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Paper

Phage display technology for fabricating a recombinant monoclonal ScFv antibody against tetanus toxin

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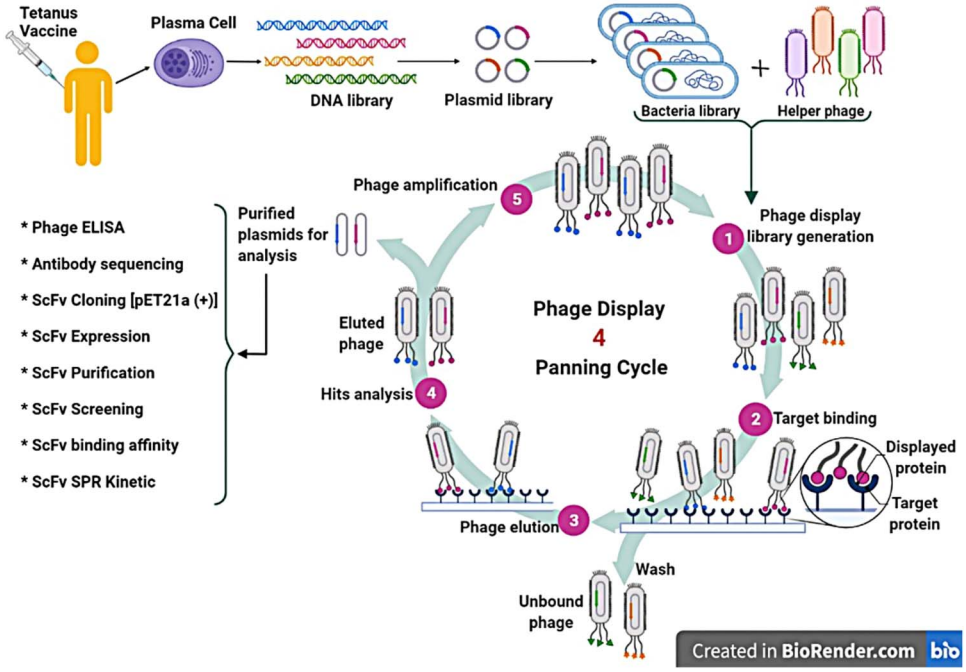
Tetanus is a specific infectious disease, often associated with lower immunization in developing countries and catastrophic events (such as earthquakes). Millions of people, especially children, die every year from tetanus disease. Therefore, it is necessary to devise a rapid and sensitive detection method for tetanus toxin to ensure an early diagnosis and clinical treatment of tetanus. The current study looks at developing a novel, high specific, low-cost, and sensitive ScFv antibody. It is capable of tetanus detection immunoassays in clinical diagnosis, suspicious foods, and water monitoring. For this regard, a high-quality phage display antibody library (8.7×10^7 PFU/ml) was constructed. Tetanus-specific antibodies with high affinity retrieved from libraries. After phage rescue and four rounds of biopanning, clone screening was performed by phage ELISA. Recombinant antibodies expressed from the AC8 clone showed the highest affinity for tetanus. SDS-PAGE and western blotting confirmed the presence of a high-quality, pure ScFv band at 32 kDa. ELISA was used to determine the affinity value, estimated to be around 10^{-8} M. The results suggest that the proposed detection method by ScFv antibodies is an alternative diagnostic tool enabling rapid and specific detection of the tetanus toxin.

Graphical Abstract



Key words: tetanus; ScFv; antibody; detection; phage display.

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Introduction

Clostridium tetani is a common soil bacterium and present in human and animal feces.[1] Tetanus is a disease caused by a potent neurotoxin known as tetanospasmin produced by this bacteria.[2] Although *C. tetani* are sensitive to heat and oxygen, its spores are highly resistant (remains on soil for many years) and survive in boiling temperature (40–60 min). The spores are ubiquitous worldwide and can be found mainly in agricultural areas.[3] Infection begins when tetanus spores infiltrate into a wound. Then, *C. tetani* can grow and produce a potent toxin and cause severe symptoms of fatal tetanus disease.[4] Tetanus has remained one major cause of mortality in developing countries for inadequate immunization, and millions of people, especially children, die every year from tetanus disease. Also, tetanus disease is often associated with catastrophic events such as earthquakes, traumas, and war wounds.[5]

Detection of *C. tetani* is generally predicted on microscopic analysis of wound exudates and anaerobic cultivation, followed by biochemical identification.[6] The direct smear method is fast and inexpensive, but it has low specificity and sensitivity. Hence, it is complicated to separate *C. tetani* from other Clostridium species by this technique. The "gold standard" method for detecting *C. tetani* is anaerobic cultivation followed by analytical profile index identification.[7] But anaerobic cultivation is a time-consuming method, which needs comparatively high standard laboratory equipment. Consequently, this causes a delay up to the optimal time of the therapeutic window.[6]

