic and the resultant antibodies response interferes with the interaction of homologous flagellin with TLR5 [22]. This reduces the ability of the host to clear homologous, but not heterologous, flagellin-expressing P. aeruginosa. To circumvent this problem, Saha et al. [21] engineered a DNA vaccine encoding a mutant FliC 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 FliC- and FlaA-expressing P. aeruginosa. Flagella vaccine efficacy showed to be superior to that of the flagellin vaccine [21]. Besides, antibodies to flagellin monomers inhibited TLR5 activation and associated activation of innate immunity [23]. Doring et al. [24] 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 patients in the per-protocol group, who received all four vaccinations or placebo treatments, revealed 37 of 189 patients with infection episodes in the vaccine group compared with 59 of 192 patients with such episodes in the placebo group (P=0.02; relative risk risk [RR]: 0.66; 95% Confidence Interval [CI]: 0.46–0.93). P. aeruginosa strains, exhibiting flagella subtypes included in the vaccine, were significantly less frequently isolated from vaccinates than from placebo controls (P= 0.016, RR:

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0.319; 95% CI: 0.12–0.86). 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 [25,26,27]. 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 [18,29]. 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.

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[31]. 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 [32]. Audette et al. [33] and Kao et al. [34] 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 [35]. 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 [36]. Some studies in mice using purified pili protein or pilin peptides conjugated to carrier proteins has showed good results. Hertle et al. [37] 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. [38] 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 [36]. 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.

T3SS

Pseudomonas aeruginosa uses a Type III secretion system (T3SS) analogous to that of Yersinia 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 needlelike 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. 955 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” [39,40]; 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 [41]. Vaccine targeting PcrV showed protective response in mouse model, reduced lung inflammation and injury in a murine model and in a burn mouse model [42]. Interruption of the translocation of type III effectors by anti-PcrV was pointed as the possible mechanism for the protection [42,43,44]. In a recent study, a multivalent T3SSbased protein vaccine, including P. aeruginosa PcrV and needle tip proteins from other Gram-negative bacteria, proved to be immunogenic [45]. 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. 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

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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%)[46]. 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.

При создании генно-инженерных вакцин обязательным условием является наличие достоверной информации об иммуногенности определенных белков и нуклеотидных последовательностей, которые кодируются геном P. aeruginosa (6260000 нуклеотидных пар). Он полностью отсеквированный и есть в базе данных Gene Bank (Accession Number AE 004091). Поэтому сегодня зарубежными учеными проводится работа по созданию таких вакцин.

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 a important platform for anti-pseudomonas vaccines [47]. Adenovirus (Ad) vectors are attractive delivery vehicles [48] 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) [49]. 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 [49,50,51]. Adenoviral vaccine and DNA vaccines represent a safe, flexible, and widely characterized vaccine types [52]. However more P. aeruginosa clinical trials are needed since other adenoviral vaccine trials have been demonstrating safety and effectiveness [53,54].

Vaccine with extracellular proteases and exotoxin A

Despite the considerable interest to the extracellular products with proteolytic activity of *Pseudomonas aeruginosa*, the exact number of such products has not yet been established. By this time only two of them have been fully characterized: elastase and alkaline protease. Both enzymes have specificity. Causing a number of pathological changes in the body, extracellular proteases play a significant role in the pathogenesis of *P. aeruginosa* infection [55]. Although patients with *P. aeruginosa* infection showed an increase in antibody titres against protease and elastase, the role of these enzymes in the immune response has not been fully clarified [56].

Given important role of exotoxin A in the pathogenesis of Pseudomonas infection [57, 58], attempts have been made to obtain the nontoxic forms of this component, with the goal of creating on its basis an immune preparation for the prevention and treatment of Pseudomonas infection. Several methods of detoxification of exotoxin A have been developed [59, 60]. The data obtained in the study of the protective activity of exotoxin A detoxified forms are contradictory.

