Immunology Letters 212 (2019) 125–131

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Passive immunotherapy with specific IgG fraction against autolysin: Analogous protectivity in the MRSA infection with antibiotic therapy

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ARTICLE INFO

Keywords:
Methicillin-resistance *staphylococcus aureus*
Autolysin
Passive immunization

ABSTRACT

Staphylococcus aureus is a leading infectious cause of life-threatening diseases in human beings, with no effective vaccine available to date against this bacterium. Treatment of methicillin-resistant *S. aureus* (MRSA) infections has become increasingly difficult because of the emergence of multidrug-resistant isolates. Immunotherapy represents a potential approach to prevent *S. aureus*-related infections.

Autolysin is one of the virulence factors, which controls the growth, cell lysis, daughter-cell separation, and biofilm formation. Our study focused on passive immunization against MRSA infection. Herein, rabbit polyclonal IgG was produced following the preparation of r-autolysin. Specificity of IgG against r-autolysin was investigated by ELISA and western blotting assays. IgG fraction was prepared using sulfate ammonium precipitation, and the ability of antiserum to promote phagocytosis of bacteria was assessed by opsonophagocytosis assay. Then, passive immunization of mice was carried out with polyclonal IgG fraction and, mice were sacrificed three days after challenge and their kidneys, liver, and spleen were collected. Results exhibited that the passive immunization with rabbit polyclonal anti-IgG fraction tremendously improved survival rates of mice challenged by *S. aureus* as well as vancomycin treatment compared with the negative control groups. In addition, a remarkable decrease in bacterial numbers was observed in mice treated with rabbit polyclonal anti-IgG. Importantly, our findings demonstrated that passive immunotherapy and antibiotic therapy lead to decreased histopathological damage in mice infected by *S. aureus* as compared with control groups. Our results suggested that the passive immunization may result in the introduction of excellent strategies to control infections caused by MRSA, like antibiotic therapy.

1. Introduction

Staphylococcus aureus, the major bacterial pathogen in the hospital and the community, has become progressively resistant to multiple antibiotics. In fact, the wide use of antibiotics led to the generation of multiple antibiotic-resistant strains of Staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA). Thus, the problem of MRSA treatment is an important challenge to the current antimicrobial strategy. The generation of specific anti-serum and their clinical application were discovered and successfully used at the end of 19 century [1]. Resistance to vancomycin [2,3], linezolid [4], daptomycin [5], and mupirocin [6] has all been identified as clinical concerns [7]. This increasing saga of antimicrobial resistance in *S. aureus* and the slow production of well-functioning antimicrobials are reminiscent of similar clinical concerns with *Haemophilus influenzae type b*, which is resistance to ampicillin and chloramphenicol, and *Streptococcus pneumoniae*, which is resistance to penicillin. This revealed serious concerns about the treatment of infections caused by these principal pathogens; however, the incidence of MRSA is stable or decreasing in some parts of the world such as in many European countries [8,9].

Non-antimicrobial approaches to control *S. aureus* have recently attracted potential interests in immunotherapy [10]. Because a number of individuals who are susceptible to staphylococcal infections are not competent to mount an effective immune response, passive as well as active immunization strategies have been explored [10,11]. However, serum sickness may occur in the passive immunotherapy.

Surface proteins are considered to be crucial factors for *S. aureus* colonization and virulence [12,13]. Therefore, recombinant cell wall-anchored antigens have been recommended as potential *S. aureus* vaccine candidates.

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https://doi.org/10.1016/j.imlet.2018.11.010
Received 8 March 2018; Received in revised form 20 November 2018; Accepted 25 November 2018
Available online 26 November 2018

In the previous studies, we reported that the autolysin protein exhibits protective immunity against *S. aureus* infections [14,15]. Autolysin, as a surface-associated protein, has both enzymatic (amidase and glucosaminidase) and adhesive functions [16–18], involved in initial attachment of the cells to a polymer surface [17], Excretion of cytoplasmic Proteins [19], biofilm formation [20,21], and separation of daughter cells after cell division [16,22]. Autolysin also binds to vironectin (Vn), suggesting its role not only in colonizing polymer surfaces, but also in colonizing host factor-coated materials and host tissues [17,21]. Recent studies have well documented the importance of autolysin in *S. aureus* pathogenicity [17,23]. Active immunization with this protein could trigger specific antibodies and humoral immune responses, resulting in reduced bacterial loads and inflammation responses, as well as improved survival times and rates in mice [14,15].

