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Evaluation of the New Outer Membrane Protein A Epitope-based Vaccines for Mice Model of *Acinetobacter baumannii* Associated Pneumonia and Sepsis Infection

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ABSTRACT

Nosocomial infections caused by *Acinetobacter baumannii (A. baumannii)* are considered as a global serious problem in hospitalized patients because of emerging antibiotic resistance. Immunotherapy approaches are promising to prevent such infections. In our previous study, five antigenic epitopes of outer membrane protein A (OmpA), as the most dangerous virulence molecule in *A. baumanii*, were predicted in silico. In this study, the investigators evaluated some immunological aspects of the peptides.

Five peptides were separately injected into C5BL/6 mice; then the cytokine production (interleukin-4 and interferon-gamma) of splenocytes and opsonophagocytic activity of immunized serum were assessed. To identify the protective function of the peptides, animal models of sepsis and pneumonia infections were actively and passively immunized with selected peptides and pooled sera of immunized mice, respectively. Then, their survival rates were compared with the non-infected controls.

Based on the results, activated spleen cells in $P1_{27}$ peptide-immunized mice exhibited an increased level of IFN-γ compared with the other experimental groups, but not about the IL-4 concentration. The results of the opsonophagocytic assay revealed an appropriate killing the activity of produced antibodies against *A. baumannii* in a dose-dependent manner. Further, the survival rates of the mice under passive immunization with the immunized sera or active immunization with P127 peptide were significantly more than those in the control group. Moreover, the survival rate of the P127 peptide immunized group was considerably higher than that of the other peptide-immunized group.

In conclusion, findings indicated that peptides derived from OmpA can be used as a

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promising tool for designing the epitope-based vaccines against infections caused by *A. baumannii*.

Keywords: *Acinetobacter baumannii*; Outer membrane proteins; Peptides; Pneumonia; Sepsis; Vaccines

INTRODUCTION

Nosocomial infections consist of acquired infections that occur in hospitals or health care services more than 48 h post-admission or 30 days after discharge.[1] Numerous microorganism are involved in the development of nosocomial infections. Among these, *Escherichia coli*, *Staphylococcus aureus*, and *Acinetobacter baumannii (A. baumannii)* are the most common bacteria isolated from nosocomial infections.[2] The last one is a gram-negative opportunistic pathogen and one of the *Moraxellaceae* family members that can be isolated from a wide range of sources including some foods, hospital supplies, and devices.[3] In recent years, some researchers have reported an increase in the incidence of nosocomial infections caused by *Asintobacter* strains[4] with the superiority of *A. baumannii* (80%). In addition, this strain is one of the most common causes of death in the intensive care unit (ICU) of hospitals and one of the most prevalent ventilator-associated pneumonia (VAP)-causing pathogens.[5] Respiratory system is the favorite region for this pathogen to be colonized due to transient pharyngeal colonization in healthy individuals and high levels of colonization in patients with tracheostomy. Furthermore, the presence of severe sepsis or septic shock is observed in this bacterial infection.[6]

In immune responses against *A. baumanni*, the innate immune cells especially alveolar macrophages and neutrophils are considered as the first line of defense in respiratory infections and play a critical role in the patient's fate.[7]

Several reports have shown that T helper 17 (Th17) cells and interleukin (IL)-17 mediated immunity play a critical role in defense against *A. baumannii* infections.[8]

Recent studies on efficient immunotherapies are considered as promising approaches against *A. baumannii* infection due to its virulence factors, pathogenicity, and antibiotic resistance features. One of the most important pathogenic virulence factors of *A. baumannii* is outer membrane protein A (OmPA) with a molecular weight of 38 kilodaltons (KD). Some studies have indicated that this molecule can be also secreted as extracellular vesicles and has a vital role in multidrug-resistant (MDR) infections.[9,10] Further, OmPA can induce apoptosis in the host cells and make them resistant to the performance of the complement system by forming a serum-resistant shape.[11] Furthermore, it increases the potential capacity of the host epithelial cells to simultaneously stimulate the innate and adaptive immune responses.[12]

According to the importance of humoral immune responses against *A. baumannii*, designing an efficient vaccine to control the nosocomial infections caused by this microorganism is a big challenge.[13] The selection of appropriate antigenic peptides in terms of antigenicity and folding, is one of the critical design strategies for peptide-based vaccines.[14]

In a previous study, we designed a few OmpA derived peptide-based vaccines based on *A. baumannii* isolated from antibiotic-resistant) hospitalized patients.[15]

In addition, the strain harbored *blaOXA-51-like*, *blaOXA-23-like*, and *blaOXA-24-like* genes. The nucleotide sequence data reported in this study have been submitted to the GenBank sequence database and assigned under the accession number: KY052003.1 for the *OmpA* gene.[15] In this study, we performed a pre-clinical study on our predicted epitopes derived from OmpA for evaluating their potential roles in immune protection and survival rate in pneumonia and sepsis models in C57Bl/6 mice. Together, the findings of this study suggest that the designed peptide vaccines may have clinical application for protecting against nosocomial infections caused by *A. baumannii*, although more experimental studies are required.