It was shown that obtained for exotoxin A antitoxic antibodies in the experimental animals blood serum had a pronounced protective effect: the introduction of antitoxic serum to mice quite protected them from subsequent infection with a lethal dose of the toxigenic RA-103 strain of *P. aeruginosa* [61].

Cryz S. J. et al. (1981) and Pavlovskis, A. R., et al. (1981) also found that stable exotoxin A toxoids have poorly antigenic properties when used without an adjuvant.

From the presented above results, it follows that although the role of exotoxin A as one of the main pathogenetic factors of *P. aeruginosa* is recognized, the possibility of using it like anatoxin or serum preparations derived from it is not yet fully established.

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 [62]. Tanomand et al. [63] 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 [63]. None of these vaccines with exotoxins or enzyme components have yet been tested in humans.

Vaccines based on P. aeruginosa porins

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 surfaceexposed and antigenically preserved in wild-type strains [64, 65]. 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. 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

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elicited a long lasting systemic and lung mucosal antibody response, with higher levels of systemic IgG and mucosal IgA [66]. 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 [67, 68]. Sorichter et al. [69] and Ding et al. [70] 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-, 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 cellpulsed, viral vectors or heterologously expressed in bacterial vectors with good results in preventing infections [71,72,73,74,75]. Although Opr may be one of the most promising antigens, controlled clinical trials are needed to evaluate the real protection against P. aeruginosa.

At the beginning of the research protective ability of the *P. aeruginosa* pore-forming protein (protein F), the following was established: anti-pseudomonas vaccines based on LPS are reactogenic and allow to obtain a protective effect only for the strain of the same serotype from which LPS was isolated; in patients with pseudomonasic lung fibrosis, antibodies to the F protein predominate; protein F is the main protein of the outer membrane, it is conserved and antigenically linked to all serotypes of *P. aeruginosa*. Based on these data, N. Gilleland et al. [76] conducted a series of experiments to study the protective effect of protein F.

The advantages of protein F as a "candidate" in vaccines for the prevention of chronic pseudomonasic pulmonary infection N. Gilleland and co-authors are justified by the fact that as a result of the transition of the rough form *P. aeruginosa* to smooth form during colonization of lung tissue, the causative agent loses O-antigen, stimulates specific immunity, produce alginate (the main component of mucoid secretion). At the same time, antibodies to the protein F (OprF) have a bactericidal effect not only on the rough form of the pathogen, but also after its transition into the smooth one. Another "candidate" for use as an antigen in the vaccine-alginate-did not have a sufficient protective effect in similar experiments [77,78].

The receptor of this F protein is the pyoverdin receptor, which is called ferrisiderophore (Fsv). Its mutations have been well studied for the assertion that the structure is conservative and promising for vaccine development [80].

Thus, it can be concluded that the metabolic exogenous products of *P. aeruginosa* listed above should be considered as promising components of new vaccine preparations intended for the prevention of *P. aeruginosa* infection [81,82,83].

In the Mechnikov Institute of Microbiology and Immunology in previous years, the immunogenic properties of *P. aeruginosa* various strains were studied. Seized in the 80s and a deposited strain of *P. aeruginosa* 66-16, which produces a much higher concentration of exotoxin A, was taken for experimental studies that were performed in 2011-2013. The accumulation dynamics of *P. aeruginosa* extracellular compound was studied depending on various nutrient media [84].

The known methods of isolation *P. aeruginosa* extracellular substances provide obtaining only heterogeneous substance by composition and biological properties. *P. aeruginosa* produces at least two extracellular antigens: O-antigen and protein nature antigen [85]. Extracellular proteinous antigen, that is common for different O-serotyming strains *P. aeruginosa* [86], was obtained using chromatographic methods.

The most active and immunogenic substances, namely the exogenous toxin in soluble form and its high molecular weight conjugates with the cell wall, are also derived from *P. aeruginosa* strain 66-16. The conditions for the isolation of proteinous components from the *P. aeruginosa* cell's wall were determined, which allow preserving their antigenic properties [87,88,89].

