A Novel Fully Human Recombinant Antibody Neutralizing the α -hemolysin of Staphylococcus Aureus

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Abstract

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to almost all β -lactam antibiotics. Hence, new ways to control MRSA infection, such as antibacterial antibodies, need to be explored. α -Hemolysin is the most important virulence factor widely expressed in *S. aureus*. This study aimed to develop a new fully human antibody against α -hemolysin of *S. aureus* and explore its neutralizing effect.

Results

The single-chain antibody fragments—scFvs—against *S. aureus* were screened from a fully human scFv library using phage display technology with purified α -hemolysin protein. The selected scFvs had good binding affinities to α -hemolysin and were highly specific to *S. aureus*. The IgG-like scFv-Fc inserted into the pcDNA3.1 or pMH3 vector was expressed in HEK293F suspension cells to extend the antibody's *in vivo* half-life and restore Fc function. The size of purified scFv-Fc was about 55 kDa. The functions of expressed scFv-Fcs against α -hemolysin were validated. The results of A549 cytotoxicity assays showed that scFv10-Fc and scFv555-Fc had better protective effects than other scFv-Fcs on A549 cells. The results of anti-rabbit erythrocyte lysis assay confirmed that scFv555-Fc had a significant neutralizing effect on toxins. The scFv555-Fc was used to construct the docking model of antigen–antibody complexes using Discovery Studio software. It predicted that the key binding sites of α -hemolysin were TYR28, LYS37, PHE39, ARG56, and LYS58, which might be the key toxic sites of α -hemolysin.

Conclusions

A novel fully human scFv-Fc antibody neutralizing the α -hemolysin toxin of *S. aureus* was successfully developed. The findings might provide a new theoretical basis and treatment method for preventing MRSA infection.

Background

Staphylococcus aureus (*S. aureus*) is a versatile pathogen that can cause different degrees of diseases, such as soft tissue infection, pneumonia, and even sepsis [1]. The emergence and spread of multi-drug-resistant strains aggravate the disease. Methicillin-resistant *S. aureus* (MRSA) has become the leading pathogen of nosocomial infections worldwide. Invasive diseases such as bacteremia caused by MRSA are associated with high mortality [2]. The degree of bacterial resistance increases in parallel with the development of antibiotic drugs [3]. Therefore, it is imperative to discovery of new effective drug targets and development of revolutionary anti-*S. aureus* drugs.

Monoclonal antibody drugs have the advantages of high specificity and good targeting. They can inhibit pathogenic microbial infection by neutralizing toxins and killing pathogenic bacteria via phagocytosis and other ways [4, 5]. At present, some antibacterial antibodies has been marketed worldwide, including

raxibacumab, which is for treating inhalation anthrax [6], as well as several others that are at the clinical stage or preclinical stage for treating *S. aureus* and *Pseudomonas aeruginosa* infections [7]. By the end of 2018, more than 30 anti-infective monoclonal antibodies were under clinical investigation worldwide. The targets of antibacterial antibodies are mainly related to the toxin or inhibiting the invasion of pathogenic bacteria [8].

Alpha-hemolysin (α -HL or AT) is a key virulence factor of *S. aureus*. The main pathogenesis of α -HL is as follows. First, α -HL can damage red blood cells and platelets in humans and animals and promote vascular smooth muscle contraction, spasm, capillary blood flow block, and ischemic necrosis. Second, α -HL can also induce apoptosis via proinflammatory responses. The genes encoding α -HL of *S. aureus* are highly homogeneous, and almost all the strains of *S. aureus* encode α -HL.[9-11] Evidence shows that α -HL expression directly affects the virulence of *S. aureus*, including MRSA, which has been demonstrated in rodent models of *S. aureus* infection. The pathogenicity of *S. aureus* lacking α -HL expression in mice was significantly lower than that of the normal strain of *S. aureus*. [12] Moreover, the mortality of mice with pneumonia and infected with the α -HL mutant of *S. aureus* was significantly reduced, indicating that α -HL was an effective target for MRSA infection.[13, 14]

However, no therapeutic monoclonal antibody against the α -HL of *S. aureus* is currently available. In this study, the fully human recombinant antibodies neutralizing the α -HL of *S. aureus* were developed, and the key amino acid bound to the antibody that determined the virulence of α -HL was predicted. The findings might provide a new way of immunotherapy for the control of MRSA infection.