MATERIALS AND METHODS

All experimental procedures in the current study have been approved by the Ethics Research Committee of Shahid Beheshti University of Medical Sciences with the ethical code of 8223.

Prediction and In Silico Evaluation of the Peptides

All procedures of peptide prediction and epitope selection have been described in our previous study.[15] Briefly, the complete *OmpA* gene was amplified by PCR method on the *A. baumannii* isolated from samples of hospitalized pneumonia patients. Then, the amplified sequence was aligned with the multiple sequence alignment tool FlorenceCorpet to be compared with other *A. baumannii* OmpA sequences. Later, the Garnier-Osguthrope-Robson server was used to recognize the secondary structures of OmpA. The OmpA structure then was modeled using I-TASSER (Iterative Threading Assembly Refinement). The potent antigenic epitopes of OmpA were identified using LBtope, ABCpred, SWMTrip, BcePred, BepiPred, BCPred, and CBTope tools. The ProtParam tool was applied to predict various physio-chemical features of the selected peptides. Finally, five potent peptides were synthesized by the 1ST BASE Company [Order ID: 35747/ Commodity number: 3822.00.90 (www.base.asia.com)]. OmpA-derived epitopes predicted by various online servers are listed in Table 1.

Table 1. Characteristics of previously developed OmpA-derived peptides injected into the 3-week old C57BL/6 mice

Peptide vaccine	Length sequence injected (AA)	Position number of amino acid (AA)In OMPA	Code in experience
P1	27	24-50	$P1_{27}$
P2	18	122-139	$P2_{18}$
P3	16	236-245	$P3_{16}$
P4	16	317-332	$P4_{16}$
P5	26	197-222	P_{26}

* OMPA: Outer membrane protein A

The immunogenicity of OmpA derived peptides from *A. baumannii* was examined on their spatial structures using the PEP-FOLD server. The binding capacity of the peptides to human Immunoglobulin G (IgG) antibodies was investigated using the HPEPDOCK server. For each peptide, 10 different models of the best way of binding to the antibodies were predicted.

Animals

Female 3-week old C57BL/6 mice immediately after weaning (n=6, for each group) were purchased from the animal facility of Iran Pasteur institute animal resource center and were kept in the SPF room of the immunology department. In addition, female 8-10 week old C57BL/6 mice were purchased for nosocomial models (n=5, for each group).

Experimental and Control Groups

In this study, the female 3-week old C57BL/6 mice were divided into 8 experimental for active immunization. We had 2 main groups: Control as without injecting peptide group (n=6), and as shown in Table 2, five experimental groups for injecting the peptides (n=6, for each group).

For passive immunization, two nosocomial models of sepsis and pneumonia were induced in 8-10 week old C57BL/6 mice (n=5, for each group).

Preparation of the Specific Pathogen-free (SPF) Room

Before vaccination, an isolated microorganism-free room was prepared to prevent any infections from interfering with the test results. Therefore, the room was disinfected with paraformaldehyde, and an air conditioner with 5 individual filters (cold catalyst filter, active carbon filter, anti-bacterial filter, high-efficiency particulate absorbing (HEPA) filter, and UV light) was used to provide a germ-free airflow in the SPF room. Then, 3 weeks old C57B/6 mice (60 female mice) were transported to the SPF room using transport cages, kept in the autoclaved cages, and fed under sterile conditions.

Vaccination

The immunization protocol of the mice has been previously described.[15] In brief, the mice were evaluated in terms of SPF condition and absence of bacterial colonization on the body surface of the mice. To do so, skin and saliva samples of the mice were

Table 2. Categorizing the 3-week old C57BL/6 mice into the control and experimental groups. Different OmpA-derived peptide-based vaccines were injected into the corresponding experimental groups

Control Injection	Experimental Injection Vaccines
C1 - No Injection	$P1_{27}$ + Adjuvant
	$P2_{18}$+ Adjuvant
	$P3_{16}$ + Adjuvant
	$P4_{16}$ + Adjuvant
	$P5_{25}$ + Adjuvant

cultured in MacConkey and blood agar and tested for bacterial growth at 24 and 48 h post-sampling. The mice that were negative for bacterial infection were selected to be immunized. Based on the peptide dosing from our previous study,[15] 50 µg of each peptide was dissolved in PBS and emulsified in aluminum hydroxide gel adjuvant (InvivoGen, USA) in a ratio of 1:1. Then, 100 µL of the prepared solution was subcutaneously injected into the back of the neck of each mouse at days 0, 14, and 28 (the latter two doses as boosters). After two weeks from the last injection, the sera were collected by cardiac puncture blood samples from the mice after deep anesthesia. Then, the mice were sacrificed and their spleens were removed for further evaluation.

