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Specific Single Chain Antibodies (Scfvs) Against Influenza A Virus Using Phage Display Technique

Research Article

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Abstract

Introduction: 2009 H1N1 swine-origin Influenza A virus with human pandemic potential emerged in 2009 and caused a number of mortality. Two highly conserved regions 173-181 and 227-239 has recognized in Hemagglutinin of H1N1 Influenza virus. Immunity to the different subtypes of Influenza virus could obtain with high affinity antibodies against conserved epitopes of the virus. In the present study, a phage antibody display library of scFv was applied for the selection of scFv antibodies against the conserved sequences of the hemagglutinin.

Materials and Methods: Panning process was used to isolate specific scFvs against highly conserved neutralizing epitopes of Hemagglutinin. PCR and fingerprinting were done on the selected clones. Phage ELISA was performed to evaluate the specificity of the clones selected against the epitopes.

Results: Two common patterns gainst each peptide were obtained which represented the isolation of two specific scFvs, scFv-I and svFv-II with the frequencies of 25% (5/20) and 55% (11/20) against peptide 1 and scFv-I' and scFv- II' with the frequencies of 20% (4/20) and 75% (15/20) against peptide 2. Phage ELISA showed the reactivity of the scFv antibodies with the related epitopes while no reaction with unrelated epitopes and other negative controls were obtained.

Conclusion: Results demonstrated the isolation of two specific scFvs against two conserved neutralizing sequences of Hemagglutinin of Influenza virus A. Due to several advantages of scFv, these antibodies are introduced as valuable agents in clinics. Results suggest further evaluation of the specific scFvs against Influenza A virus for their use in clinical applications.

Introduction

Influenza infection in humans is one of the most important respiratory diseases in all over the world. The infection could be fetal specially in children [1]. Influenza virus A, is responsible for prevalence of pandemics and the most of the yearly flu epidemics [2]. This type is further categorized into the subtypes according to the properties of Hemagglutinin (HA) and neuraminidase (NA) antigens on the surface of viral particle [3]. The unexpected variability of the Influenza A virus makes annual epidemics in population and is the principal reason of the lack of impressive prevention against Influenza infection to date [4].

In April of 2009, a swine-origin influenza H1N1 virus (S-OIV) has appeared. 2009 pandemic H1N1 influenza A virus is a reassortant strain of the influenza virus containing gene segments from swine, avian and human [5]. 2009 pandemic H1N1 influenza A virus is antigenically similar to the 1918 pandemic H1N1 Influenza A virus [6]. Until 2013, pandemic H1N1 has led to more than 1.4 million infections with about 25,000 deaths worldwide [7]. The most of the serious consequences caused by influenza infection occurred in individuals with underlying medical conditions. The highest mortality rates were found in immunocompromised individuals, patients with chronic neurological disease and respiratory disease [8]. Pregnant women were also under threat [9]. Due to Ser31Asn substitution in the transmembrane Matrix 2 protein, the 2009 pandemic H1N1 Influenza A virus is resistant to Amantadine antiviral drug. 2009 pandemic H1N1 Influenza A virus is sensitive to Oseltamivir and Zanamivir antiviral drugs [10,11].

Hemagglutinin is the main antigen responsible for the attachment of virus to the specific receptors on the surface of host

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cells, providing entrance into the host cell via endocytosis. It defines the virulence, host ranges and transitivity of influenza viruses. The precursor of hemagglutinin (HA0) is synthesized in the infected host cells and undergoes a enzymatic cleavage to obtain two subunits, HA1 and HA2 [12]. HA exhibit conserved and variable domain [13]. Hu et al [14] reported seven conserved epitopes of HA1 of 2009 pandemic H1N1 Influenza A virus containing 5-23, 63-81, 142-158, 178-195, 201-216, 246-269, and 277-287, which are responsible for attachment of the virus to the swine cells. According to the previous researches conducted by Kubota-Koketsu et al [15] and Yamashita [16], two highly conserved epitopes 173-181 and 227-239 were reported which antibodies against these epitopes could neutralize the different subtypes of influenza virus. This glycoprotein also has a principal role in the emergence of human pandemic influenza viruses [17]. The HA protein is the main antigenic stimulator of the acquired immunity [18] and is the major target for virus-neutralizing antibodies [19]. The prominent immune response against influenza HA is presumed to be conducted to the head of the glycoprotein (amino acids 52-277) particularly against determined antigenic zones that enclose the receptor binding site [20]. It is well documented that antibodies against HA in the circulation protect against intensive viral pneumonia following the secretion of antibodies from the blood into the lungs of mice [21,22].