Results

Expression, purification, and identification of α -HL

The cDNA of α -HL was obtained by reverse transcription using total RNA as the template and Oligo dT15 as primers. The fragment of the α -HL gene was amplified using nested PCR. The results showed that the size of the α -HL gene fragment was about 900 bp (Fig. 1A). The soluble α -HL fusion protein was expressed with IPTG induction at low temperatures. The expression of the α -HL fusion protein was detected by western blot analysis, and the expressed protein was confirmed to be the target protein α -HL (Fig. 1B). After purification, the results of SDS-PAGE showed that the target α -HL fusion protein was purified successfully, and its size was about 90 kDa (Fig. 1C). In the N-terminal of expressed α -HL, a trigger factor of 48 kDa was connected, which was mainly used to increase the solubility of protein expression.

Preliminary screening of fully human scFvs against α -HL

The purified α -HL was used for phage screening. Specific single-chain antibodies were enriched after four rounds of phage display. More than 1000 clones were randomly selected by enzyme-linked immunosorbent assay (ELISA). The results showed that some clones had better binding reactions with the antigen α -HL, in which the positive scFvs with OD₄₅₀ > 0.8 accounted for about 34%. Eighty scFv

clones were randomly selected for expression, and the binding between α -HL and scFvs was detected by ELISA (Fig. 2).

Expression and identification of scFvs against α -HL

The scFvs gene with the highest ELISA OD values was inserted into the prokaryotic expression vector pLZ16 for soluble expression. The binding activity and specificity of expressed scFvs to *S. aureus* were determined by ELISA. The results showed that 15 scFvs could specifically bind to *S. aureus*, but not to *S. albicans* (Fig. 3A). The 15 scFvs were identified by western blot analysis, and the scFv protein was the target protein with a size of 30 kDa (Fig. 3B). The 15 scFvs also could bind well to the α -HL of *S. aureus* (Sigma, USA). (Fig 3C). The sequencing results showed that the sequences of 15 scFvs were correct, all of which were open reading frames, and their CDR regions were different (Fig. 3F).

Construction, expression, and purification of scFv-Fcs

The toxicity test of α -HL on A549 showed that three scFvs (scFv10, scFv555, and scFv802) showed better neutralizing activity. Due to the short half-life of scFv *in vivo*, the Fc segment was added to the three scFvs that performed well in the hemolysis blocking experiment, thus generating an IgG-like scFv-Fc antibody. The SP-scFv-Fc/pcDNA3.1 and SP-scFv-Fc/pMH3 recombinant vectors were constructed to examine the eukaryotic expression of soluble scFv-Fcs (Fig. 4A and 4B). The three scFv-Fcs were expressed in pcDNA3.1 and pMH3. Western blot results showed that the size of scFv-Fcs was about 55 kDa, and the expression level of scFv-Fcs in PMH3 was significantly higher than that in pcDNA3.1 (Fig. 5A). The purified expressed α -HL (HLA-EX) and the commercial hemolysin (HLA-Sigma) were used as antigens in ELISA. The result showed that the expressed proteins could bind well to α -HL (Fig. 5B and 5C). The three scFv-Fcs were highly expressed in the pMH3 vector and were purified (Fig. 4D) for subsequent functional verification of scFv-Fcs.

Functional verification of scFv-Fcs

The neutralization properties of the three purified scFv-Fcs were further verified. A549 cytotoxicity tests showed that all three scFv-Fcs could neutralize cytotoxicity, reduce cytotoxicity, and protect A549 cells to varying degrees compared with the control group. Among these, scFv555-Fc has a better neutralizing effect than the other two. (Fig. 6A). The results of the anti-rabbit hemolysis test showed that scFv555-Fc had the most obvious anti-hemolytic effect (Fig. 6B). The results show that the three scFv-Fcs screened had a certain neutralization effect on hemolysin.