Evaluation of IL-4 and IFN-γ

Immediately after removing the spleens, they were transferred into a Dulbecco's Modified Eagle medium (DMEM) and cut into smaller parts. Then, spleen cells were isolated using a cell strainer, washed with DMEM, and incubated with RBC lysis buffer (Sigma, German) for 2 min at room temperature followed by two times washing with DMEM. To activate the spleen cells with the designed peptides, 1×10^6/mL of the spleen cells were placed into a 24-well culture plate, treated with 2 µg of each peptide, and incubated at 37℃ and 5% CO2 in a humidified incubator for 24 and 48 h. Finally, the concentrations of IL-4 and INF-γ in the supernatants were measured at three time-points (days 0, 24, and 48h after treating by peptides) s using ELISA kits (Invitrogen, USA).

Opsonophagocytic Assay

To investigate the efficiency of produced antibodies against the designed peptides,[16] peritoneal macrophages were isolated from 7 weeks old C57BL/6. Briefly, mice were sacrificed by cervical dislocation, abdomen skin was cut to expose the abdominal muscles, and the peritoneal cavity was lavaged (4-5 times) with a 5 mL cold medium (DMEM, Zigma). After washing, the harvested cells were suspended in a complete medium (DMED+10% FBS) and cultured in a T-25 tissue culture flask. Following incubation, non-adherent cells were removed and the attached cells were detached using a scraper, then the cell count and viability were determined. Thereafter, the cells were cultured in a complete medium and activated with 50ng/mL of Phorbol 12- Myristate 13-Acetate (PMA). In the next step, the sera of immunized mice with the highest titers of antibodies (injected with$P1_{27}$, $P2_{18}$, or $P3_{16}$ peptides) and a control serum were diluted as 1:10, 1:100, and 1:1000 and then incubated at 56°C for 30 min to inactivate the complement proteins. For the opsonophagocytic assay, 2×10^5 macrophages and 7×10^4 *A. baumannii* were placed into each well of a 24-well culture plate and then, incubated with the diluted sera. Following 1h incubation on a shaker incubator (37°C, 80 RPM), the bacterial growth in the test and control wells was evaluated by using the colony count method.[16,17]

***A. baumannii* Sepsis Model**

To induce a sepsis model, 500 µL of 2×10^7 (CFU) of *A. baumannii* isolated from hospital admitted patients as shown in our previous study,[15] was administered intraperitoneally into the 8-10 weeks old C57BL/6 mice. After 24 h the sepsis induction in mice was confirmed by showing the signs of anorexia, abdominal breathing, increased body temperature, and lack of response to environmental stimuli. Moreover, histopathological evaluations and bacterial culture were carried out on liver and blood samples of infected mice compared to the PBS-injected control mice.[17]

A. baumannii Pneumonia Mice Model

The pneumonia mice model was induced as follows:

The mice (8-10 weeks old) were anesthetized with isoflurane (TerellTM, NDC 66794-011-10 USA) inhalation followed by intranasal administration of 50 µL PBS containing live bacteria (10^8 CFU/mL of *A. baumannii*). 12-18 h after bacteria inoculation, the infected mice displayed the signs of pneumonia including wet breathing sound, fever, reduces the desire to eat, and no response to environmental movements. Moreover, lung histopathology and mucus culture were carried out to confirm the pneumonia model.[17]

Histopathological Analysis

To evaluate the protective effect of the designed vaccines, the mice were initially inoculated intraperitoneally or intranasally with *A. baumannii* to induce sepsis and pneumonia, respectively. Later, histological evaluations were carried out to confirm the infection models. For preparing the histological sections, the mice were anesthetized with the overdose of pentobarbital sodium (Sigma Aldrich, Louis, USA) and after losing the footpad-pinch response, the lungs and livers were removed and fixed in paraformaldehyde (10%) and formalin (10%). A 4 µm-paraffin section of organs were stained with hematoxylin and eosin (H & E). By using standard pathology scales of severity, the main criteria for this assessment were: Acute inflammation., the presence of bacteria in the tissue. Infiltration of neutrophils, hyperemia (Excess of blood in the vessels supplying tissue), hemorrhages, and Edematous.

Passive Immunization

100 µL of pooled sera containing the high titers of antibody collected from previously immunized mice,[15] were injected intraperitoneally into the infected mice, twice with an interval of 24 h).

Active Immunization

We allocated four experimental groups containing five 8-week old C57BL/6 mice in each to evaluate active immunization and protection against *A. baumannii*. All mice were assessed to be SPF (4 days before the immunization) and received peptides 1 (P_{27}), 2 (P_{18}), and 5 (P_{26}) were injected into the 8-week old mice. These three peptides were selected based on their higher immunogenicity found in our previous study.[15] The booster doses were also injected subcutaneously into the back of the neck on days 14 and 28 after the first injection. Five weeks after the last injection, the mice were treated *A. baumannii* in the volume of 100µl in PBS, intranasal and intraperitoneally as described, to investigate the protective capacity of peptide-based vaccines to prevent pneumonia and sepsis in vaccinated mice, respectively. Finally, the survival rate in the immunized mice was compared with the control group.