To avoid potential threats to human and animal health, reliable and sensitive tetanus toxin analysis is critical in clinical diagnosis, suspicious food analysis, and water monitoring. During the past decades, monoclonal antibodies had an intense effect on diagnostics.[8] Currently, the generation of single-chain fragment variable (ScFv) has become an established method used to produce a functional antigen-binding fragment, possible to clone desired antibody genes in bacteria by using antibody phage display technology.[9] ScFv is a monomeric structure with interesting physicochemical properties, such as solubility and stability, and high production yields in *E. coli*.[9] The main goal of this study was to use phage display to create a high tetanus specific and sensitive ScFv for the first time. In this regard, a specific ScFv antibody was generated and purified from an immunological human phage library. This ScFv antibody is inexpensive, more selective, and sensitive in recognizing tetanus toxin than traditional antibodies. The other techniques are beneficial in laboratory diagnostic immunoassays or biosensor antibodies for detecting a negligible amount of tetanus toxin.

Material and methods

Construction of phagemid antibody library against tetanus toxin

Antibody library construction was performed in our previous work.[10] Briefly, an individual was vaccinated, his serum was isolated. The number of antibodies from a vaccinated person compared before and after vaccination by ELISA. Then total RNA was extracted using an RNA extraction kit (Roche, Germany) following the manufacturer's guidelines. Consequently, cDNA was synthesized by reverse transcription process with cDNA synthesis kit (Roche, Germany) according to the supplier's instructions. In the next step, human variable heavy (V_H) and light chain (V_L) of antibody against tetanus was amplificated by primer library set (contain enzyme restriction site for NcoI & HindIII (V_H), MluI & NotI (V_L)). After that, V_H and V_L genes were cloned in the pSEX81 phagemid vector. Colony PCR was performed, ensuring the accuracy of cloning. Transformed *E. coli* XL1-Blue cells were inoculated in 1 ml of 2x yeast extract-tryptone (2 × YT) medium (2% glucose) and incubated at 37 °C for about one hour. Transformed cells again plated on SOB agar plates (super optimal broth of 20 g/liter glucose and 80 g/ml ampicillin) and incubated at 37 °C overnight. The growing colonies were collected and immersed in a 2 × YT medium (100 g/ml ampicillin and 2% glucose). A portion of the new medium combination was diluted to reach an OD of .5 at 600 nm for phage recovery and biopanning.[11]

Rescue of phagemid library

Phage particles displaying ScFv antibody rescued after infection with M13K07 helper phage.[11] The above-diluted culture was grown, and a helper phage was added at a multiplicity of infection (MOI) of 10. The culture was then incubated (30 min without shaking and 30 min with shaking) and then centrifuged at 3,000 RPM for 10 min. The pellet was resuspended in 5 ml 2 × YT medium with 80 μg/ml ampicillin and 60 μg/ml kanamycin and incubated at 37 °C overnight. Afterward, the culture was centrifuged at 3,000 RPM for 20 min at room temperature, and the supernatant containing recombinant phage particles transferred to a new tube. The resulting particles were precipitated with polyethylene glycol-NaCl on ice for one hour and collected by centrifugation at 9,000 RPM for 20 min at 4 °C. Precipitated recombinant phage particles resuspended in a 2× YT medium, and their titer determined for biopanning.

Biopanning and screening of phage antibody library

Phage-displayed tetanus-specific ScFv antibodies isolated by panning.[12] In biopanning, tetanus-specific clones are enriched by binding to an immobilized toxin, followed by eluting and repropagation of phage. The tetanus toxin suspension (2 μg/ml) was coated in the panning plates and incubated overnight at 4 °C. After that, plates were blocked with 100 μl of blocking buffer (TBS containing 5% (w/v) skim milk) at 4 °C overnight. Block wells were washed with 5% TBST (TBS with .05% Tween-20 (v/v)) for four times. Then, suspension of recombinant phage particles was diluted with 5 ml of 10% MPBS (phosphate-buffered saline containing 5% (w/v) skim milk) and incubated at room temperature for 1 hour. The diluted recombinant phage particles (100 μl) were added to the tetanus-coated plate and incubated for 2 hours at 37 °C (phage particles that did not display ScFv considered negative control). After discarding the supernatant, wells were washed with .05% TBST 10–15 times. The recombinant phages were eluted by adding 20 μL glycine-HCL buffer (pH 2.2) and neutralized it with 1 M Tris (pH 9). They incubated at room temperature for 10 min, then supernatant harvested. Instantly, the new exponentially growing culture of *E. coli* XL1-Blue infected with the eluted recombinant phages, and half of them amplified for the next rounds of selection. After four rounds of panning, 25 clones were randomly selected for further analyses.

