

Isolation of Specific Human Recombinant Antibodies Against Glycoprotein 41 of HIV

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Abstract

Background: Blocking of gp41 of HIV virus, which is involved in the virus entry has been introduced as an effective strategy against HIV infection. In this study we used phage display technology to select specific single chain antibody (scFv) against gp41 HIV for its application in clinical use.

Methods: Single chain antibodies against an epitope located in C-terminal part of gp41 were selected using the panning process which enriched a phage antibody display library of scFv. Following panning, 20 clones were amplified by PCR and fingerprinted. To test the specificity of the selected antibodies phage ELISA was performed.

Results: PCR of the library clones demonstrated the presence of VH-linker-VL inserts. Fingerprinting of the clones showed a diverse library with different patterns. Fingerprinting of selected clones after panning revealed two specific single chain antibodies with frequency of 25% and 20%. These clones were preserved for further investigations. Phage ELISA results showed specificity of the two scFvs against the immunodominant epitope of gp41. The absorbance of the scFv1 and scFv2 were 0.72 and 0.63 while the absorbance of the no peptide were 0.18 and 0.12, respectively.

Conclusions: In this study we successfully selected two specific recombinant antibodies against gp41. These libraries are human antibodies with high affinity and specificity and have the potential to be used for diagnosis and treatment. Further investigations are needed to show the effects of the antibodies in vitro and in vivo.

Keywords: HIV virus, scFv, gp41 Panning, Recombinant antibody, Phage display

Introduction

Infection with human immunodeficiency virus type I (HIV-I) continues to exist as a global pandemic, placing significant burden on the healthcare system. Developing effective treatments and vaccines to prevent the spread of HIV-I infection persists as a major global and public health challenge. HIV-I targets the immune system preferentially targeting the CD4+ T-lymphocytes, which hold a critical role in the activation of the immune response. Infection with HIV-I can therefore lead to significant depletion of these critical immune cells, eventually leading to the development of acquired immunodeficiency syndrome (AIDS). AIDS can develop into a life-threatening condition due to the inability of the

immune system to successfully combat pathogens. Many individuals with HIV infected developed an influenza or mononucleosis-like illness the most common symptoms of that may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophageal sores, and may include, but less commonly, headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms. Infected persons may experience all, some, or none of these symptoms. The duration of symptoms differs, averaging 28 days and usually lasting at least a week (1). Zidovudine (AZT) was the first anti-HIV drug in 1987 for treatment of HIV infection that interferes with viral replication (1).

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Received: 13 Oct, 2018; Accepted: 18 Jun, 2019

Nowadays five different groups of anti-HIV drugs are implicated including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), integrase Inhibitors, non-nucleoside Opposite Transcriptase inhibitors (NNRTIs), and entry/fusion Inhibitors (3). Due to high mutation rate and generation of different HIV mutant strains, that are resistant to immune responses and anti-retroviral drugs, the HIV virus is changing permanently. But, currently new strategies including immunotherapy through vaccination and neutralizing antibodies have been developed as the best way for HIV prevention and therapy (4). However, some of neutralizing antibodies against viral cover proteins have demonstrated effective neutralization of different strains of the virus *in vitro* (5).

The glycoproteins of HIV envelope such as transmembrane glycoprotein 41 (gp41) and external glycoprotein 120 (gp120) play major roles in virus entrance and initiation of infection (6). These glycoproteins have conserved and neutralized epitopes that are common among different HIV strains. The presence of variable regions on envelope gp41 enables the virus to escape from immune response, but production of neutralizing antibodies against conserved epitopes causes inhibition of different strains of entry into the cells and eradicate the infection (7-9).

Novel monoclonal antibodies (mAbs) have been considered that target the gp41 nowadays. These mAb that targets the CD4 binding site on gp120 and the membrane proximal external region of gp41 (10). The majority of these neutralizing antibodies are largely strain-specific that is one of the problems for the use of them, and therefore would not create protecting reaction against globally circulating viral variants (11). Human single chain variable fragment (scFv) antibodies consist of heavy (VH) and light (VL) chain variable regions joined by a flexible peptide linker (12-14). These antibodies offer several advantages over mAbs. They can carry toxic drugs and radioactive materials to the target cells and due to their small size they have greater infiltration. The high specificity and affinity of these recombinant antibodies are additional benefits of scFvs that offer their clinical potential as effective and new agents (15-19). ScFv fragments could gain entrance to neutralizing epitopes and provide highly virus neutralization effect (9).

In this investigation we selected specific scFvs against gp41 of HIV-1 using phage display technology and evaluated the specificity and reactivity of the selected antibodies against the gp41 epitope by ELISA.