Statistical Analysis

In this study, all statistical analysis was performed using SPSS version 25.0 (Inc Chicago.il, USA). Continuous variables were checked for the normality based on the one-sample Kolmogorov–Smirnov test and were presented as median with inter-quartile range (IQ25-75) for variables with skewed distribution. The Kruskal-Wallis test was applied to compare variables with skewed distribution. Kaplan-Meier analysis and log-rank test were used to determine the survival rate. The p value<0.05 was considered to be statistically significant

RESULTS

In silico Evaluation of the Peptides

Early assessments on bioinformatics and the spatial structure of each peptide were performed using PEP-FOLD software (Figure1).

For each peptide, the best way to bind to the Fab portion of human IgG antibody in 10 different models was determined by the HPEPDOCK server. A negative docking number for each peptide (the same range from -190 to -230) indicates a lower energy level and greater stability for binding to the Fab antibody (Figure 2). AllerTOP server did not identify the peptides as allergens.

Immunization with P27 and P18 Ameliorates the IFN- γ Production in Spleen Cells

To ensure the immunogenic function of peptides after vaccination, we tested splenic lymphocytes to measure cytokines.

Mice were vaccinated to evaluate the effect of active immunization by peptides showed results. The levels of IL-4 and IFN-γ in the activated spleen cells were measured; using an ELISA kit. We found that those cells activated by peptides no. 1 and 2 showed a

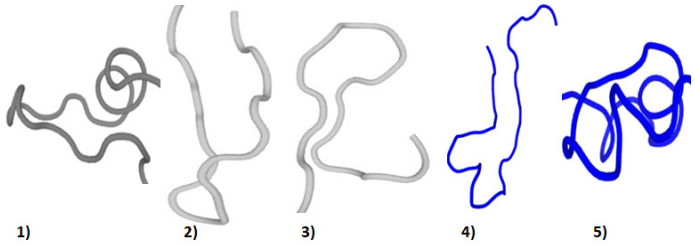

Figure 1. Three-dimensional (3D) structure of peptides. Peptide 1 with 27 amino acid (aa), peptide 2 with 18aa, peptide 3 with 16 aa, peptide 4 with 16 aa and peptide 5 with 26 aa.

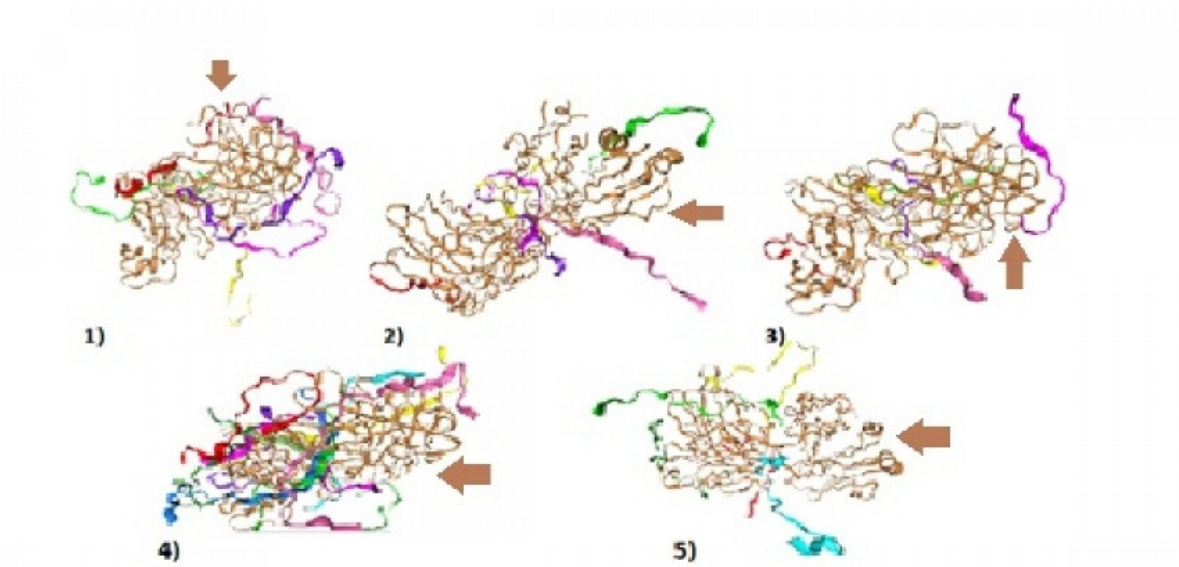

Figure 2. Interaction between antigen-binding fragment (Fab) of human immunoglobulin G (IgG) and predicted peptides. Brown arrow keys show the Fab fragment and peptides were shown in different colors (red, blue, yellow, purple, and green).

higher amount of IFN-γ compared with the control group ($p \leq 0.01$). This peptide showed a significant difference in cytokine stimulation compared to other peptides ($p \leq 0.05$). However, no significant differences were observed in the IL-4 levels between control and all experimental groups (Figure 3).

Remarkable Opsonophagocytic Killing Activity of Antisera from P27-immunized Mice

The results obtained from the opsonophagocytic assay confirmed the killing activity of produced antibodies against *A. baumannii* in in a dose-dependent manner. Accordingly, the sera collected from the mice immunized with peptide 1(P_{27}) exhibited the killing activities of 82%, 62%, and 11% at the concentrations of 1:10, 1:100, and 1:1000, respectively. Serums with 1:10 dilution showed the highest opsonophagocytic killing rate compared to controls ($p \leq 0.01$). Also in 1:100 dilution with significant difference ($p \leq 0,05$). However, no significant differences were found in the killing activities of antibodies against peptides 2 ($P2_{18}$) and 3 ($P3_{16}$) with the control group in all concentrations. (Figure 4).