However, *S. aureus* usually causes an acute infection with rapid progression, in which 60% of the patients with invasive infections die within 7 days, indicating that active immunization is not one of the potential approaches for the prevention of such acute infections [24]. Contrastingly, passive immunization is well-known to activate immediate and effective protection [24,25]. Thus, the aim of the present study was to evaluate the therapeutic effect of anti-r-autolysin IgG as an opsonin for bacterial phagocytosis, and the protective efficacy of passive immunization in infected mice. In addition, further investigations were conducted to explore the mechanisms that might contribute to reduce bacterial loads in the internal organs.

2. Materials and methods

2.1. Bacterial strains, plasmid and protein

The *S.aureus* strain COL was kindly gifted by Dr. Mohammad Emaneini (Tehran University of Medical Science, Tehran, Iran). The *E. coli* strain BL21 (DE3) (Novagen, Wisconsin, USA) was used for the expression of recombinant protein. Luria-Bertani (LB) broth or agar (Merck, Germany) was used for culture.

2.2. Recombinant autolysin preparation

Plasmids containing the sequence encoding autolysin protein (amino acids 72–424) were constructed and maintained in our laboratory. The recombinant protein was expressed in E. *coli* BL21 (DE3) under induction with 1 mM of IPTG and purified using nickel affinity chromatography in denature conditions. The expressed protein was characterized by SDS-PAGE and Western blot as described previously [14].

2.3. Production and characterization of polyclonal antibodies (PcAbs) specific to r-autolysin

Polyclonal antibodies (PcAbs) were generated in New Zealand white rabbits (Pasture Institute of Iran, Karaj, Iran) based on a previously-published method [26]. Briefly, female rabbits were immunized subcutaneously with 500 µg of purified r-autolysin emulsified in an equal volume of complete Freund's adjuvant (Sigma, USA) at 2-week intervals (in seven sites and 70 µg injection per site). Boosting was performed two times with 2-week intervals while the antigen was formulated in the incomplete Freund's adjuvant; subsequently, blood samples were collected prior to immunization and 2 weeks after each immunization (at days 0, 14, 28, and 42). Approximately 30 mL of blood was collected in each step and incubated at 37 °C for 2 h. Sera were collected from the retracted clot and clarified by centrifugation (6500 × g). An optimized ELISA was conducted on experimental sera and confirmed the high level of specific IgG antibodies.

2.4. IgG Fractionation, characterization, and reaction against r-autolysin

When sufficient r-autolysin antibodies were prepared, the IgG-rich fraction was pooled and precipitated with a saturated solution of ammonium sulphate to a final concentration of 50%. Sera obtained prior to immunization were precipitated in the same method to obtain the control non-immune IgG fraction. The samples were dialyzed against phosphate-buffered saline (PBS) buffer and protein concentration in IgG fractions was quantitatively measured using a Bradford protein assay kit (Bio-Rad, USA). Anti r-autolysin and non-immune IgGs were adjusted in sterile PBS to 1 mg/mL and finally stored at −20 °C until use. The potency of specific IgG antibodies to binding to r-autolysin was determined by the ELISA method.

2.5. Western blot analysis

The purified r-autolysin was electrophoresed in a 12% SDS–PAGE and then transferred into PVDF membrane (Hi-bond Amersham Biosciences, USA) using a Mini-PROTEIN tetra cell (Bio-Rad, USA) at 100 mA for 1 h. The membrane was then blocked for 60 min in 2% bovine serum albumin (BSA) in PBS. After blocking, the primary antibody, rabbit polyclonal anti r-autolysin IgG, diluted 1:1000 in blocking buffer was added and the plates were incubated for 3 h at room temperature (RT) with shaking. After washing three times with TBST (Tris-buffered saline with 0.05% Tween 20), the goat anti-rabbit IgG secondary antibodies, conjugated with HRP (Sigma, USA) at a 1:5000 dilution in blocking buffer, were added and allowed to incubate for 1 h at RT with shaking. The membrane was then washed three times for 5 min. Finally, the color development was carried out by the addition of 3, 3′-diaminobenzidine (DAB) solution (Sigma, USA), and the plates were incubated until bands were clearly visible.

2.6. Opsonophagocytic killing assay

The opsonophagocytic assay of IgG fractions was carried out according to previously-published methods [14,27]. Briefly, bacterial suspensions (*S.aureus* strain COL) were prepared at an approximate concentration (~10^8 CFU/mL) in 1% BSA. Mouse macrophages were used at a final concentration of 2×10^7 mL. A 3-week-old rabbit (Pasteur Institute of Iran, Karaj, Iran) was bled, and prepared sera were pooled and used as a complement source. Four different dilutions (1:2, 1:4, 1:8, 1:16 and 1:32) of anti r-autolysin IgG were used. Complement activity of antisera was eliminated by heating at 56 °C for 30 min. For the opsonophagocytic assay, the bacteria (2×10^9 cells per well) were first incubated with an equal volume of diluted and heat-inactivated (at 56 °C for 30 min) polyclonal IgG at 22 °C for 60 min and then washed twice with BSA (1% (w/v)) for elimination of unbound antibodies. After suspending with 200 µL of 1% BSA, 100 µL of mouse macrophages was mixed with 100 µL complement in sterile microtube and then incubated in a shaker at 37 °C for 90 min.