Establishment and evaluation of the scFv555 homologous model

The Discovery Studio 2016 software predicted CDRs of the scFv555 antibody. A total of 25 templates were obtained using the “identify framework templates” module. 4M6O LH was selected as the overall template, 4R4B A as the light chain template, and 3NH7 H as the heavy-chain template. The scFv555 model, including the frame region and the loop region, was constructed. Then, scFv555-M0019 was selected as the best 3D model using PDF total energy, PDF physical energy, and DOPE score (Fig. 7A).

The Ramachandran diagram was used to evaluate the model scFv555-M0019. The results showed that more than 90% of the amino acid residues fell in the allowable region (purple) and the optimal region (blue), indicating that this model conformation conformed to the rules of stereochemistry (Fig. 7B). The profile-3D image showed that most of the Verify Score was > 0. The scFv555-M0019 was matched with its own amino acid sequence, and therefore the model had great credibility (Fig. 7C).

Antibody–antigen docking and key residue analysis

The scFv555 model as the receptor and α -hemolysin monomer from the MEDI4893·AT complex (PDB code: 4U6V) as the ligand were docked using the ZDOCK module. A total of 26 poses were chosen using the ZDOCK score (ZDOCK score > 20) and the spatial structure to optimize RDOCK. The best E_RDOCK score for docking was pose 2 (Fig. 8A). Then the “analysis protein interface” module was used. The analysis results suggested that the key amino acid residues of interface prompt for α -hemolysin were VAL26, TYR28, HIS35, LYS37, PHE39, ARG56, LYS58, THR60, HIS144, ASN214, and GLY223 (Fig. 8B). Meanwhile, the binding region of scFv555-M0019 was mainly located in the heavy-chain CDR3 region.

In the meantime, the scFv555 model as the receptor and α -hemolysin monomer from the LTM14·AT complex (PDB code: 4IDJ) as the ligand were docked using the ZDOCK module. A total of 23 poses were chosen using the ZDOCK score (ZDOCK score > 20) and the spatial structure to optimize RDOCK. The best E_RDOCK score for docking was pose 6 (Fig. 8C). Then, the analysis interface module was used. The analysis revealed that the key binding sites of the α -hemolysin monomer for this binding model were ILE7, TYR28, LYS37, PHE39, ARG56, LYS58, ILE142, LYS147, and SER222 (Fig. 8D). Meanwhile, the binding region of scFv555-M0019 was mainly located in the heavy-chain CDR3 region.

The two α -hemolysin monomer models from different complex crystals [PDB code]4U6V and 4IDJ[were used to simulate the antigen-antibody binding with the model of scFv555 , and it was found that some key binding sites (TYR28, LYS37, PHE39, ARG56, and LYS58) of α -hemolysin bound to scFv555 model could be overlapped.

Discussion

Infections caused by MRSA have received worldwide attention. The development of antibacterial drugs with different therapeutic mechanisms has become an urgent topic for human health maintenance to better solve the problem of MRSA drug resistance, while the targeting and high efficiency of antibody drugs have become an inevitable trend and object of concern in the development of antibacterial drugs. Based on the key role of α -hemolysin in *S. aureus*, this study screened the anti-*S. aureus* α -hemolysin-specific single-chain antibodies from the fully human antibody library and constructed fully human IgG-like scFv-Fc antibodies. The study also preliminarily identified that the purified scFv-Fc antibody had the characteristics of neutralizing α -hemolysin.