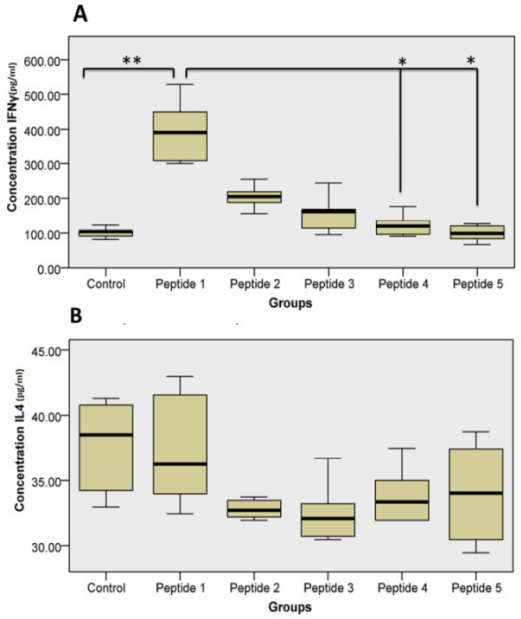

Figure 3. Cytokine measurement from activated splenocytes immunized mice with selected peptides A) significantly higher levels of IFN-γ were observed in splenocytes of immunized mice with peptide No.1 compared with other peptides and control group. B) No significant differences were observed in IL-4 concentration between the experimental and control groups. (*p<0.05, **p<0.01)

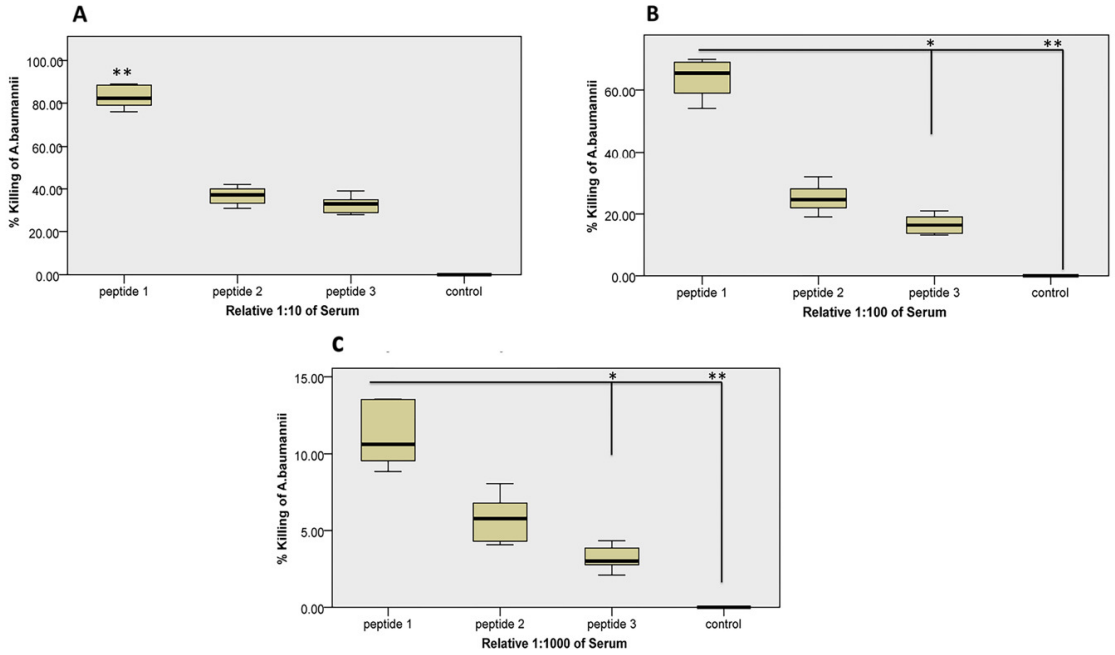

Figure 4. Results of the opsonophagocytic assay at different concentrations. A) At 1:10 concentration, the killing activity of antibodies against peptides 1 (P_{27}) was significantly higher than those against peptides 2 (P_{18}) and 3 (P_{16}) as well as the control group. B) The killing activity of antibodies against peptide 1 (P_{27}) at a concentration of 1:100 showed a higher amount than peptide 3 (P_{16}) and the control groups. C) Peptide 1 (P_{27}) group also showed the higher killing activity compared with Peptide 2 (P_{18}) and the control group at 1:1000 concentration. (*p<0.05, **p<0.01).