Shortly thereafter (time 0) and after 90 min, 25 µL of the mixture was removed, diluted in saline and finally plated for bacterial enumeration. Non-immune rabbit serum (NRS) was used as pre-immune serum (control IgG). The opsonic killing activity of immune sera was compared with the pre-immune sera. Omitted antibodies, complement, or macrophage substituting with 100 µL of BSA were components of the control tubes. This experiment was performed in triplicate for each quantity. The following formula was used for the calculation of the percentage of killed bacteria;

Percentage of killed bacteria = [1- (CFU of the immune sample/CFU of the Pre-immune sample)] × 100

2.7. Mice and passive immunotherapy

Six- to 8-week-old female BALB/c mice (20–22 g) were purchased from the Pasteur Institute of Iran (Karaj, Iran). The animals were

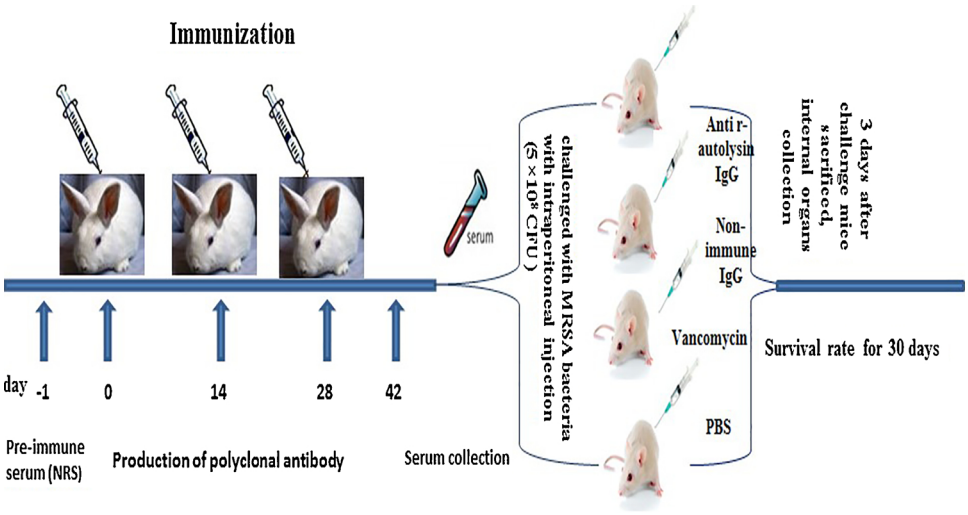

Fig. 1. Diagram showing the passive immunization schedule of the study. After preparation of polyclonal antibodies (PcAbs), Four groups, each consisting of 16 mice, were infected intraperitoneally (i.p) with 100 µL of a sub lethal dose of MRSA COL (5×10^8 CFU per mouse). Two hours before and 24 h after infection, the mice were passively immunized intraperitoneally with 500 µL of IgG fraction against r-autolysin (500 µg) (group 1) or the same volume of normal rabbit serum IgG fraction (NRS) (group 3), PBS (group 4) for control subjects and vancomycin (15 mg/kg) for the witness group (vancomycin treatment control group 2). The survival rates for all mice were monitored for 30 days after infection.

handled according to national animal care ethics. Four groups, each consisting of 16 mice (shown below), were infected intraperitoneally (i.p) with 100 µL of a sublethal dose of MRSA COL (5×10^8 CFU per mouse) [28,29]. Two hours before and 24 h after infection, each mouse was passively immunized intraperitoneally with 500 µL of IgG fraction (500 µg) against r-autolysin (group 1) or the same volume of normal rabbit serum IgG (500 µg) fraction (NRS) (group 3), PBS (group 4) for control subjects, and vancomycin (15 mg/kg) for the witness group (vancomycin treatment control group 2) (Fig. 1), as following. The survival rates for all mice were monitored for 30 days after infection.