Materials and methods

Selection of specific scFv antibody against gp41

Phage rescue was done on a library of phage transformed *E. coli* scFv developed before (15) clones displaying scFv were selected from the library after four series of panning. Momentarily, immunotube (Nunc, Roskilde, Denmark) was coated with the gp41 peptide as the epitope at 4 °C overnight. The phage-rescued supernatant diluted with blocking solution (skimmed milk 2%), added to the tube and incubated for 1 h at room heat.

Following washing log phase *E. coli* were added and incubated at room temperature for 1hr with random shaking. The tube was centrifuged and the pellet was developed and rescued with helper phage M13KO7 (Amersham, Biosciences). Four rounds of panning were done to remove nonspecific scFvs and select the specific and high affinity binders.

PCR and DNA Fingerprinting of the certain clones

The existence of VH-Linker-VL inserts of the selected clones got after panning was confirmed by PCR on the clones and the single chain fragments were amplified. DNA fingerprinting of the selected clones were resolute by using MvaI restriction enzyme (Roche Diagnostic GmbH, Mannheim, Germany). The common patterns were discovered by electrophoresis. One clone with the most frequent pattern was selected against the epitope and phage-rescued for further evaluations.

Determination of phage-antibody concentration

Phage antibody supernatant was added to one ml of log phase *E. coli* and incubated with shaky at 37 °C for 1 h, ongoing dilution of bacteria was cultured on 2TY Agar/Ampicillin medium at 30 °C overnight. Numeral of colonies per dilution was determined and phage concentration titer per milliliter was calculated.

Assessment of reactivity of scFvs by phage ELISA

Specificity of the selected scFv was assessed by phage ELISA. The ELISA plate well was covered with the

peptide (dilution: 100 µg/ml in PBS) at 4 °C overnight. An unrelated peptide was used as a negative controller. The wells were coated with 2% skimmed milk for 2 h at 37 °C. The plate was washed with PBS/Tween 20 and PBS, the phage-rescued supernatant containing the selected scFvs was added to the wells. M13KO7 helper phage was used as a negative antibody control.

After incubation and washing, anti-fd bacteriophage antibody was added and incubated for 1 hr at room temperature. Following washing, HRP-conjugated anti-Rabbit IgG (Sigma, UK) was added and left at room temperature for 1 h. The plate was washed and 150 µl of the substrate (1 µl H₂O₂ with 0.5 mg/ml ABTS in citrate buffer) was added and the optical density of each well was determined at 405 nm by an ELISA reader.

Statistical analysis

To compare the mean ratio of the phage ELISA outcomes between scFvs against the peptide and of the controls (unrelated peptide, M13KO7, Unrelated scFv and no peptide), Mann-Whitney test was used.

Results

Anti-gp41 selected scFv

Figures 1 and 2 show PCR and DNA-Fingerprinting of 20 clones against gp41 peptide separately. The presence of VH-Linker-VL are shown by 950 bp PCR product (Figs. 1 and 2).

As shown in Figures 3 and 4 DNA fingerprinting of the selected clones after panning demonstrated: pattern 1, scFv1, (lanes 1, 9, 10, 12, and 14) with frequency of 25%, pattern 2, scFv2, (4, 5, 16, and 20) with frequency of 20%. Dominant pattern (pattern 1, 2) were selected as our desired samples for evaluation (Figs. 3 and 4).

Phage ELISA

Phage ELISA assess confirmed the specificity of the selected scFv to the peptide. The obtained OD presented that the scFv antibody responded with related peptide 4-5 fold higher than the wells with no peptide (Table 1). While, the M13KO7 helper phage, unrelated peptide and unrelated scFv presented no reactivity to the peptide (Figs. 5 and 6).