Evaluation of panning progress by polyclonal and monoclonal phage ELISA

Tetanus binding clones were selected by phage ELISA. An ELISA plate was coated with 100 μl of tetanus toxin (2 μg/ml) and BSA in coating buffer by incubation at 4 °C overnight. The coating solution was discarded and washed once with the washing buffer (TBS-T). All wells were blocked with 100 μl of blocking buffer (TBS containing 5% (w/v) skim milk) and incubated at 37 °C

for 1 hour. The plate was washed four times with the washing buffer after discarding the blocking solution. Phage solution (Phages prepared after each panning round) (100 μl) was added per well and incubated at room temperature for 2 hours. Then, the plate was washed with the washing buffer five times over. Afterward, 100 μl of diluted HRP-conjugated anti-M13 antibody (Roche, Germany) with the dilution of 1/5,000 was added per well and incubated at 37 °C for 1 hour. Then, the plate was washed with the washing buffer 5 times again. Bounded recombinant phage particles were detected once treating with an anti-M13 HRP-conjugated antibody. The colorimetric reaction was performed by adding 100 μl of TMB (substrate solution) per well and incubating at 37 °C for 30 min. This reaction stopped by adding 100 μL of sulfuric acid (1 M). The absorbance of the wells was read by the ELISA microplate reader at wavelengths from 450 to 630 nm. After 4 rounds of panning, single colonies from the dilution series for antigen-binding assay employing the monoclonal ELISA (all steps performed the same as described above).

Analysis of antibody sequences

The anti-tetanus Scfv sequence was cloned into the pSEX81 phagemid vector and then forwarded for sequencing. The resulting sequences were analyzed using Snap gene (version 7.1.0) software. The antitetanus Scfv sequence data have submitted to the GenBank database, and the nucleotide sequence was compared with databases using BLAST, with the statistical significance of matches estimated.

Cloning of ScFv antibody gene into pET21a (+) expression vector

PCR-amplicon of ScFv antibody genes were cloned into pET21a (+) vector between NotI and NcoI (Sigma) sites and transformed into *E. coli* strain BL21(DE3) (Novagene). Transformed cells were cultured on LB agar plates containing 80 mg/ml ampicillin (Sigma) and incubated overnight at 37 °C. The colonies were picked and cultured in LB medium and then extracted by bioflux plasmid extraction kit. Finally, the accuracy of cloned genes in the recombinant plasmid of pET21a (+) - Scfv was checked and confirmed by restriction endonuclease digestion and colony PCR.

Expression, purification, and screening of Scfv antibody

In the antibody expression step, the positive clones were transferred into 5 ml of LB broth and incubated overnight at 37 °C with shaking at 200 RPM. About, 500 μl of this culture was added to 15 ml LB broth and incubated at 37 °C with shaking at 250 RPM until the A600 reached .6, afterward, 14 μl IPTG (1 mM) added, and the culture incubated overnight at 37 °C. Later, centrifugation was performed at 7,000 RPM for 10 min. The bacterial pellet resuspended in ice-cold TES (.2 M Tris hydrochloride, .5 mM ethylenediaminetetraacetic acid, .5 M sucrose) at the rate of 2% of initial culture volume. After that, the mixture was incubated on ice for 45 min to induce osmotic shock. The resulting mixture was centrifuged at 10,000 RPM for 10 min, the supernatant containing the soluble antibodies stored at −20 °C. Anti-tetanus ScFv antibody was resolved on a 12% SDS-PAGE gel and stained with Coomassie brilliant blue R-250. Antibodies resolved by SDS-PAGE blotted on a polyvinylidene difluoride (PVDF) membrane. After that, the membrane was blocked with 5% MPBS, anti-tetanus ScFv was detected by incubating the blot with an anti-His-tag/HRP conjugated antibody. The blotting developed using diaminobenzidine DAB-H_2O_2 solution. Antibodies purified by nickel sepharose affinity chromatography. The total protein concentration was estimated using the Bradford method.[13]