Group 1: Anti r-autolysin IgG fraction;
Group 2: The witness group (Vancomycin treatment control group);
Group 3: Non-immune IgG fraction (control IgG); and
Group 4: PBS (control group)

2.8. Quantitative bacterial load in the internal organs

To evaluate bacterial loads in the internal organs, followed by passive immunization and experimental challenges, the mice (four in each group) were sacrificed 3 days after challenge. The liver, spleens, and kidneys from the animals were removed, weighed, and homogenized in 1 mL of PBS. Then, appropriate serial dilutions were placed on LB agar containing 50 µg/mL of oxacillin for 24 h at 37 °C. Colony-forming units were counted and the bacterial burden was calculated as CFU per organ. Histopathological analysis was conducted 3 days after infection. The organs were fixed with 10% phosphate-buffered formalin and embedded in paraffin. Four-micrometer-thick sections were prepared and stained with hematoxylin and eosin (H&E) for microscopic examination.

2.9. Statistical analysis

Data are presented as the means ± SD. GraphPad Prism v 6.01 Software was used for statistical analysis. The non-parametric log rank test was used to determine the differences in the survival rates. The Student's *t*-test was used to calculate the differences in antibody responses in the experimental groups. Otherwise, the non-parametric Mann–Whitney test was used for analysis the bacterial burdens. In all analysis, p-values less than 0.05 were considered as a significant difference.

3. Results

3.1. Total specific IgG ELISA assay

IgG-specific polyclonal antibodies produced in the immunized

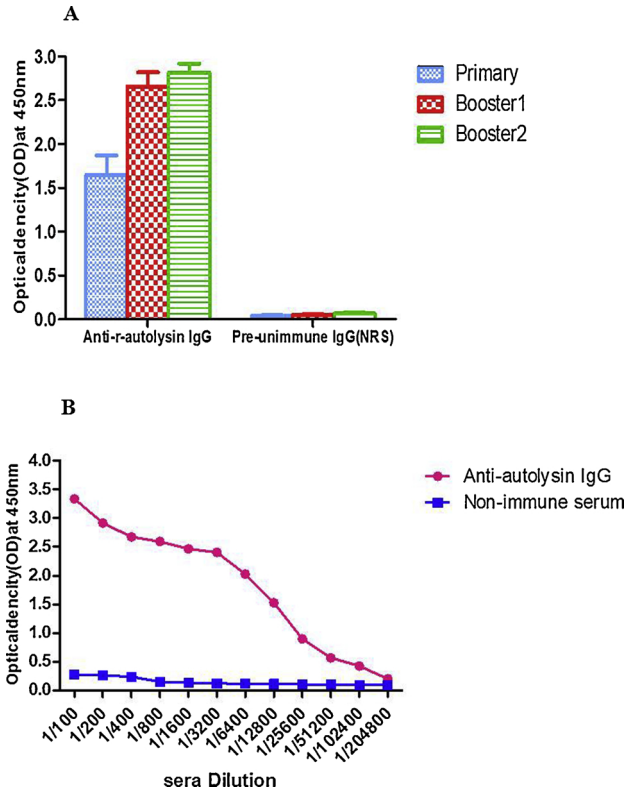

Fig. 2. A The Vaccination and passive immunisation antibodies (PcAbs) in the serum of rabbit immunized with the recombinant protein vaccine and the negative control (NRS) after each course of immunization. Bars represent means of duplicate/triplicate determinations and the error bars represent S.D. Results were verified to be significant at $P < 0.05$.**B** Characterization of polyclonal antibodies (PcAbs) generated from rabbits immunized with r-autolysin. ELISA was used to determine the titer of antigen-specific IgG for the indicated pcAbs. Data are presented as the mean from two separate experiments, each conducted individually in triplicate.

rabbits were assessed with an optimized indirect ELISA. Results of ELISA (Fig. 2A) showed that a significant level of antibody responses was induced after each immunization of rabbits with r-autolysin, as compared with the control group (NRS) ($P < 0.003$). As shown in Fig. 2B, the pcAbs were interacted with r-autolysin with high titers, ranging from 1/100 to 1/51200 after the third immunization, and significant differences were observed as compared with the control group ($P < 0.0001$).