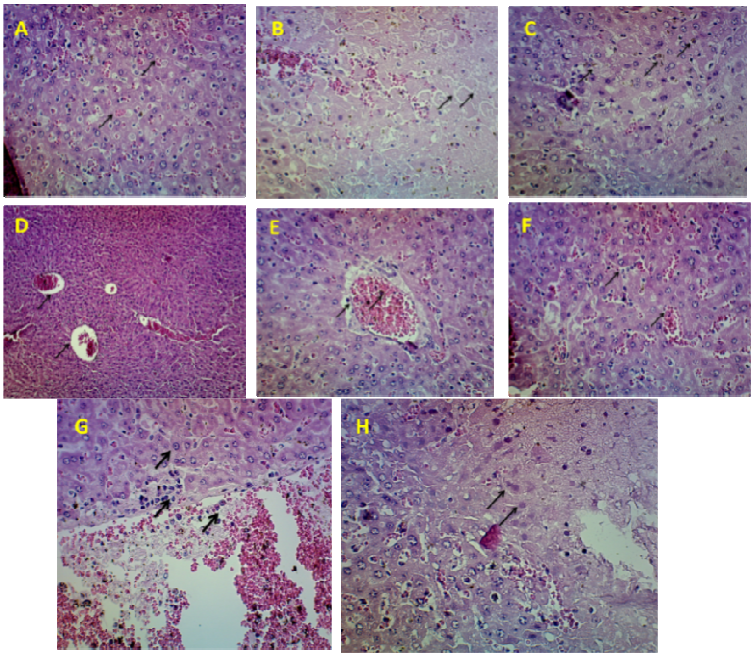

Figure 5. Photomicrographs of liver from sepsis model of *A. baumannii* infection (H & E staining). A: Kupfer cells. B: Hepatocyte necrosis. C: The presence of bacteria in the tissue. D: Hemorrhages. E: Portal vein inflammation. F: Inflammation of sinusoids. G: Infiltration of neutrophils. H: Kariolysis (Magnification100x)

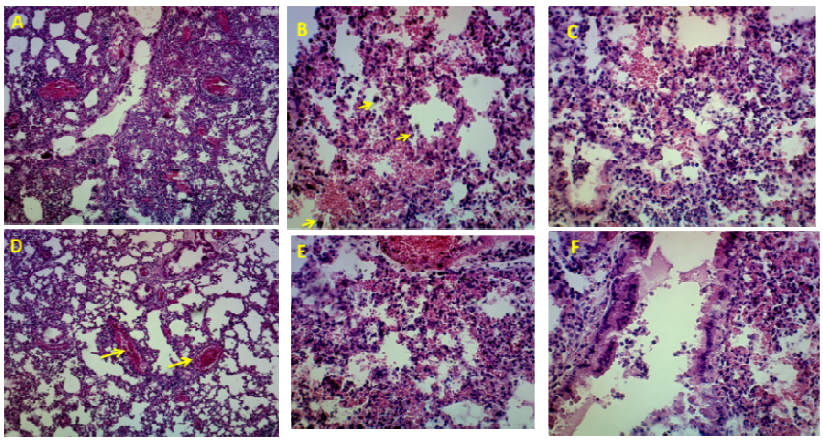

Figure 6. Histological evaluations of lung tissue samples following induction of *A. baumannii* pneumonia model (H & E staining). A: Acute lung inflammation. B: The presence of bacteria in the lung tissue. C: Infiltration of neutrophils. D: Hyperemia (Excess of blood in the vessels supplying tissue). E: Hemorrhages. F: Lung edematous. (Magnification 100x)

Histopathological Assessments of Pneumonia and Sepsis Mice Models

As shown in Figure 5, the stained liver sections showed severe hyperemia and edema of interstitial tissues, degeneration of granular tissue, and penetration and marginalization of neutrophils. Moreover, focal lytic necrosis, scattered foci of single-nucleus cells, and necrosis were observed in the liver of the infected mice. In addition, pneumonia was confirmed by observing the following items in H & E staining of the formalin-fixed sections of the lung: incidence of congestion, hemorrhage, the severity of neutrophil recruitment, and proportion of airspace area (Figure 6).

Evaluation of Survival Rate Following Passive Immunization

Passive immunization with sera obtained from the mice immunized with peptide 1 (P_{27}) showed considerable protection against *A. baumannii* in both pneumonia and sepsis models (p<0.01). Our findings showed that the mice under passive immunization exhibited a survival rate of 60% in both sepsis and pneumonia models, while 100% of the mice in the control group died from pneumonia and sepsis infections (Figure 7A and B).

Evaluation of Survival Rate Following Active Immunization

The survival rates of the experimental groups after active immunization in pneumonia model were as following: peptide 1 (P_{27})=81.7 (stable disease or SD=28), peptide 2 (P_{18})=41.5 (SD=23.9), peptide 5 ($P5_{26}$)=48.2 (SD=29.3), and control=1.7 (SD=0.3). All mice in the sepsis model and control group died from an infection in less than three days (Figure 8).