In this study, the specific human single-chain antibody against *S. aureus* α -hemolysin was screened from the fully human single-chain antibody library using phage display technology[15]. However, the molecular

weight of the single-chain antibody was low, and the serum half-life was short *in vivo* [16]. Therefore, it was difficult to achieve a therapeutic effect. The Fc fusion protein is composed of the Fc region of the IgG antibody (Hinge-CH2-CH3) and scFv. The Fc fragment of the antibody is mainly responsible for pharmacokinetic properties, which can reduce the renal clearance rate and improve serum half-life [17]. At the same time, Fc can be combined with many Fc receptors on immune cells to activate the complement system by activating C1q and regulate the interaction between antibody Fc and its receptor in circulation, so as to participate in complement-dependent cell-mediated cytotoxicity and complement-dependent cytolysis response[18]. Based on the structure and function of Fc fusion protein, the human IgG1 fragment was added to the single-chain antibody, and an IgG-like recombinant antibody was constructed[19, 20]. MEDI4893, an anti- α -hemolysin monoclonal antibody obtained by hybridoma technology in immunized VelocImmune mice, was transformed into human IgG, which was later modified by the Fc fragment [21]. Although the chimeric antibody can partially solve the heteroprotein rejection problem, it may still induce human antimouse antibody response reaction and interfere with the efficacy of the antibody because it also contains a mouse source, which limits its clinical application to some extent. A large number of studies have reported the disadvantages of chimeric antibodies. Until 2018, 11 antibodies produced using the phage display technology were approved by FDA/EMA. Compared with chimeric antibodies prepared by mice, the fully human antibodies produced using the phage display technology could theoretically maximize the potential therapeutic effect, thus reducing or even eliminating the immunogenicity of antibodies [22, 23]. The added Fc segment could prolong the half-life and had a certain biological function. This advantage might be reflected in later clinical trials.

α -Hemolysin is cell specific and species specific in that it has a hemolytic effect by relying on metalloproteinase ADAM10 [24]. In the present study, the scFv555-Fc performed well in the preliminary functional screening and had a neutralizing effect on α -hemolysin. The simulated model of scFv555 was built using computer simulations, and then two α -hemolysin monomers were chosen to participate in simulation docking and analysis. The results showed that the key sites of AT were TYR28, LYS37, PHE39, ARG56, and LYS58. The two α -hemolysin monomers from LTM14-AT and MEDI4893-AT formed a complex crystal structure[25]. In the study on an LTM14-AT complex, the epitopes on α -hemolysin were concentrated in the R66 region, while the MEDI4893-AT complex showed that the epitopes were concentrated in the regions comprising amino acids 101–110, 224–241, and 248–277 [26, 27]. The prediction region of α -hemolysin epitope displayed by the simulated docking was relatively close to the region of the LTM14-AT complex, which might be related to the method used for preparing the antibody. LTM14 is also a human antibody prepared using the phage display technology, while MEDI4893 is a mouse and human chimeric antibody. However, the sites differed from the aforementioned structures, indicating new binding epitopes on α -hemolysin. In previous studies on the structure of α -hemolysin, PHE39 was thought to be an important part of the hydrophobic core that stabilized the amino latch conformation. The results suggested that the antibody bound to hemolysin at sites not previously reported, and the antibodies neutralized the toxic effects of α -hemolysin via a new mechanism.

Here, we prepared scFv-Fc antibodies that neutralized α -hemolysin of *S. aureus*, and predicted the binding of the scFv555 and α -hemolysin monomer by using computer simulation to reveal key residues

associated with the binding. The computer simulations results might provide the basis for subsequent mechanisms by which antibodies neutralize toxins, while the neutralizing scFv-Fc against α -hemolysin might provide a new way to control MRSA.

Methods

Acquisition, expression, and purification of α -HL

Total RNA of *S. aureus* was extracted, and cDNA was obtained by reverse transcription using total RNA as the template and Oligo dT15 as primers. The sequence of the *S. aureus* α -HL gene was downloaded from the National Center for Biotechnology Information gene database. The primers were as follows: α -HL F1-Forward (5'-CACCATGCTATTGCTAGGTTCC-3') and α -HL R1-Reverse (5'-GGT ACC TTT CCA ATT TGT TGA AGT CCA ATG-3'); α -HL F2-*Xho*I (5'-CCGCTCGAGATGACACGTATAGTCAGCTCA-3'); and α -HL R2-*Eco*RI (5'-CCG GAA TTC TTC TGA AGA ACG ATC TGT CCA-3'), designed according to the gene sequence; α -HL was amplified using nested PCR. The α -HL gene was cloned into the p-Cold TF fusion expression vector. The His-tagged α -HL recombinant protein was expressed in *E. coli* BL21 at low temperature for 24 h, and then the bacterial precipitate was collected by centrifugation. The bacteria were sonicated, and the supernatant was collected after centrifugation. The expression of the fusion protein was detected using Western blot analysis, and the protein was purified using an Ni column (Qiagen, Germany) using standard techniques and verified using SDS-PAGE.