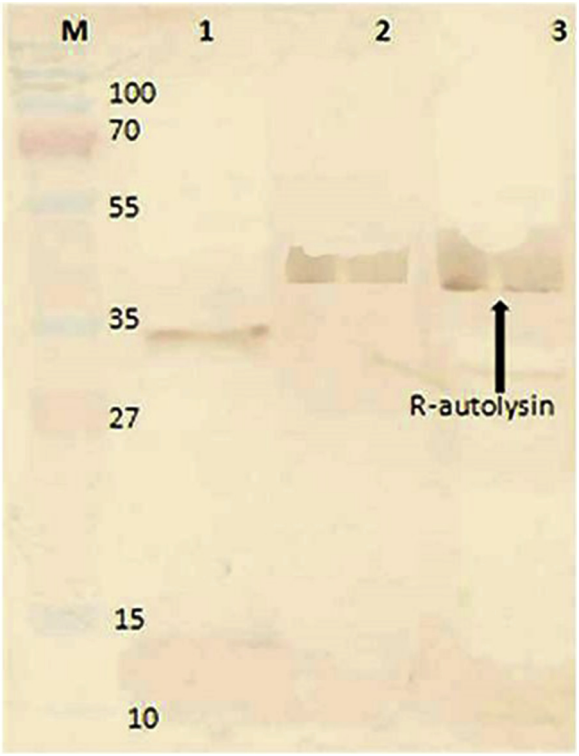

Fig. 3. Western blot analysis of the expressed r-autolysin protein in *E. coli* BL21. After running the SDS-PAGE, the protein were transferred into PVDF membrane and detected with an anti-r-autolysin IgG fraction. Lane M, Pre-stained Protein Ladder; lane 1, Non-induced sample; lane 2, induced sample; and lane 3, purified recombinant autolysin of MRSA by NI-NTA agarose. Molecular weight of the protein was about 43 kDa.

3.2. Specificity of pcAbs against recombinant autolysin

Western immunoblot analysis was performed to determine the specificity of IgG fraction against purified recombinant autolysin. Non-induced and induced colonies as well as purified recombinant autolysin were immunoblotted and then hybridized with anti r-autolysin IgG fraction. A strong reaction was observed between purified anti r-autolysin IgG and purified r-autolysin (Fig. 3; lanes 2 and 3). No band was developed when the non-induced sample was probed with anti r-PBP2a IgG (Fig. 3; lane 1). Overall, our results indicated that anti r-autolysin IgG was highly specific to detect the recombinant autolysin isolated from *S. aureus* strain COL.

3.3. Opsonophagocytic killing activity

To determine its bioactivity *in vitro*, the ability of anti r-autolysin IgG to promote phagocytosis of bacteria was evaluated by incubating the *S.aureus* strain *COL* with diluted antiserum (1/2 to 1/32) and mouse macrophages in the presence of rabbit complement. In the presence of normal rabbit serum (control group), a marginal opsonic killing activity was observed which is most likely an indicator of non-opsonic phagocytosis (4.5%). Our results also showed that the addition of anti r-autolysin promoted phagocytosis of *S. aureus* strain COL, and the number of viable bacterial cells decreased over 66.5% after 90 min compared with the control group (Fig. 4). These data indicated that anti–autolysin IgG can serve as an opsonin for killing of the *S.aureus* strain COL.

3.4. Survival rate

A mouse model of sepsis against lethal doses of the *S.aureus* strain COL was used to study the protective efficacy of anti r-autolysin

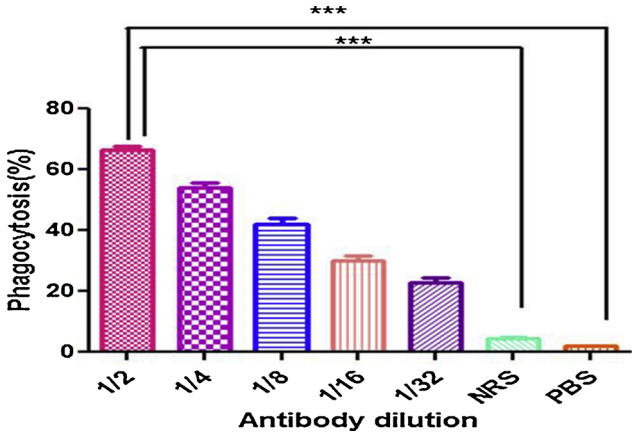

Fig. 4. The opsonic killing activity of five different dilutions of anti r-autolysin antiserum against *S.aureus* strain COL. For this purpose, the strain was incubated with different dilutions of anti r-autolysin and mouse macrophages in the presence of rabbit complement. Bars represent means of duplicated determinations and error bars indicate S.D. Results were confirmed to be significant at $P < 0.05$.