Because of the production of vaccines and new anti-viral drugs are time-consuming, passive immunization by neutralizing antibodies provides urgent treatment for influenza pandemic emergency, particularly, for acutely exposed individuals [23]. Neutralizing monoclonal antibodies successfully applied for therapy and prophylaxis of viral infection [7,24]. Due to HAMA (human anti-mouse immunoglobulin antibody) responses induced by monoclonal antibodies, limited protections are reported [25,26]. Single chain fragment variable are small fragments (~25-30 kDa) comprised of variable heavy (VH) and light chain (VL) of antibody, which are joined with a flexible peptide linker contains 15-20 amino acids [27]. Human scFv antibodies have advantageous properties including: human origin, low molecular weight, higher tissue penetration, lower retention times in non desired tissues, better clearance of the immunocomplexes, preservation the binding specificity of the intact monoclonal antibody and minimally immunogenic. Large quantities and cost-effective production of scFv antibodies in bacteria is possible [28-30]. Many scFvs are used successfully in clinical trial and treatment [30,31]. Highly specific scFv antibodies already produced to several important pathogens, for example, against H5N1 Influenza viruses [32,33], Urease and lysate of H.pylori bacteria [34], p24 of HIV-1 [35], thermolabile hemolysin (TLH) of Vibrio parahemolyticus [36], Varicella-zoster virus [37] and Human Papillomavirus type 16 [38].

In the present study, a phage antibody display library of single chain variable fragments (scFv) was utilized for isolation scFv antibodies against the conserved sequence of the hemagglutinin of 2009 pandemic H1N1 influenza virus. The antigenic specificities of these antibodies were evaluated by enzyme-linked immunosorbent assay (phage ELISA).

Materials and Methods

scFvs selection

A phage antibody display library of scFv was developed as described previously [39]. Phage clones displaying scFv were selected from the library after four rounds of panning. Briefly, immunotubes (Nunc, Roskilde, Denmark) were coated with peptides as epitopes (amino acids 173-181 and 227-239 of hemagglutinin of influenza virus at 4°C overnight. The phage-rescued supernatant (10^{10} PFU/mL) diluted with blocking solution was added to the tubes and incubated for 1h at room temperature. The recombinant phagemid was introduced into competent E. coli TG1 cells, and rescued with helper phage M13KO7 (Amersham, Biosciences). Four rounds of panning were performed. To confirm the existence of VH-Linker-VL inserts, PCR was performed on the clones obtained after panning. DNA fingerprinting of the scfv from individual E. coli clones were determined by using MvaI restriction enzyme (Roche Diagnostic GmbH, Mannheim, Germany). The common patterns were revealed by electrophoresis. One clone with the most frequent pattern was selected against each epitope and phage-rescued for further evaluations.

Determination of phage-antibody concentration

For determination of phage-antibody concentration of each rescued phage, phage antibody supernatant was added to 1ml of log phase TG1 E. coli and incubated with shaking at 37°C for 1 h. Serial dilution of bacteria was prepared and cultured on to 2TY Agar/Ampicillin medium at 30°C overnight. Number of clones per dilution was determined and phage concentration titer per milliliter was calculated.

Evaluation of reactivity of scFvs by phage ELISA

Specificity of individual scFv was assessed by phage ELISA. The 96 well ELISA plate was coated with peptides (dilution: 100 µg/ml in PBS) at 4°C overnight. An unrelated peptide was used as a negative control. The wells were blocked with 3% skimmed milk for 2 h at 37°C. Following washing with PBS/Tween 20 and PBS, the phage-rescued supernatant of each clone containing the selected scFvs was added to the wells. M13KO7 helper phage was added to peptide coated wells as a negative antibody control. After incubation and washing, anti-fd bacteriophage antibody (Sigma, UK) was added and incubated for 1hr. Following washing, HRP-conjocated anti-Rabbit IgG (Sigma, UK) antibody was added and left at room temperature for 1 h. 100µl of TMB solution was added to each well and the reaction was stopped with 100 µl of 1 M Sulfuric acid. The optical density of each well was detected at 450 nm by ELISA reader (BP-800, Biohit, USA).

Statistical analysis

Mann- Whitney test was used to compare the mean ratio of the phage ELISA results of phage display scFvs and of the control (no peptide).