Preliminary screening of fully human scFv against α -HL

The human scFv phage library was used to select scFvs that specifically bound α -HL of *S. aureus* using previously published methods [28]. Afterward, 20 μ g biotinylated α -HL was serially incubated with the phage library at room temperature (RT) for 1 h and with streptavidin magnetic beads (Dynabeads M-280, Invitrogen, USA) for 30 min. After washing, the bound phage scFvs were eluted with 0.1M HCl (adjusted to pH 2.2 with glycine). The eluted phages were amplified in TG1 cells and used for the next rounds of selection with 15 μ g biotinylated α -HL. A total of four rounds of panning were performed. scFvs were initially screened for their ability to bind α -HL using ELISA.

Expression and specificity detection of scFv against α -HL

The 18 strains of anti- α -HL scFvs with the highest OD₄₅₀ value were expressed in large quantities, and the scFvs were extracted and sequenced. Among these, 15 scFvs with correct sequencing results were inserted into vector pLZ16 using *Not*I and *Nco*I restriction enzymes and transformed into *E. coli* DH5 α for expression. The pLZ16 vector was constructed based on the pUC vector and contained a Flag and His-tag label. Western blot identified the scFv/pLZ16 recombinant vector, and the secondary antibody was the anti-Flag-HRP-labeled antibody. The binding activities of scFvs to the antigen and their specificity against *S. aureus* were detected using ELISA. The absorbance value (A) was read at 450 nm.

Construction of scFv-Fc recombinant plasmids

In this study, on the basis of the pcDNA3.1 vector, the heavy-chain signal peptide sequence—the initiation codon, Kozak expression element, and Fc fragment—including Hinge + CH2 + CH3, were added to construct a general expression vector of SP-Fc/pcDNA3.1 with multiple restriction endonuclease sites between SP and Fc, so as to insert scFv into SP-Fc/pcDNA3.1. Based on the SP-scFv-Fc/pcDNA3.1 eukaryotic expression vector, the SP-scFv-Fc fragment was amplified and inserted into the pMH3 vector to construct the recombinant expression vector SP-scFv-Fc/pMH3.

Expression and purification of scFv-Fc

The recombinant vectors, SP-scFv-Fc/pcDNA3.1 and SP-scFv-Fc/ pMH3, were transfected into HEK 293F suspension cells. The cells were collected by centrifugation after 48 h and lysed using cell lysate. After completion of the cell lysis, the supernatant was collected at low temperature for verification using western blot analysis. The supernatant was directly loaded onto an SDS-PAGE gel, and the primary antibody was a goat anti-human IgG HRP-labeled antibody. The recombinant protein was expressed using the aforementioned method; the expression was verified using ELISA. The cells were resuspended in 1× PBS, mixed with protease inhibitor PMSF, and lysed by sonication on ice. After sonication, the supernatant was collected by centrifugation at a low temperature. α-HL (Sigma) and purified α-HL were coated in the wells of the microtiter plate. The primary antibody was the collected supernatant, and the secondary antibody was the goat anti-human IgG HRP-labeled antibody (Abcam, UK). The antibody protein was purified using protein A affinity chromatography.

A549 cytotoxicity assay

The lung epithelial cell line A549 was cultured in a 37 °C, 5% CO₂ incubator in RPMI-1640 medium with 10% fetal bovine serum and 1% double antibody (penicillin and streptomycin). A549 cells were seeded in 96-well plates at a density of 0.5×10^4 cells/well. After 24 h, 10 μL of scFv-Fc expressed proteins were preincubated with 10nM α-HL for 20 min at RT and then added to A549 cells for incubation for an additional 24 h. Cytotoxicity was measured with the Cell Counting Kit-8 (Dojindo, Japan). The results were graphically presented, and statistical analysis was performed using GraphPad Prism v.5.0.