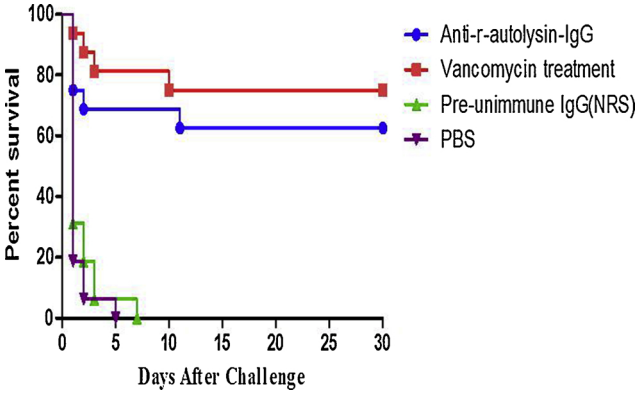

Fig. 5. The percent survival of infected mice following passive immunization with polyclonal antibodies (PcAbs). Antibody against r-autolysin enhanced the survival rate following challenge with a sublethal dose of COL strain (5×10^8 CFU) as well as mice treated with vancomycin and compared with the control groups. No protection was found in the control groups and all dead mice. Additionally, 75% (n = 12) of the mice in the witness group (treated with vancomycin) and 62.5% (n = 10) of the mice treated with anti r-autolysin IgG fraction survived.

antisera on the survival rate of infected mice. When anti r-autolysin IgG fraction was administered and boosted intraperitoneally in COL infected mice, 62.5% of the mice survived ($P = 0.0003$ vs. control groups). The control groups (groups 3 and 4) and dead mice exhibited no protection against the *S.aureus* strain COL. In addition, 75% of the mice survived in the witness group (vancomycin treatment) (Fig. 5). In addition, passive immunization prolonged the survival time of the experimental mice. In the PBS control group, mice failed to survive 5 days post-infection. Further, no higher survival rate was observed in mice immunized with the negative control (NRS) compared with those immunized with PBS, indicating that the protection efficacy observed was directly provided by antigen-specific pcAbs.

3.5. Quantitative bacterial loads in infected tissues and pathology

To assess the ability of the anti r-autolysin IgG fraction to eliminate infections caused by the *S.aureus* strain COL, we examined the clearance rate of viable bacteria from the spleen, liver and kidney of immunized mice 72 h after intraperitoneal challenge with the *S.aureus* strain COL. The results showed that passive immunization with anti r-autolysin

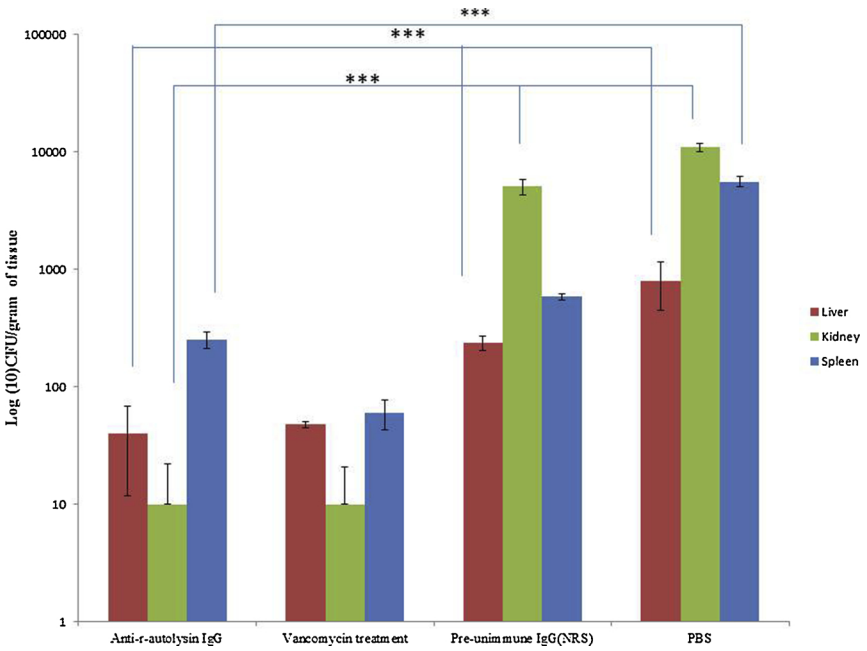

Fig. 6. The number of viable bacteria in the homogenate organs of immunized mice. Mice were sacrificed as described above 72 h after infection, and then the bacterial loads in the kidney, liver and spleen homogenates were determined. Anti r-autolysin IgG fraction significantly cleared bacterial loads in the liver, kidney and spleen of the immunized mice infected with COL strain, compared with the control groups. The CFUs were calculated after enumerating the number of colonies on each LB plate. Bars represent the mean, and the error bars show the S.D. The results were confirmed to be significant at $p < 0.05$.

resulted in a decrease in the bacterial load in the internal organs as well as vancomycin treatment compared with the control groups ($p < 0.0001$) (Fig. 6). Meanwhile, bacterial loads in mice that were passively NRS were similar to those immunized with PBS, indicating that antigen-specific pcAbs were directly correlated with reduced bacterial burden rather than nonspecific stimulation of immune responses by foreign antibodies. These results suggested that anti r-autolysin IgG fraction was able to partially inhibit *S. aureus* growth and colonization *in vivo* as well as treatment with vancomycin.