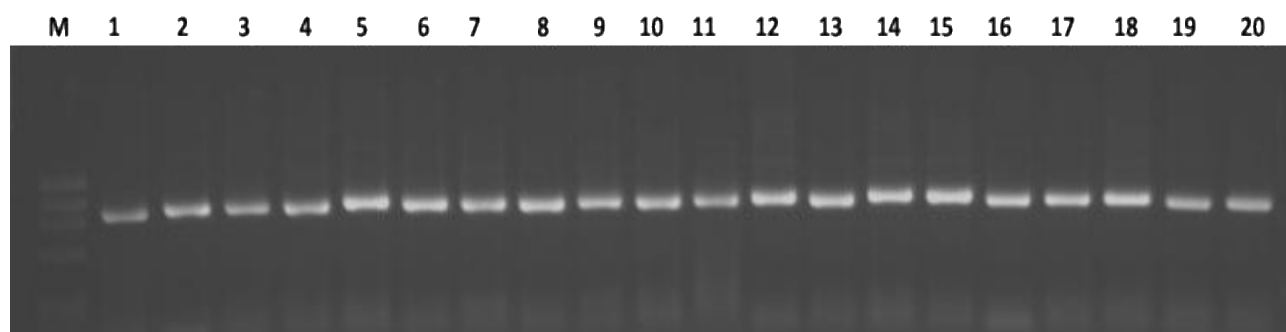


Fig. 1. PCR result of the randomly selected library clones before panning. 950 bp bound was obtained. M: X174 DNA marker.

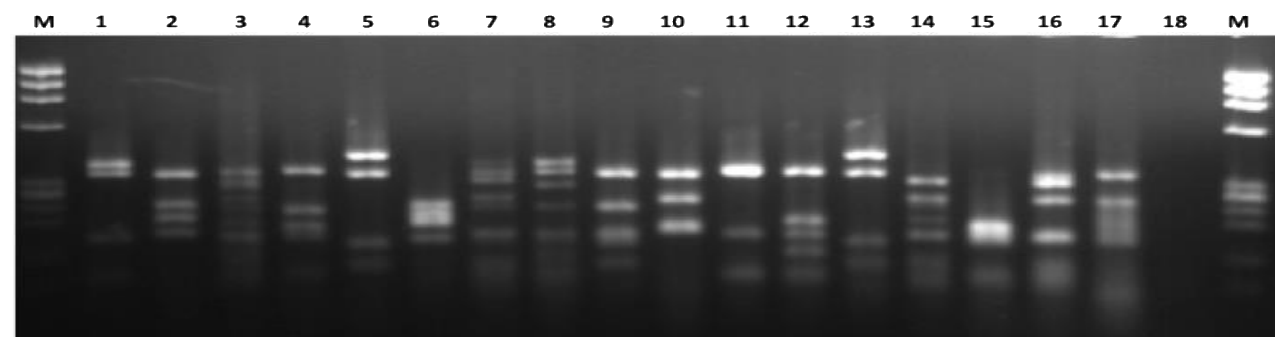


Fig. 2. DNA Fingerprinting of the randomly selected library clones before panning. M: X174 DNA marker.

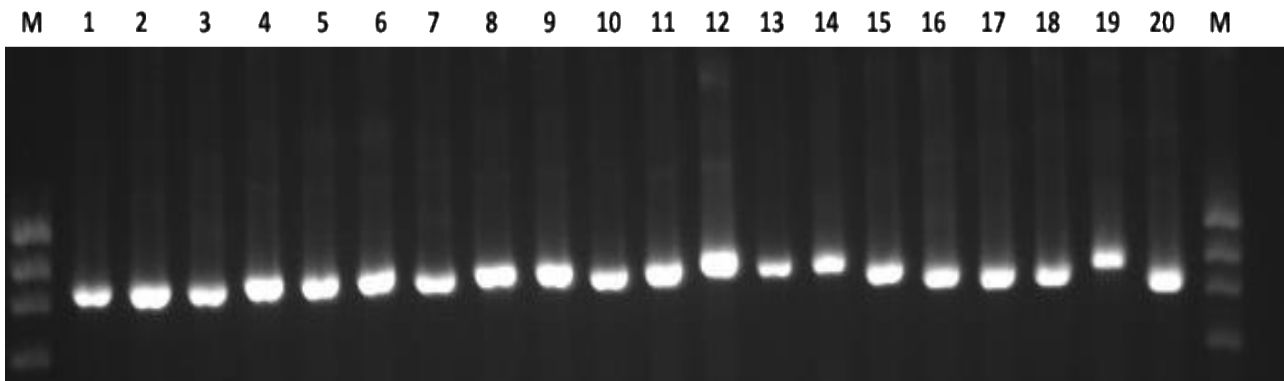


Fig. 3. PCR result of the selected clones after panning. 950 bp bound was obtained. M: X174 DNA marker.