Determination of binding assay and affinity for antitetanus ScFv antibody

The tetanus toxin binding assay for the antitetanus ScFv antibodies was performed by the ELISA method. ELISA plate wells coated with 500 ng tetanus toxin in bicarbonate buffer (pH 9.6) by an antigen-binding activity test. After blocking with 5% MPBS, different concentrations (0, 1, 2, 3, 4, 5 μg/mL) of antitetanus ScFv antibodies were incubated for 1 hour at room temperature. Bound tetanus-ScFv was detected with anti-E-tag HRP-conjugated antibodies, and affinity was determined by the method described by Beatty et al.[14]

ScFv SPR kinetic measurements

The affinity of AC8 bound TeNT was measured by surface plasmon resonance (SPR) spectroscopy instrument (Metrohm Autolab, Utrecht, the Netherlands). Briefly, a monolayer of substrate assembled on sensor chip, activated by the manufacturer's instruction. TeNT (25 μg/mL) fixed on the sensor surface by the instruction. To evaluate the antibody affinity, different concentrations of AC8 nanobody (20, 40, 60 ng/mL) encountered with immobilized TeNT. The next steps were performed according to Aghamollaei et al. (2021).[15] To gain the kinetic parameters, data were fitted to simple 1:1 Langmuir interaction model using "kinetic evaluation software 5.4" (Kinetic Evaluation Instruments BV, Leusden, the Netherlands).[15]

Results

Phagemid library construction

A phagemid antibody library on the order of 8.7×10^7 PFU/ml in size was obtained by cloning the ScFv genes in the pSEX81 phagemid vector. By cloning the ScFv genes in the pSEX81 phagemid vector, a phagemid antibody library with a density of 8.7×10^7 PFU/ml was created. The phage titer and library size calculated as follows:

$$\textit{Phage titration} = \frac{\left(\textit{Plaque number (pfu)} \times \textit{Dilution factor } (\mu l/ml)\right)}{\textit{Volume of phage added to bacteria culture } (v) \times \textit{Phage dilution}(D)}$$

$$\textit{Library size} = \frac{\textit{Total volume of medium (ml)} \times \textit{Plaque number (pfu)}}{\textit{Volume of culture (ml)}}$$

Screening of biopanning progress by phage ELISA

Four rounds of biopanning enriched the phages expressing tetanus-specific Scfv. Following each round of panning, polyclonal phage ELISA was performed (Fig. 1). After four rounds of biopanning, 25 clones were randomly selected for binding with tetanus toxin by monoclonal phage ELISA (Fig. 2). Clone number 8 (AC8), as shown in Fig. 2, exhibited the highest affinity for tetanus toxin and therefore were selected for expression and further analysis.

Analysis of antitetanus Scfv antibody sequence

Amino acid sequences of the Scfv antibody (AC8) were aligned with human genomes. Analysis of the amino acid sequences

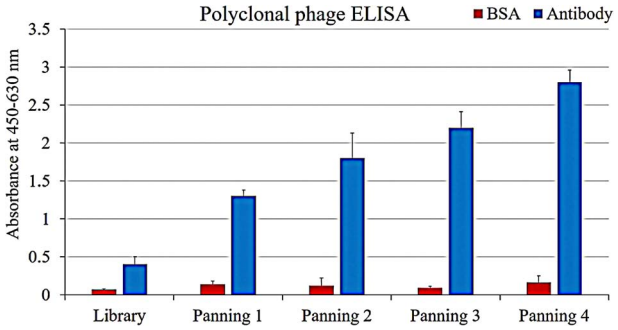

Fig. 1. Polyclonal phage ELISA. The results were obtained from the polyclonal phage after every panning step.

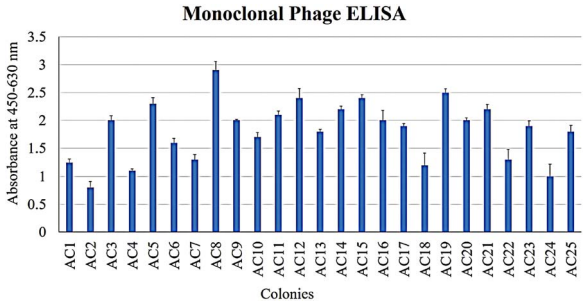

Fig. 2. Monoclonal phage ELISA. Twenty-five clones were selected from the fourth panning and used in monoclonal phage ELISA. Results indicate that antitetanus Scfv can recognize the tetanus toxin, and AC8 had possessed the highest affinity for tetanus toxin.