In addition, as shown in Fig. 7, passive immunization with anti r-autolysin IgG reduced the pathology of *S. aureus* infection. Consistently, histological analysis showed that mice immunized with anti r-autolysin IgG fraction exhibited reduced inflammatory cell infiltration, bleeding, and tissue injury as well as vancomycin when compared with the control groups (Fig. 7 A–H). These results further confirmed a protective efficacy of anti r-PBP2a IgG fraction against *S. aureus* infection.

4. Discussion

The emergence of extensive inherent and acquired antibiotic resistant strains led to introduction of immunoprophylaxis for *S.aureus* infections by active and passive immunization [11]. Passive immunization has long been implemented as an alternative and effective treatment approach for a variety of infectious diseases [26,30].

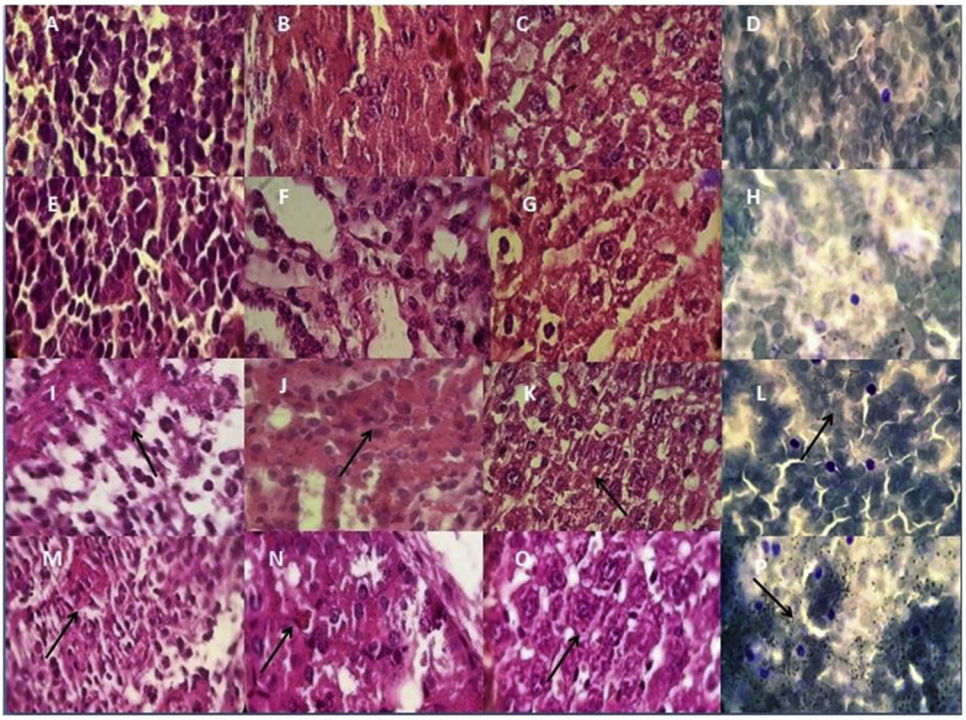

Fig. 7. Histological analysis of mice after challenge with *S.aureus.* Passive immunization of mice with anti r-autolysin IgG fraction and treatment with Vancomaycin were correlated with reduced bacterial burden and decreased pathology. The differences between vaccinated and control mice were indicated as p-value. (A) Spleen histopathology from mice passively immunized with anti r-autolysin IgG, as well as treatment with Vancomaycin (E), NRS (I) and PBS (M). The black arrow indicates moderate infiltration of mononuclear cells around the central veins. Kidney histopathology of mice passively immunized with anti r-autolysin IgG (B), as well as treatment with Vancomaycin (F), NRS (J) and PBS (N). Liver histopathology of mice passively immunized with anti r-autolysin IgG (C), as well as treatment with Vancomaycin (G), NRS (K) and PBS (O). Blood smears of mice passively immunized with anti r-autolysin IgG (D), as well as treatment with Vancomaycin (H), NRS (L) and PBS (P).
Three days after passive immunization and experimental challenge, the kidneys, liver and spleen were harvested and stained with hematoxylin-eosin. Blood samples were stained with giemsa. Representative histopathological sections from 4 mice per group were shown (magnification = 100 X). Arrows indicate inflammatory cell infiltration.