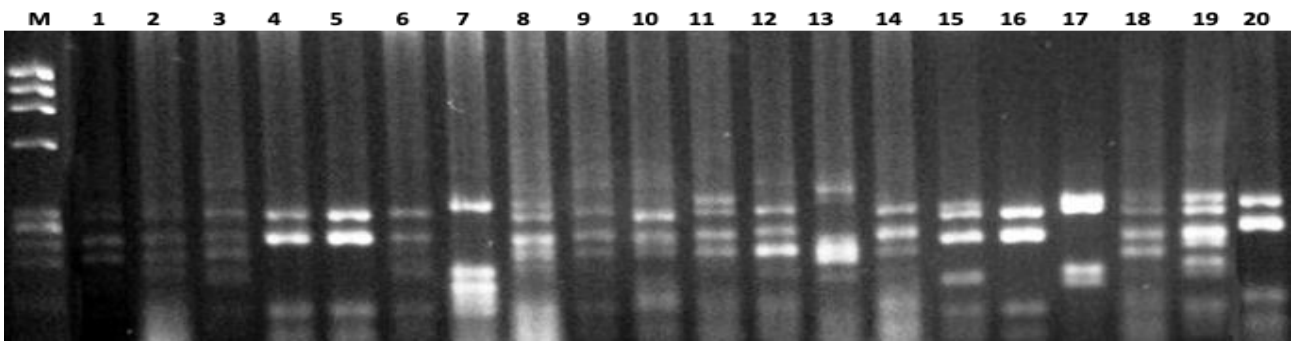


Fig. 4. DNA Fingerprinting outcomes of certain clones after panning. M: X174 DNA marker.

Table 1. Phage ELISA results of scFv1, scFv2-Absorbance at 405 nm

	scFv1	scFv2
Related Peptide	0.72	0.63
Unrelated Peptide	0.19	0.14
Unrelated scFv	0.22	0.13
No peptide	0.18	0.12
M13KO7	0.09	0.12

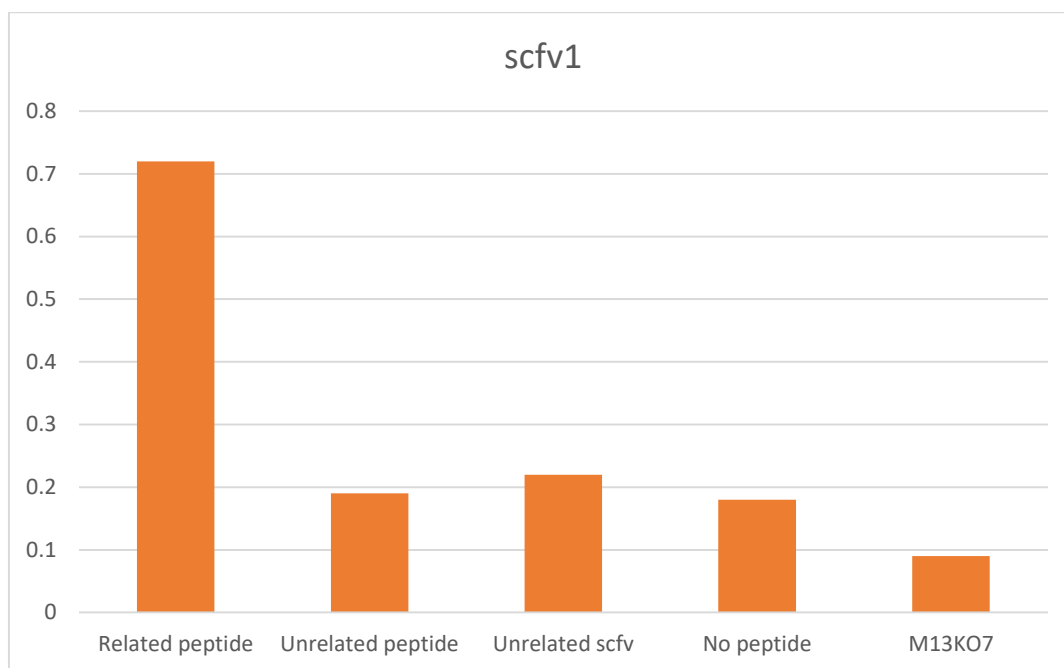


Fig. 5. Phage ELISA results of scFv1 (OD at 405 nm).