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 [90,91]. 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 [92]. In a Phase I clinical trial, the authors examined the safety and immunogenicity of an oral, whole-cell vaccine administered to a healthy population [93]. Thirty subjects received an oral dose of Pseudostat® in two timed, measured doses with serological follow-up to 56 days postvaccination. 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 wholecell 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 [94]. An attenuated aroA mutant of S. enterica serovar Typhimurium (strain SL3261) was used to express OprF/I 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 [95]. A live attenuated P. aeruginosa vaccine was safe, highly immunogenic and capable, when nasally administrated in a serogroup-specific manner, of

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protecting immunized mice against lethal pneumonia [96]. It can also protect immunized mice against corneal infections, even that caused by different serogroups, and probably could be used to prepare therapy reagents [97]. 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 [98]. Development of different methods to attenuate virulence while maintaining immunogenicity will help support such vaccines for clinical trials.

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 [99]. 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 [100]. 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 Oantigens have demonstrated protection against infection in animal models [101]. In a Phase I trial [102] there was described the generation and preclinical characterization of a fully human IgM/ 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. [102] 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 [103,104]. So far, no passive immunotherapy has been successful enough in clinical trials to warrant licensure.

Table 1. Summary and main characteristics of Pseudomonas aeruginosa vaccines.

Antigenic fragment		Advantages	Limitations	Phase	References
Vaccine Type	Vaccine target	Long-lasting protective systemic or localized antibodies.	Need of including additional flagellar types. Variants without flagella	III	Campodonico et al. [30], Doring et al. [24], Honko et al. [29], Rosok et al. [22]
	Flagella	Clinically effective and immunogenic (high level of opsonic antibodies)	Serological heterogeneity, LPSassociated toxicity, cost and complexity of development of lipid free multivalentconjugates	III	Kashef et al. [11], Lang et al. [13], Zuercher et al. [12]
	LPS	Safe and well tolerated	Only for Cystic Fibrosis.	I	Campodonico et al. [19], Theilacker et al. [18]
	Opr	High immunogenicity	Variable efficacies to reduce bacterial adherence mainly because of the difficulty of generating specific RBD antibodies	Pre clinical	Audette et al. [33], Horzempa et al. [38], Kao et al. [34, 36]
	Pilin	Safety	Eliciting long term immunity	I	Cripps et al. [90, 93]
Killed		Safety	Eliciting long term immunity	I	Cripps et al. [90, 93]
Live attenuated		Highly immunogenic	Residual virulence	Pre clinical	Priebe et al. [96], Zaidi et al. [97]

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Vector based		Safe, flexible, effective and widely characterized vaccine	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	Pre clinical	Lanzi et al. [50], Krause et al. [51], Sharma et al. [49], Worgall et al. [73]

LPS= Lipopolysaccharide; MEP= Mucoid Exopolysaccharide; Opr - Outer Membrane Proteins.

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 lipolysaccharides, 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. Recently, the antigenic drift of the *P. aeruginosa* actual strains has changed significantly, and no one has studied such changes in Ukraine, despite the high mortality from pseudomonas in the intensive care unit. The development of a multi-strain vaccine based on the actual strains of *P. aeruginosa*, in our opinion, can significantly reduce the formation of nosocomial circulation of multidrug-resistant strains, and contribute to reducing morbidity and mortality rates from nosocomial pseudomonosis.

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IMMUNOPROPHYLAXIS OF PSEUDOMONOSIS: ACHIEVEMENTS AND PERSPECTIVES

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The review article presents a retrospective analysis of attempts to develop effective vaccines for pseudomonas prophylaxis. *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 lipolysaccharides, 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*. Recently, the antigenic drift of the *P. aeruginosa* actual strains has changed significantly, and no one has studied such changes in Ukraine, despite the high mortality from pseudomonas in the intensive care unit. The development of a multi-strain vaccine based on the actual strains of *P. aeruginosa*, in our opinion, can significantly reduce the formation of nosocomial circulation of multidrug-resistant strains, and contribute to reducing morbidity and mortality rates from nosocomial pseudomonosis.

Keywords: pseudomonosis, vaccines, development, protective properties

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