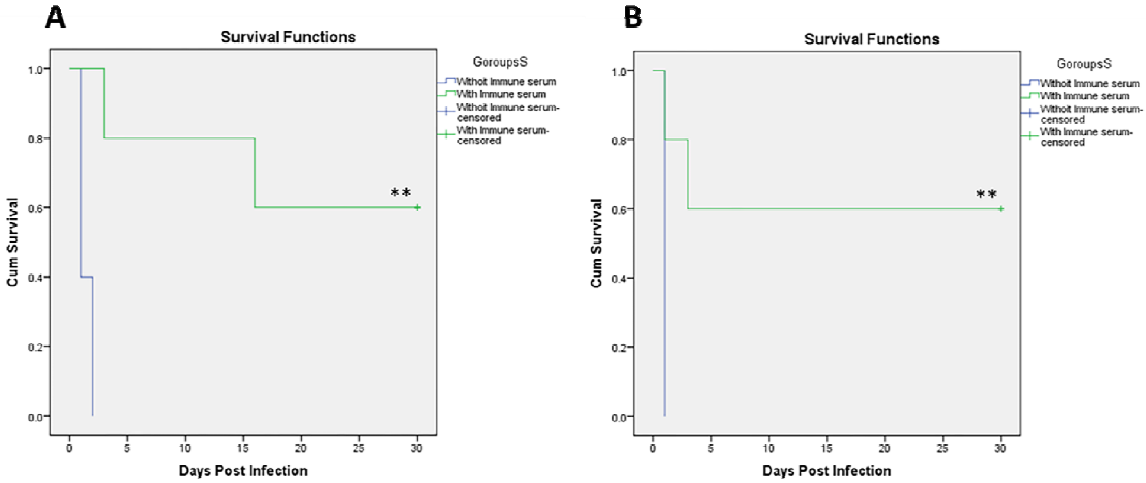

Figure 7. Evaluation of survival rate after passive immunization in A) sepsis model and B) pneumonia model (**p<0.01)

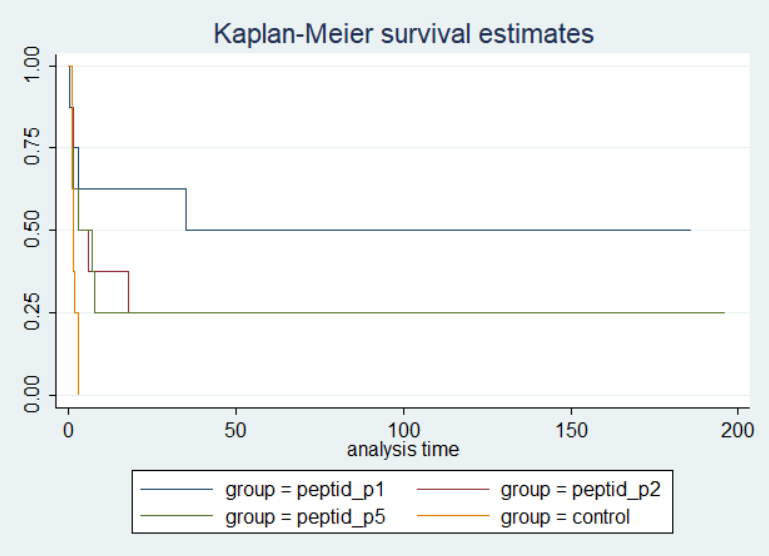

Figure 8. As shown in the chart, the survival rate of the pneumonia mice model immunized with peptide number 1 was significantly higher than that in peptide numbers 2 and 5 as well as the control group (p=0.04).

DISCUSSION

In this study, animal models of pneumonia and sepsis were induced to mimic human nosocomial infections. C57BL/6 mouse is a good model for inducing an inflammatory condition caused by an infection.[18] In both infected mouse models, the most important clinical signs of infection were observed (see materials and methods). According to ethical considerations, in the immunization group, we started serum therapy immediately after confirming the bacterial culture results. To prevent the vaccine-associated infection, the actively immunized mice did not receive any treatment. The duration of vaccine exposure was determined based on previous experience in the maximum antibody production time.[15] In the passive immunization group, the mice were inoculated by antisera collected from previously immunized mice. Then the therapeutic effectiveness of the vaccine was assessed based on the reduction of symptoms and signs as well as the survival rate compared to the control groups (without injecting the antisera or receiving the vaccine). We did our best to determine the survival time of the treated animals as much as possible.

Clinical findings and failure of treatment options for Gram-negative strains such as *A. baumannii* infections emphasize a need for designing new preventive and therapeutic approaches. Thus non-antibiotic-based protocols including antibody-based therapies and vaccine strategies are under development.[15] Considering the high mortality rate of nosocomial infections caused by *A. baumannii*, various vaccine platforms have been established ranging from whole-cell organisms to recombinant antigens belonging to the cell surface of the bacteria.[18] Ainsworth et al used a mutant strain of *A. baumannii* (ΔtrxA) to produce a live attenuated vaccine.[19] They reported that the mice immunized with the vaccine showed remarkable protection against sepsis. However, little immunoglobulin class switching was found due to exciting IgM as a predominant immunoglobulin in the immunized mice. Recently, the ghost of *A. baumannii* named Ali190 was developed by extracting the DNA and proteins of this strain, making it an appropriate vaccine candidate against *A. baumannii* infections.[20] Despite favorable results, there is a global concern regarding live attenuated vaccines because of their potential to revert to the wild type in the vaccinated individuals. Further, live attenuated vaccines contain various types of proteins that are not only needless for the immunization process but may also induce allergic reactions.[21] Furthermore, polysaccharide-based vaccines using conserved exopolysaccharide parts of *A. baumannii* have been successfully examined for favorable protection against this pathogen. Choi et al developed a vaccine based on poly-β-1-6-N-acetylglucosamine (PNAG) as a pivotal polysaccharide structure for maintaining the integrity of *A. baumannii*-formed biofilms. They reported that passive immunization using anti-PNAG sera enhanced the opsonization capacity against some MDR strains of *A. baumannii*. However, active immunization with this antigen was not preferred because of inducing the T-cell independent immune response. Moreover, Polysaccharide-based vaccines in a pure and non-protein conjugated form failed to induce memory cells of the immune system.[22]