Rabbit erythrocyte lysis assay

Afterward, 50 μL of scFv-Fcs expressed proteins were incubated with 50 μL of 1.25 ng/μL α-HL for 30 min at RT. The mixture was then added to 1% Rabbit erythrocyte (Sibjio, China) and incubated for 30 min at 37 °C in a 96-well plate. The plate was subsequently spun at 4000g for 5 min to collect the supernatant. The absorbance of the supernatant was read at 405 nm to quantify the amount of released hemoglobin as a measure of lysis. The results were graphically presented, and statistical analysis was performed using GraphPad Prism v.5.0.

Prediction of the key sites of antigen binding

The model antibody framework protocol was used to model the antibody model and loop structure with the DS 4.5 system (Discovery studio visualizer version v16.1.0. 15350, NeoTrident Technology Ltd.).

Then, the model was evaluated using the Ramachandran plot and Profile-3D. The α -hemolysin monomer structure and antibody model were docked with dock proteins using ZDOCK, and then the high ZDOCK score and reasonably structured poses were chosen to refine with RDock. The best E_RDOCK score pose was used to calculate mutation energy and find the key residues with the analysis protein interface.

Abbreviations

scFv: single-chain antibody fragment; MRSA: Methicillin-Resistant *Staphylococcus aureus*; α -HL ,AT : alpha-hemolysin; ELISA: enzyme linked immunosorbent assay; *S. aureus* :*Staphylococcus aureus*; IgG: immunoglobulin G

Declarations

Author's contributions

QY and LP, CL designed the study. CL, TW, CY Expressed and identified α -HL. CL, LP constructed the recombination vector, expressed recombination antibody and identified the function. HC, HZ, LP completed the molecular simulation. LP, YW, HY and CY analyzed the data. All authors contributed to drafting of the manuscript and approved the final version.

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Availability of data and materials

All data generated and analyzed during this study are included in the published data.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. Lowy FD. Staphylococcus aureus infections. N Engl J Med 1998; 339: 520-532.
2. Cosgrove SE, Sakoulas G, Perencevich EN et al. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis 2003; 36: 53-59.
3. Howden BP, Davies JK, Johnson PD et al. Reduced vancomycin susceptibility in Staphylococcus aureus, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 2010; 23: 99-139.
4. Cheung GY, Otto M. The potential use of toxin antibodies as a strategy for controlling acute Staphylococcus aureus infections. Expert Opin Ther Targets 2012; 16: 601-612.
5. Chow SK, Casadevall A. Monoclonal antibodies and toxins—a perspective on function and isotype. Toxins (Basel) 2012; 4: 430-454.
6. Kummerfeldt C. Raxibacumab: potential role in the treatment of inhalational anthrax. Infection and Drug Resistance 2014.
7. <https://clinicaltrials.gov/ct2/show/results/NCT02296320?term=MEDI4893&draw=2&rank=1>. In. Accessed on 27,September,2020
8. Rodgers KR, Chou RC. Therapeutic monoclonal antibodies and derivatives: Historical perspectives and future directions. Biotechnol Adv 2016; 34: 1149-1158.
9. Seilie ES, Bubeck Wardenburg J. Staphylococcus aureus pore-forming toxins: The interface of pathogen and host complexity. Semin Cell Dev Biol 2017; 72: 101-116.
10. Wiener-Kronish JP, Pittet JF. Therapies against virulence products of Staphylococcus aureus and Pseudomonas aeruginosa. Semin Respir Crit Care Med 2011; 32: 228-235.
11. Montgomery CP, Boyle-Vavra S, Adem PV et al. Comparison of virulence in community-associated methicillin-resistant Staphylococcus aureus pulsotypes USA300 and USA400 in a rat model of pneumonia. J Infect Dis 2008; 198: 561-570.