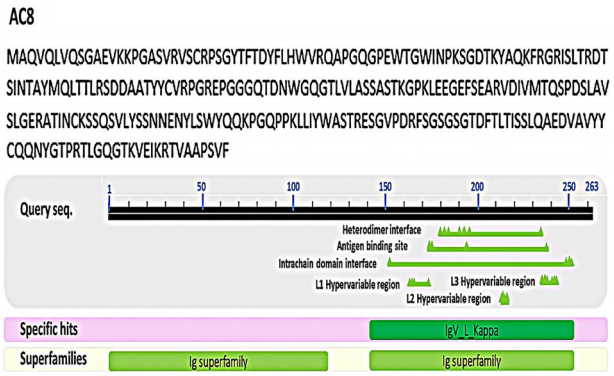

Fig. 3. Amino acid sequence analysis. Amino acid sequences of variable regions of AC8 ScFv aligned with the GenBank databases. The NCBI accession number for the AC8 ScFv sequence is MG725617.

using NCBI Blast revealed that the variable region of AC8, 97.81%, identical to the human immunoglobulin genes (Fig. 3).

Expression of soluble antitetanus ScFv antibody and specificity detection

The antitetanus ScFv antibody was expressed in soluble form in AC8-infected XL1-Blue cells after induction with IPTG. A protein band at ~32 kDa, corresponding to the expected size of anti-tetanus ScFv, was detected by SDS-PAGE (Fig. 4). Antibody bands with a molecular weight of 32 kDa were present in the XL1-Blue cells with high purity of about 90%. The production yield was 45 μg/ml. The total protein concentration was estimated using the Bradford method. The expression of an anti-tetanus ScFv confirmed by western blotting using an anti-His-tag/HRP conjugated antibody and revealing band at 32 kDa (Fig. 5).

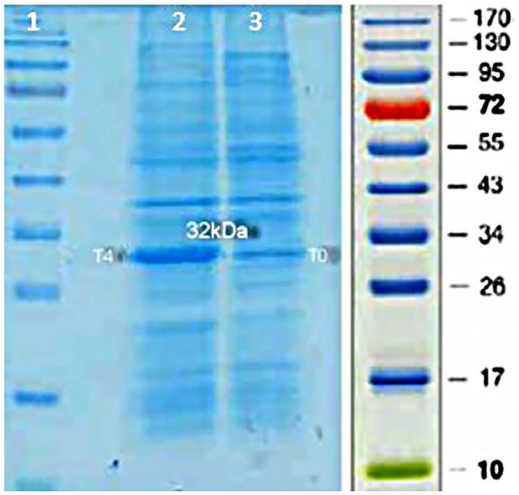

Fig. 4. SDS-PAGE results, showing expression of antitetanus antibody in a soluble form. Lane 1: molecular weight markers. Lane 2: overnight incubation after induced culture of AC8-infected BL21 cells with IPTG (T4). Lane 3: 1-hour incubation after induced culture of AC8-infected BL21 cells with IPTG (T0).

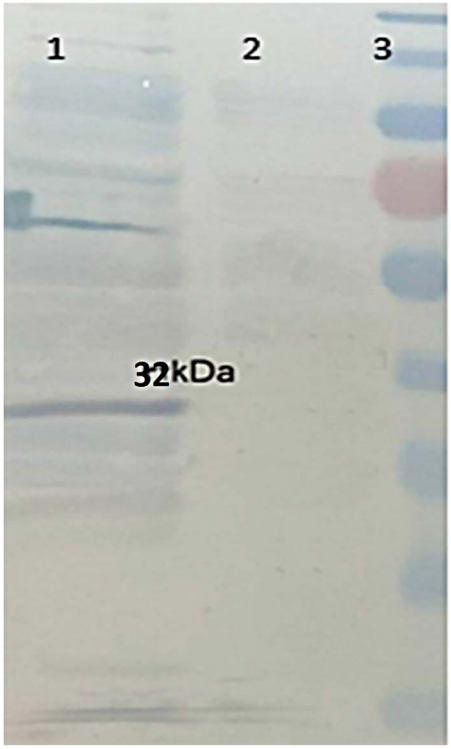

Fig. 5. Western blot results confirmed expression of ScFv antibody. Lane 1: induced culture of AC8-infected BL21 cells, showing the antitetanus antibody band at 32 kDa (overnight induction). Lane 2: uninduced culture of AC8-infected BL21 cells. Lane 3: molecular weight markers.

Tetanus binding activity and affinity determination of antitetanus ScFv antibody

The tetanus binding assay of different dilutions of antitetanus ScFv was studied by ELISA. It was observed that with an increase or a decrease in the concentration of antitetanus ScFv, there was an increase or a decrease in binding with tetanus as revealed by OD values. The affinity constant measured by ELISA revealed around 10^{-8} M. Drawing the binding curve and estimating the affinity of the ScFv antibody were performed using the Beaty et al.