Results

Anti- hemagglutinin of 2009 pandemic H1N1 influenza A virus scFvs

Figure 1 shows DNA-Fingerprinting of 20 panned clones against peptide 1(A) and peptide 2(B). Two common patterns obtained against each peptide, scFv-I and svFv-II with the frequencies of 25% (5/20) and 55% (11/20) against peptide 1 and scFv -I' and scFv II' with the frequencies of 20% (4/20) and 75% (15/20) against peptide

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2. The common patterns are shown by common symbol in Figure 1. Lanes (1, 5, 8, 9, 17) and lanes (2, 3, 6, 7, 10, 11, 12, 13, 16, 19, 20) show the common patterns of scFv-I and scFv-II against peptide 1, respectively. Lanes (3, 8, 9, 17) and lanes (1, 2, 4, 5, 6, 7, 10, 12, 13, 14, 15, 16, 18, 19, 20) represent common patterns of scFv-I' and scFv-II' which were selected against peptide 2, respectively.

Phage ELISA

The binding specificity of the selected scFvs to the related peptides was measured by phage ELISA assay. All of four isolated scFvs (scFvI,

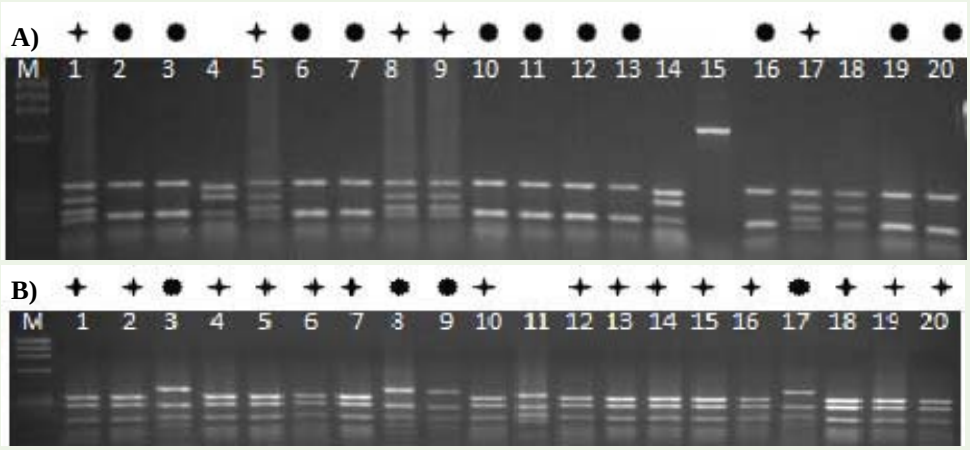

Figure 1: DNA fingerprinting of 20 panned clones (lanes 1-20) of peptide 1 (A) and peptide 2 (B). M: Marker.