12. Tkaczyk C, Hamilton MM, Datta V et al. Staphylococcus aureus alpha toxin suppresses effective innate and adaptive immune responses in a murine dermonecrosis model. PLoS One 2013; 8: e75103.
13. Diep BA, Hilliard JJ, Le VT et al. Targeting Alpha Toxin To Mitigate Its Lethal Toxicity in Ferret and Rabbit Models of Staphylococcus aureus Necrotizing Pneumonia. Antimicrob Agents Chemother 2017; 61.
14. Hilliard JJ, Datta V, Tkaczyk C et al. Anti-alpha-toxin monoclonal antibody and antibiotic combination therapy improves disease outcome and accelerates healing in a Staphylococcus aureus dermonecrosis model. Antimicrob Agents Chemother 2015; 59: 299-309.
15. Wenzel EV, Roth KDR, Russo G et al. Antibody Phage Display: Antibody Selection in Solution Using Biotinylated Antigens. Methods Mol Biol 2020; 2070: 143-155.
16. Raag R, Whitlow M. Single-chain Fvs. Faseb j 1995; 9: 73-80.
17. Jafari R, Zolbanin NM, Rafatpanah H et al. Fc-fusion Proteins in Therapy: An Updated View. Curr Med Chem 2017; 24: 1228-1237.
18. Sondermann P, Szymkowski DE. Harnessing Fc receptor biology in the design of therapeutic antibodies. Curr Opin Immunol 2016; 40: 78-87.
19. Hwang WYK, Foote J. Immunogenicity of engineered antibodies. Methods 2005; 36: 3-10.
20. Wan L, Zhu S, Zhu J et al. Production and characterization of a CD25-specific scFv-Fc antibody secreted from Pichia pastoris. Appl Microbiol Biotechnol 2013; 97: 3855-3863.
21. Tkaczyk C, Hua L, Varkey R et al. Identification of anti-alpha toxin monoclonal antibodies that reduce the severity of Staphylococcus aureus dermonecrosis and exhibit a correlation between affinity and potency. Clin Vaccine Immunol 2012; 19: 377-385.
22. Hwang WY, Foote J. Immunogenicity of engineered antibodies. Methods 2005; 36: 3-10.
23. Frenzel A, Schirrmann T, Hust M. Phage display-derived human antibodies in clinical development and therapy. MAbs 2016; 8: 1177-1194.
24. Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in Staphylococcus aureus alpha-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A 2010; 107: 13473-13478.
25. Oganessian V, Barnes A, Tkaczyk C et al. Crystallization and preliminary X-ray diffraction analysis of the complex between a human anti-alpha toxin antibody fragment and alpha toxin. Acta Crystallogr Sect F Struct Biol Cryst Commun 2013; 69: 302-305.
26. Foletti D, Strop P, Shaughnessy L et al. Mechanism of action and in vivo efficacy of a human-derived antibody against Staphylococcus aureus alpha-hemolysin. J Mol Biol 2013; 425: 1641-1654.
27. Oganessian V, Peng L, Damschroder MM et al. Mechanisms of neutralization of a human anti-alpha-toxin antibody. J Biol Chem 2014; 289: 29874-29880.
28. Yuan Q, Huang L, Wang X et al. Construction of human nonimmune library and selection of scFvs against IL-33. Appl Biochem Biotechnol 2012; 167: 498-509.

Figures

Figure 1

Expression, purification, and identification of α -HL. (A) Identification of amplified α -HL cDNA using PCR. M, DNA marker 1000 bp; lane 3, α -HL cDNA. (B and C) Identification of α -HL fusion protein using western blot analysis (B), and analysis of purified α -HL fusion protein using SDS-PAGE (C). M, Protein molecular weight marker; lanes 1–3, Expressed protein of α -HL fusion protein.

Figure 2

Preliminary screening of fully human scFv against α -HL. Lanes 1–82, different clones. The absorbance reading at 450 nm was taken as the result. Noncoated antigen-labeled wells were used as negative control. The negative control had OD450 = 0.05.