Generally, three classifications of antibodies are used for passive immunization, including (i) monoclonal antibody or its genetically-modified derivatives, (ii) homologous or heterologous pcAbs, and (iii) IgG purified from pcAbs (pcAb-IgG) [24]. In this study, r-autolysin IgG pcAb fraction was used as a passive immunotherapy for the treatment of *S.aureus* infection in a murine model.

There are many features making the *S. aureus* autolysin a very potential target for anti *S. aureus* vaccine research [31]. First, autolysin is highly conserved among staphylococci. For example, glucosaminidase (Gmd) is greater than 95% conserved across all strains of *S. aureus* in the public database, and approximately 85% conserved among other staphylococci [32]. Second, autolysin is a crucial enzyme for complete separation of daughter cells following binary fission [16,22]. *S. aureus* bacteria deficient in autolysin still divide, but daughter cells fail to separate, causing the generation of large clusters that fall out of suspension [33]. Third, Gmd is located on the extracellular surface of the bacteria, where it can be easily recognized by antibodies from the host immune system and eliminated by phagocyte cells [22]. Fourth, autolysins may play an essential role in biofilm formation [34]. For example, previous studies have shown that autolysins are involved in the initial attachment of *Staphylococcus* bacteria to a polymer surface [17,35], and that murein hydrolysis is regulated by effector genes that control bacterial death and lysis in the case of biofilm formation [34,36]. Fifth, elevated levels of anti-Gmd antibodies are detected in the serum from mice that survived a challenge with *S.aureus* [14,31,32]. Overall, these data have determined potential evidence for the selection of this antigen for the present investigation. In the previous study, the protective effect of the r-autolysin was demonstrated against the methicillin-resistant *S.aureus* strain COL in an active immunization strategy, and the potency of autolysin was well-established in the induction of humoral immune responses and protection against experimental MRSA infections [14,15].

Herein, pcAb-IgG against the r-autolysin of MRSA was produced in rabbit after three times of immunization. Then, the pcAb-IgG was precipitated with a saturated solution of ammonium sulfate. Bioactivity of fractionated pcAb-IgG was studied by western blotting, demonstrating that the purified IgG is able to bind to the r-autolysin. Further, the bioactivity of purified IgG was analyzed using *in vitro* bioassay methods for measuring the functional activity of antibodies in the opsonophagocytosis test. This study showed that the anti-autolysin IgG obtained by active immunization in rabbit could opsonize bacteria and facilitate their phagocytosis. In the presence of anti r-autolysin IgG and complement, the number of viable bacterial cells decreased moderately after 90 min under *in vitro* conditions (66.5% vs. 4.5% in the control IgG group). A previous study confirmed that these results, the increased levels of antibodies against r-autolysin, could intensify killing of *S.aureus* strain COL [14,31]. The principal way for *S. aureus* to enter the body is through the bloodstream because the bacteria have an easy access to all organs to cause septicemia [37].

Our present study demonstrated that passive immunization of mice with anti-autolysin IgG fraction significantly decreased the bacterial load in the internal organs and increased the survival rate as well as vancomycin treatment versus the control groups. Reduced bacterial loads in the internal organs may be associated with the efficient elimination of bacteria by specific antibodies in the opsonization process. This process may lead to efficient elimination of bacteria, and thereby reduce the bacterial loads in the internal organs. However, bacterial load decreased in the antibiotic treatment group as compared with the control group. Histological analysis showed that mice passively immunized with anti-autolysin IgG and vancomycin therapy also exhibited reduced inflammatory cell infiltration, bleeding, and tissue damage when compared with the control groups. These results are due to lower bacterial loads in the treatment groups with specific antibodies and antibiotic therapy. Furthermore, specific antibodies and antibiotic therapy groups exhibited the same pattern in bacterial loads; in addition, the pathological analysis of internal organs with immunotherapy ability could show comparable results as antibiotic therapy.

In conclusion, the results obtained in the present study demonstrated that the therapeutic use of autolysin-pcAbs resulted in reduced bacterial loads and improved protection in a mouse model of lethal *S. aureus* infection (sepsis) like antibiotic therapy. Antibody-mediated opsonophagocytosis by neutrophils, in combination with the neutralizing activity against autolysin, was essential for the high protective efficacy. However, in antibiotic therapy, non-immunologic mechanisms involved in the protection and antibiotic resistance may interfere with therapy.

Since *S.aureus* causes major therapeutic challenges worldwide, and some strains become resistant to nearly all front-line antibiotics, our results introduced basic information regarding an alternative therapeutic approach for the treatment of *S.aureus* infection by pcAb-mediated immunotherapy.

Conflict of interest

The authors declare no conflict of interest associated with the present manuscript.

Acknowledgements

This work was supported by Pasteur Institute of Tehran, Iran and Herbal Medicines Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (HMRC).

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