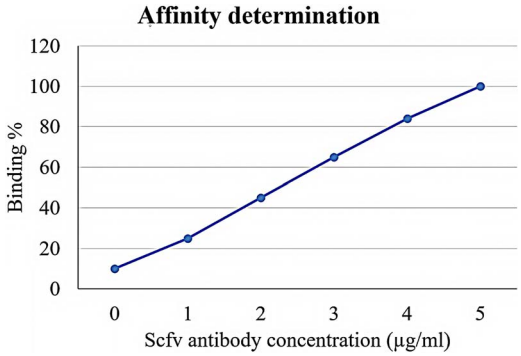

Fig. 6. ScFv-binding activity against tetanus toxin. ELISA was done with different concentrations of soluble ScFv (0, 1, 2, 3, 4, 5 μg/mL), and the binding curve was drawn using the method of Beaty et al.

method (Fig. 6), and Beatty's formula, repeatedly[14]: $\frac{[Ag]}{[Ag']} = n\,Ka = \frac{(n-1)}{2([n[Ab]-[Ab']])}$

[Ag] and [Ag´] refer to the concentrations of tetanus toxin. [Ab] and [Ab´] refer to the concentrations of ScFv antibody at half of the maximum OD of the Ag and Ag curves, respectively.

Affinity determination

Evaluation of the AC8 nanobody affinity to TeNT performed by SPR. Three various concentrations (20, 40, and 60 ng/mL) of nanobody interacted with TeNT. SPR sensor response and Langmuir plot, the computed affinity of AC8 equaled 2.1×10^{-7} M (Fig. 7). The findings represent the high interaction affinity of the selected nanobody (AC8) to TeNT.

Discussion

Tetanus is associated with a high mortality rate of 13.2% of cases overall, 5–7% of all neonatal, and 5% of maternal deaths globally.[16] Tetanus typically occurs via small puncture wounds contaminated with *C. tetani* spores, which germinate and produce toxins. Therapeutic applications may be possible by developing humanized antibodies in vitro, bypassing animal use. Recent attention has been drawn to antibody-based therapeutics, while interest in gene-based therapeutics has decreased. Creating and administering antibody-based therapies is now easier and more cost-effective. Various technologies, including phage display antibody technology, have been applied to develop antibody-containing drugs. As antibody engineering has advanced, human antibody sources have become a valuable tool for developing high-quality and high-binding antibodies. Diverse Naïve human libraries are ideal for screening for diseases and identifying antibody fragments targeting different antigens.

Phage display, a dual-purpose platform technology, can detect and neutralize toxins better, lessening healthcare expenditures. They are effective for detecting toxins because they can naturally identify selective proteins for further optimization. With phage display technology, toxins' detection and neutralization could be more precise, reliable, and comprehensive. Toxicology research will continue to be facilitated by phage display due to its near-unlimited design potential.[17]

Today, antibodies are widely employed to diagnose various toxins immunologically, but they also have limitations. This study developed a recombinant scFv antibody library to detect and neutralize Tetanus toxin and may offer an alternative to serum sickness-driving administration.[18] Recombinant antibodies have several advantages over traditional antibodies, including the lack of side effects of animal antibodies, consistent and fast production, highly specific, scalable, and customizable, in addition to their ability to penetrate tissues allows them to easily access toxins. Recombinant antibodies have been preferred due to the antiserum's limited resources, high production costs, potential risks of hypersensitivity reactions to antiserum, and potential risk of contamination of blood-derived products. Some novel recognition elements, including aptamers and short peptides, can overcome conventional antibody disadvantages, e.g. stability and production issues.[19]

Tetanus is a lethal disease, which causes a high mortality rate between 40 and 78%, generally due to respiratory failure. Most cases of tetanus result from a tiny puncture wound or rupturing, which becomes contaminated with *C. tetani* spores that germinate and produce toxins leading to the impaired muscles' function.[20] Because of the shortcomings of previous methods, the development of immunodiagnostic reagents for the successful detection of tetanus is crucial. Any immunological tetanus detection method requires specific and sensitive antibodies. Antibody phage display technology is one of the most considerable successes in antibody engineering.[21] The V_H and V_L gene repertoires are amplified and linked together by PCR before being placed into a phagemid vector. Generated phage showing the required antibody fragment, termed ScFv, on its surface after transformation into *E. coli*. Then, biopanning and phage ELISA methods were used to enrich and select the recombinant phage.[22,23]

In phage display technology, fragments of recombinant monoclonal antibodies are displayed on bacterial surfaces using bacteriophages that infect bacteria. These fragments are obtained from a library of antibodies against every imaginable molecule and then screened for their ability to bind to targets. Further engineering and modification of selected antibody fragments are possible for the required end application.[18] Here, we also used phage display technology to produced recombinant antibodies.