Figure 3

Expression and specificity detection of scFv against α -HL. (A) Identification of preliminarily screened scFvs against α -HL using ELISA. (B) Identification of scFvs by western blot analysis. The size of the protein was about 30 kDa. Note: lanes 1–15, expressed proteins of scFv2; scFv10; scFv46; scFv214; scFv511; scFv538; scFv555; scFv718; scFv722; scFv759; scFv777; scFv802; scFv845; scFv921; and scFv997. (C) The specificity of anti- α -HL scFvs detected using ELISA. Note: The absorbance reading at

450 nm was taken as the result. The negative control had OD450 = 0.05. Black horizontal bars indicate the standard deviation of each group. ***P < 0.001. (D) The CDR3 regions of 15 clones of scFvs.

Figure 4

Construction of scFv-Fc. (A) SP-scFv-Fc/pcDNA3.1 recombinant vector structure. (B) SP-scFv-Fc/pMH3 recombinant vector structure.

Figure 5

Expression and purification of scFv-Fc. (A) Identification of SP-scFv-Fc/pcDNA3.1 recombinant vector using western blot analysis. Lane 1, Expressed protein of scFv10-Fc/pcDNA3.1; lane 2, expressed protein of scFv555-Fc/pcDNA3.1; lane 3, expressed protein of scFv802-Fc/pcDNA3.1; Lane 4, Expressed protein of scFv10-Fc/pMH3; lane 2, expressed protein of scFv555-Fc/pMH3; lane 3, expressed protein of scFv802-Fc/pMH3. (B) Detection of expressed SP-scFv-Fc/pcDNA3.1 recombinant vector using ELISA.

Control, pcDNA3.1 vector. (C) Detection of expressed SP-scFv-Fc/pMH3 recombinant vector using ELISA. Control, pMH3 vector. (D) Identification of the purified scFv555-Fc protein using SDS-PAGE. Note: M, Precision Plus Protein Standard.

Figure 6

Functional verification of scFv-Fc. (A) A549 cytotoxicity assay. Note: SP-scFv10-Fc, SP-scFv555-Fc, and SP-scFv802-Fc in the experimental group were identified as proteins expressed by the SP-scFv10-Fc/pMH3, SP-scFv555-Fc/pMH3, and SP-scFv802-Fc/pMH3 vectors, respectively. SP-pMH3 in the experimental group was identified as protein expressed by the pMH3 vector. The survival rate was calculated using the CCK8 formula. (B) Rabbit erythrocyte lysis assay. scFv555-Fc was identified as the protein expressed by SP-scFv555-Fc/ pMH3 vector. Control was the indicated protein expressed by the pMH3 vector. **P < 0.01.

A

B

C

Figure 7

Homology modeling of scFv555. (a) scFv555-M0019; the three-dimensional structure of scFv555. (B) Ramachandran plots of the structure of scFv555-M0019. The blue region indicates the optimal zone, and the purple region indicates the permitted zone. Green circles indicate amino acids in the optimal zone; pink circles indicate amino acids in the permitted region. (C) Profile-3D verification of scFv555-M0019. A score >0.0 indicates that the amino acids are compatible.

Figure 8

Simulated docking model of scFv555-M0019 and α-hemolysin monomers. (A) Simulated docking model of scFv555-M0019 (purple, heavy chain; pink, light chain) and α-hemolysin monomer (green, PDB code: 4U6V). (B) Key binding sites of monomer (yellow) were VAL26, TYR28, HIS35, LYS37, PHE39, ARG56, LYS58, THR60, HIS144, ASN214, and GLY223. Shallow orange, the CDRs region of scFv555-M0019. Reddish orange, the binding sites of scFv555-M0019. (C) Simulated docking model of scFv555-M0019 (purple, heavy chain; pink, light chain) and α-hemolysin monomer (green, PDB code: 4IDJ). (D) Key binding sites of the α-hemolysin monomers were ILE7, TYR28, LYS37, PHE39, ARG56, LYS58, ILE142, LYS147, and SER222. Shallow orange, the CDRs region of scFv555-M0019. Reddish orange, the binding sites of scFv555-M0019.

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