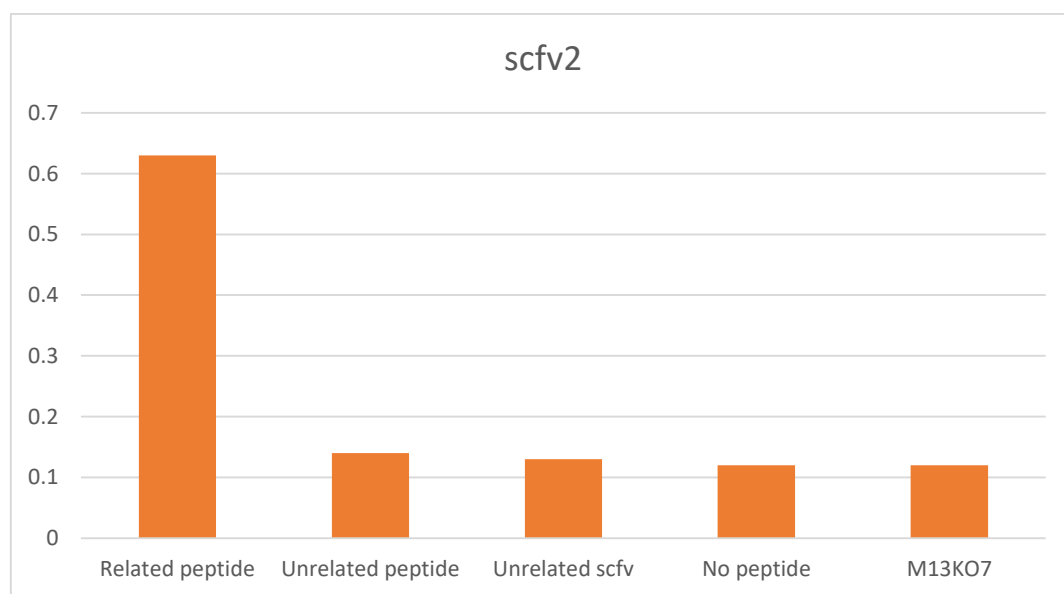


Fig. 6. Phage ELISA results of scFv2 (OD at 405).

Discussion

Anti HIV treatments nowadays prevent cellular entry, viral transcription, and maturation of newly formed virus (20), however, these agents are limited by side effects, toxicities and emergence of resistant virus strains and fail to complete protection (21, 22). Novel therapeutic approaches are being tested and demonstrated the potential to improve the immune system against the virus, currently. In this regard, Immunotherapeutic approaches are one of the exciting areas. Immunotherapy aims to assist the

natural immune system in getting control over viral infection (23). In also therapy-naïve or therapy-experienced HIV-infected persons various immunotherapy formats have been evaluated over the last 20 years. These formats consist of non-antigen specific strategies such as cytokines that suppress the viral replication or stimulate immunity, and antibodies that block viral entry and infection (24).

In this investigation, we applied a conserved neutralizing epitope of HIV-1 gp41 “ELDKWA” to select specific scFvs beside HIV virus. It has been informed that this epitope contributes to inhibit HIV env-mediated syncytium formation (25). Arnold et al (26) has shown that ELDKWA epitope induces a broad neutralizing antibody response against HIV-I and can function as an effective immunogen. Presence of reactive antibodies against this epitope has been reported in children prenatally infected with HIV (27). In this study after panning against a conserved sequence of gp41, two dominant common pattern with the frequency 25% and 20 % were detected that represented the selection of a specific scFv against gp41. It has been demonstrated that the specific scFvs are high effective antibodies and can be contribute in clinical uses for better immune-targeting results in comparison with mAbs (14, 15). In addition, mAbs have some disadvantages including time consuming preparation, high cost, and inducing of immune reactions which eliminate or reduce their therapeutic efficiency and evoke hypersensitivity or allergic reactions in patients (28, 29).

ELISA was done in order to test the specificity and reactivity of the selected scFv. The results have shown a significant higher optical density for reaction of the scFv with the corresponding epitope in comparison with the no peptide well. And the other negative controls demonstrated significant lower optical density than the related peptide. The adverse reactivity of the certain scFv with the

unrelated peptide in association with high reactivity with the gp41 antigen characterizes the specificity of the antibody. As shown by Thathaisong et al. when OD is twofold greater than negative controls, positive phage ELISA is obtained (30).

The chief common immunogenic targets of the mAbs include structurally conserved or functionally significant epitopes, such as CD4 and chemokine co-receptor binding sites on gp120 and proximal external region of gp41 (31). Approaches for vaccine design against HIV nowadays, mainly is based on conserved sequences since these sequences are thought as a promising effective immunogenic targets (32, 33). The outcomes recommend further evaluation of the selected specific scFv for its uses in clinic. The absence of broad treatment under current treatment features the great need for continued exertions in looking for innovative approaches for treatment of HIV (34). Considering the fine- recognized gains of definite scFvs in anti-viral treatments, the neutralizing properties of the selected scFv should be investigated in vivo to provide evidence for their use in anti- HIV methods.

Acknowledgment

The writers would like to acknowledge Shiraz University of Medical Sciences for financial support. The present paper was extracted from the thesis written by Mohammad Javad Aghaei Afshar Grant no 4154.

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