Phage display technology can discover novel human monoclonal antibodies against various target molecules. Phage display technology has enabled the cloning of high-affinity human monoclonal antibodies using libraries of antibodies such as Fabs and scFvs constructed from human, rabbit, mouse, and chicken cells.[18] We constructed a high-quality phage display antibody library, and retrieved from tetanus-specific antibodies with highest affinity.

Phage display technology has been used in several studies to produce recombinant antitetanus antibodies.[23] Researchers synthesized recombinant human antibodies against tetanus toxin using phage displays.[5] Investigators also used phage display technology to select antigen binding fragments (Fabs) against a Tetanus toxoid.[24] Some others developed recombinant antibodies by phage display to diagnose and treat infectious diseases and toxins.[18] As part of this project, we also produced recombinant antibodies for use in the management of tetanus.

In the current study, we report the construction of a highly reactive and specific ScFv antibody fragment from a phage display library suitable for tetanus detection. After amplifying the V_H and V_L fragments by PCR, they joined together and cloned into pSEX81 phagemid vectors. Ultimately, large repertoires of antibody libraries (8.7×10^7 PFU/ml) were obtained. The size of the antibody libraries depends on the transformation efficiency, which is the major limitation of antibody phage display technology. The screening of the phage antibody library demonstrated the expression of antitetanus ScFv on the phage surface. The pIII

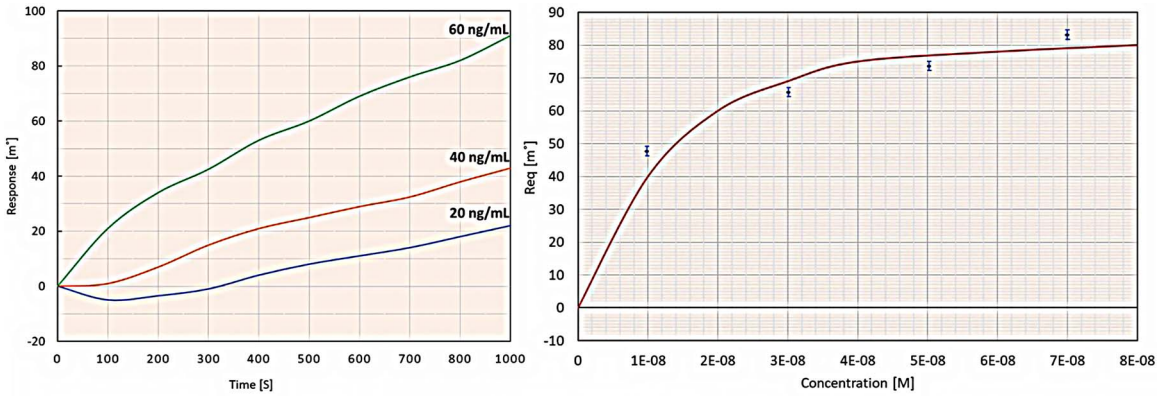

Fig. 7. Affinity of AC8 ScFv to TeNT by SPR spectroscopy. a. SPR sensor response overlay plot for the interaction of different concentrations (20, 40, 60 ng/ml) of AC8 with 25 ng/mL immobilized TeNT. b. Langmuir plot of equilibrium angle (Req) versus TeNT concentration.

protein-fused upstream of antitetanus ScFv facilitates its transportation onto the phage surface. It helps in the selection of ScFv antibodies reactive against the tetanus toxin antigens. After four rounds of biopanning, the clone AC8, showing maximum binding with tetanus, was selected for further studies. In this study, soluble antitetanus ScFv (32 kDa) was successfully expressed in the *E. coli* strain BL21. The nucleic acid and amino acid sequence analysis of the antibody heavy and light chains indicates that it belongs to the human antibody superfamily with high homology (97.81%). The affinity of the antitetanus ScFv antibody was very high (10^{-8} M). ELISA and SPR showed the AC8 antibody had high specificity and affinity to the TeNT. It concluded that tetanus antigen and its corresponding antitetanus ScFv antibody interact with high affinity.