To overcome these problems, a new generation of vaccines has been developed. Lately, Lei et al[23] investigated a DNA vaccine encoding OmpA and some other well-known antigens of *A. baumannii* to promote effective protection against pneumonia in the mice model. According to their findings, the vaccine provided acceptable humoral and cellular responses in the immunized mice. Nevertheless, DNA vaccines have some limitations especially in the route of administration for a perfect delivery and targeted gene expression which needs to be addressed before translating into the clinic.

Regarding the recent advances in bioinformatics knowledge, epitope-based vaccines have become noticed for their ability to potentiate both T and B cell immune responses. As another advantage, the risk of concomitant diseases and off-target responses are considerably lower in such vaccines in contrast to the conventional vaccines.[24] In the present study, favorable epitopes of OmpA protein were selected and their ability to induce both T and B cell responses against *A. baumannii* was identified with the. As the main component of outer membrane proteins, OmpA has a great impact on the virulence of Gram-negative bacteria through its influence on biofilm formation, resistance to antibiotics, and host immunomodulation.[25] In addition, the structure of OmpA is highly conserved among the clinical strains of *A. baumannii* and it has little homology with the human proteins. Luo et al

vaccinated diabetic mice against *A. baumannii* infections using recombinant OmpA protein (rOmpA).[26] In that study, the authors declared that vaccination of diabetic mice with rOmpA and aluminum hydroxide adjuvant had a positive effect on improving the survival rate of animals and reducing bacterial load in their tissues. They also indicated that passive immunization (with antisera) could protect the infected mice model. In this study, similar results were found on improving the survival rates of the mice immunized with OmpA-derived peptides. In another study, the survival rate of actively immunized mice 28 days post-infection with *A. baumannii*, was 80%.[27] We monitored the mice for a longer period and reported that the mice vaccinated by peptide no.1 survived approximately 81.7 days post-infection, while all control mice died due to the infection 3 days after exposure.

In a recently published paper, a new design of protein-based vaccine using B cell epitope mapping and homology modeling was introduced which may be a promising approach in generating the recombinant OmpA-based vaccines.[25]

In the present study, activated spleen cells showed a higher concentration of IFN-γ produced by peptide 1 (P_{27})-treated cells compared with the other groups, while no positive difference in the IL-4 level between all experimental groups was observed. Accordingly, It implies the tendency of selected epitope-based vaccines to activate a type 1 immune response. However, the dose of vaccines seems to play an important role in inducing the type of immune response. Lin. et al. reported that the decreasing ratio of INF-γ/IL-4 is highly associated with the increasing dose of rOmpA vaccine which polarizes the immune system into the type 2.[25] In addition, we used a single dose of vaccine to evaluate humoral and cellular responses while performing future studies on higher and lower doses will help to elucidate the type of cellular responses as well as the titer and type of the produced antibodies. The majority of host resistance is the participation of opsonophagocytosis with an essential role for both protection against infection and memory responses. Despite the effective role of antibodies in the opsonophagocytic in vitro reaction, an inconsistency was observed in the condition of the stabilized model. Although the preventive model in mice receiving the vaccine followed by the induction of respiratory infection was successful, it was failed in sepsis-induced mice leading to death in three days post-infection. This suggests the importance of antibodies in respiratory infections. However, the role of humoral immunity in sepsis as a systemic form of infection and inflammation is questionable.

In many bacterial infections including gram-negative bacteria and especially in the VAP group, complex structures like OmpA present various virulence properties, such as cytotoxicity and epithelial damages. They can stimulate an innate immune defense and elicit the inflammation cascade at the deeper levels of the body surfaces. Therefore, to avoid unfavorable reactions, it is worthy to develop subunit vaccines especially epitope-based ones using bioinformatics knowledge.

In conclusion, the results of this study showed that the production of antibodies against more immunogenic determinants of *A. Baumannii* can immunologically protect the respiratory-infected mice and create an effective therapeutic feature in both models of infections. The results can be translated into human clinical trials for treating patients in similar conditions admitted in ICUs. Preventing approaches or even performing passive immunization for the individuals at high risk of inflammatory infections may also be useful to avoid them from getting worse. More studies are required to confirm the above-mentioned strategies.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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