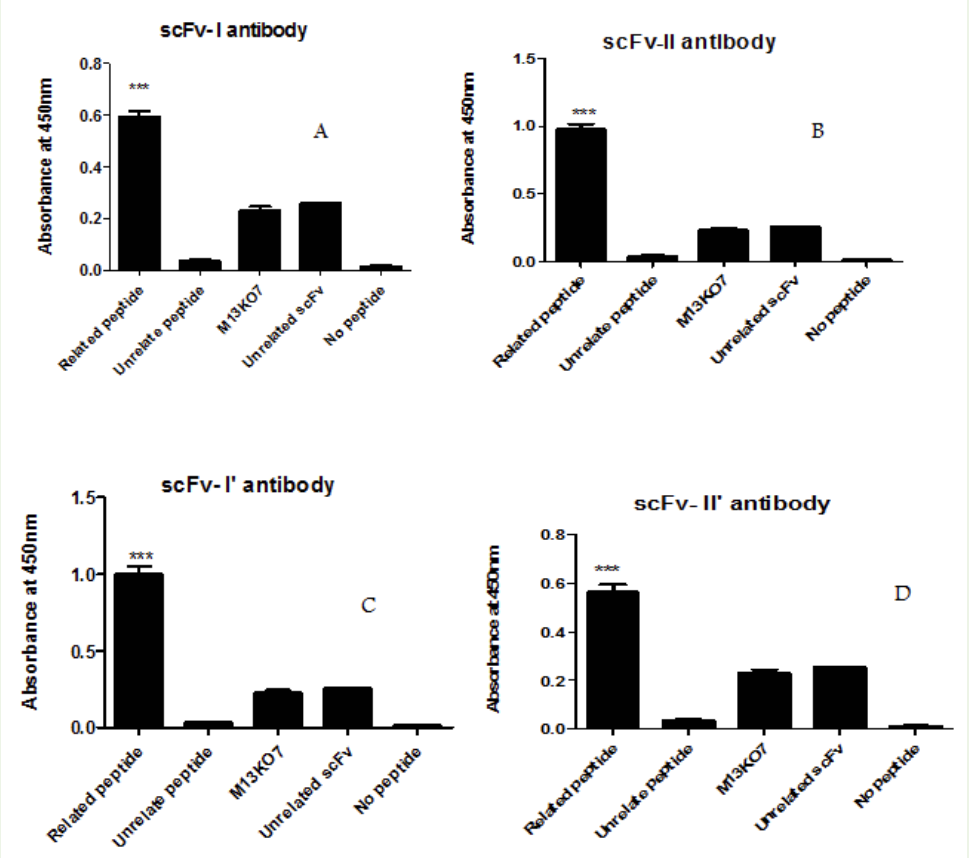

Figure 2: Phage ELISA results of scFv-I (A), scFv-II (B) against peptide 1 and scFv-I' (C) and scFv-II' antibody (D) against peptide 2. All of the four antibodies bound to the related peptides significantly higher than the wells with no peptide (*P value<0.05*). No reactivity was detected for unrelated peptide, unrelated scFv (scFv against IL25R) and M13KO7 helper phage with the peptides.

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scFvII, scFvI', scFvII') were found to bind with their related peptides (Figure 2). The absorbance obtained for reaction of scFv antibodies with related peptide were significantly higher than the wells with no peptide (P value<0.05). The M13KO7 helper phage showed no reactivity to the peptides.

Discussion

One of the major causes of the global mortality is Influenza infection and the infection remains a consistent health problem. No impressive prevention against Influenza infection has been identified till now. Immunotherapy with antibodies is a helpful tool in the treatment of infectious disease. Immunothreapy with neutralizing antibodies has been utilized against various viral infections including protection against Respiratory syncytial virus in cotton rats [40], prophylactic effect in experimentally infected chimpanzees against Hepatitis C virus [41], protection in mice and Guinea pigs inoculated with the rabies virus [42] and immunity against influenza viruses [43,44]. A strategy for defense against various viral infections is the production of antibodies to protective epitopes. Detection of these epitopes is the main step in the development of effective antibodies. Between the 11 proteins that are encoded by gene segments of influenza A virus, hemagglutinin is the major target of neutralizing antibodies as it is responsible for the attachment of the virus to the host cells and determines the virulence of influenza virus. Conserved regions of hemagglutinin of influenza virus are principal targets for immunotherapy against the virus. Highly conserved amino acids 173-181 and 227-239 were recognized in hemagglutinin of 2009 pandemic H1N1 influenza A virus [15,16,45]. These epitopes have been identified via B-1 and D-1 human monoclonal antibodies, respectively [16]. Considering the advantages of the scFv antibodies over intact antibodies, in the present study this format of antibody was used for targeting of these epitopes. We chose peptide EGRMNYYWTLVEP (amino acids resides 227-239) of hemagglutinin fragments GKEVLVLWG (amino acides residues 173-181) and H1N1 Influenza A virus as immunodominant epitopes. Hemagglutinin fragments GKEVLVLWG (amino acids residues 173-181) and H1N1 Influenza A virus as immunodominant epitopes. Maneewatch et al. [33] isolated HuscFv antibodies against hemagglutinin of H5N1 influenza virus. They reported that two of the selected HuscFvs had the highest neutralization effect against different strain of the H5N1 virus in MDCK culture and one HuscFv could rescue C57BL/6 mice that were infected with H5N1 influenza virus intranasally. In the previous studies, the selection of scFv antibodies against the related peptide was done by panning procedure. A number of specific scFvs have been selected against key antigens using panning process [36,46,47]. In this study we isolated four scFv antibodies (scFv-I, scFv-II, scFv-I', scFv-II') against peptide 1 and peptide 2 of hemagglutinin of influenza virus. The binding specificity of scFv antibodies to their related peptides were measured by phage ELISA Assay. Results demonstrated that the selected scFvs against peptide 1 and 2 specifically bound to the corresponding peptides compared to controls (Figure 2). The mean absorbance of scFv-I, scFv-II and scFv-I', scFv-II' in reaction with the corresponding peptides were significantly higher than the wells with no peptide, unrelated scFv, unrelated peptide and M13KO7 ($P.value < 0.05$). This represented the specificities of the four selected scFvs for the related peptides.

Immunotherapy via neutralizing antibodies against all species of influenza virus might be an ideal alternative for individuals who are at high risk. This group includes: newborns, the old people and the Immunodeficient patients who active vaccination probably does not help them. Neutralizing scFvs against hemagglutinin of influenza virus would yield a new tool for therapy of influenza infection since HA has a principal role in the advent of human pandemic influenza viruses. The well-known properties of scFv antibodies, specially human origin and wide penetration to the targets have made these antibodies more applicable in immunotherapy. The high affinity specific scFv antibodies against highly conserved neutralizing sequences of hemagglutinin of influenza were isolated in this study suggest further evaluation of these antibodies to select high neutralizing scFvs to prevent against influenza infection in high risk individuals.

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