Reports on the construction of recombinant diagnostic antibodies for the detection of toxins are scanty.[25] In a study by Neelakantam et al., a specific Fab antibody fragment was constructed from a phage display library that binding with tetanus toxin.[23] In other studies, researchers have constructed ScFv antibodies against different toxins and pathogens.[26]

Several sensitive and specific clinical tests are based on immunochemical reactions, known as immunoassay reactions. These reactions are basically mediated by antigens and antibodies interacting and forming Ag–Ab complexes. The complex is caused by weak interactions (such as hydrogen bonds, electrostatic interactions, hydrophobic interactions, and van der Waals interactions). The types of immunoassay techniques can be classified as qualitative, semi-quantitative, and quantitative.[27] Immunoassays can show macroscopical and visual evidence of the reaction between antigen and antibody (such as by agglutination and immunoprecipitation methods). Many early immunoassays used polyclonal antisera from immunized rabbits, providing relatively good immune responses despite limited antigens. As monoclonal antibodies became available in 1975, this method changed.[28] In spite of the fact that antibody-based immunoassays are widely used in laboratory diagnostics, they are too expensive.

It has been suggested by the search results that ScFv-based immunoassays offer advantages over current immunoassays. These immunoassays have been specifically developed for toxins detection and various applications. They also exhibit high specificity and affinity for their target antigens, making them a desirable immunoassay option.[29] A previous study developed a competitive indirect ELISA to detect IFN-γ with a high-affinity ScFv antibody. This immunoassay could determine and quantify IFN-antigens in real samples.[29] This study was the first to establish an immunoassay based on ScFv antibodies for IFN-γ detection. Another study used an anti-immune complex ScFv antibody fragment to detect HT-2 toxin in a non-competitive ELISA. Simple and specific, the assay provided a positive result. Small biomarkers were detected using a clonable ScFv antibody fragment.[30] In another study, ScFv antibodies with high specificities and affinity were used in a fluorescence polarization immunoassay (FPIA) to detect FB1 and FB2. FPIA predicted the differences between ScFv antibodies and FBs, improving understanding of immunoassay results.[30] Hence, ScFv-based immunoassays have shown promise for toxin detection, with high specificity and affinity for their target antigens. Unlike current immunoassays, ScFv antibodies can be produced in vitro without animal immunization, which makes them more ethical and sustainable.

Compared to current immunoassays, ScFv-based immunoassays have several advantages. Firstly, since ScFvs are smaller and easier to make, they are cheaper to produce. Secondly, monoclonal antibodies (mAbs) are generally expressed by mammalian cells, while ScFvs can be expressed in bacteria.[31] Thirdly, phage-displayed ScFvs can be used in clinical applications as they are more stable than mAbs.[22] Fourthly, ScFv-based immunoassays do not require animal immunity, so they are more ethical.[29] Additionally, the ScFv-based immunoassays can be engineered differently for better immobilization on the transducer's surface.[31] Furthermore, there is potential for ScFv antibodies to function as immunotoxins if they are designed as bispecific antibodies.[22] Finally, ScFvs can neutralize toxins, viruses, diseases-mediating cytokines, and growth factors.[32]

Various clinical applications of ScFv-based therapies include medical diagnosis, cancer therapy, and infectious disease treatment. A wide range of antigens, such as haptens, proteins, carbohydrates, receptors, tumor antigens, and viruses, can be targeted with ScFvs. The ScFvs penetrate tumors and other tissues more rapidly and evenly. Also, ScFvs can be delivered via viral vectors.[33]

On the other hand, ScFv-based immunoassays have some drawbacks that limit their therapeutic potential. Firstly, the small size of ScFv antibodies often results in lower affinity, lower stability, and a higher likelihood of aggregation. Secondly, ScFv antibodies' rapid clearance can also be a drawback for therapeutic uses. Thirdly, the soluble expression of a functional ScFv antibody is still a bottleneck problem.[34] Fourthly, ScFvs may elicit an immune response, restricting their clinical use. Finally, ScFvs may lack specificity compared to viral vectors.[22]

Conclusion

It can be concluded that the proposed antibody-based detection system is an alternative way to enable rapid, low-cost, and highly specific detection of tetanus toxin and could be easily applied for the detection of any other proteins.

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Conflict of interest statement: None declared.

Data availability

The datasets used and (or) analyzed during the current study are available from the corresponding author upon reasonable request.

Informed consent

For this type of study, formal consent is not required. All of the authors had the same contribution in the article and agreed to submit manuscript.

Ethical approval

Neither ethical approval nor informed consent was applicable for this study.

Authors' contribution

Conceptualization: Rouhani Nejad, and Fallah; Methodology: Rouhani Nejad, Zanganeh and Fallah; Data curation: Rouhani Nejad, Saeedi and Fallah; Data interpretation: Rouhani Nejad, Saeedi and Fallah; Resources: Rouhani Nejad, Zanganeh, Saeedi and Fallah; Supervision: Rouhani Nejad and Fallah; Project administration: Rouhani Nejad and Fallah; All authors reviewed and approved the manuscript.

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