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SELECTION OF SPECIFIC HUMAN SINGLE CHAIN ANTIBODIES AGAINST A CONSERVED NEUTRALIZING EPITOPE OF HIV-GLYCOPROTEIN 41

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ABSTRACT

Effective neutralization of different strains of HIV virus is shown by neutralizing antibodies against HIV-1 envelope glycoproteins. It has been demonstrated that gp41 is involved in virus mediated membrane fusion which results in HIV-entry into target cells. Recombinant single chain antibodies (scFvs) with high specificity and high affinity properties have been identified as useful agents in anti-viral targeted therapy. In this study we selected specific scFvs against a conserved neutralizing epitope of gp41. Four rounds of panning were performed to select the specific clones. The reactivity of the selected scFvs against the corresponding epitope was tested in ELISA. Results demonstrated that the specific clones were selected with the frequencies of 65% and 30%. The ELISA evaluation demonstrated significant higher OD of scFvs in reaction with the corresponding epitope than the negative control. Specific scFvs against conserved neutralizing epitope of gp41 of HIV has the potential to be considered for immunotherapy against HIV. Further evaluations are needed to determine the neutralizing effect and adequate safety margins of the selected scFvs.

KEY WORDS: glycoprotein 41, HIV, single chain antibody, Immunotherapy.

INTRODUCTION

HIV -1 envelop glycoprotein gp41 is involved in virus- mediated membrane fusion which leads to HIV-entry into target cells. After interaction of viral gp120 with CD4 molecule on the target cell a conformational change occurs in the gp120 which leads to binding of gp120 to a co-receptor (CCR5 or CXCR4) [1, 2]. As a result, a cascade of conformational changes occurs in the gp41 and gp120 proteins.

The core of gp41 folds into a six helical bundle structure exposing the previously hidden gp41 fusion peptides and provides fusion with the target cell. The interaction of gp41 fusion peptides with the target cell make up formation of an intermediate pre-hairpin structure which bridges and fuse the viral and host membranes together. The pre-hairpin structure has a relatively long half-life that makes it a target for therapeutic intervention and inhibitory peptides [3, 4]. It has been shown that neutralizing antibodies against these parts of the gp41 have been able to clear HIV virus in the blood of patients. The monoclonal antibody 2F5 recognizing the C-domain of gp41 (aa 669 - 674) could inhibit binding of gp41 to monocytes and lymphocytes and neutralized many HIV strains [5, 6]. The epitope ELDKWA on the C-terminal region of gp41 is a conserved epitope among HIV different strains. Therefore the antibody was able to neutralize the African, Asian, American and European Strains and a significant neutralization (90%) has been reported [7- 9]. It has been shown that specific monoclonal antibodies against the epitope-peptide CELDKWAGELDKWA containing the conserved neutralizing epitope ELDKWA, was able to inhibit HIV envelope mediated membrane fusion [10].

Single-chain variable fragment (scFv) antibodies which are composed of VH and VL domains offer several advantages over monoclonal antibodies. Due to scFvs properties including human origin, small size, high affinity, high specificity and exhibiting better tissue penetration, these antibodies have been introduced as highly effective agents for virus-targeted therapy [11-13].

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ScFv fragments could gain access to neutralizing epitopes of microorganisms and provide highly virus neutralization effect. A number of neutralizing single chain antibodies against different target antigens are introduced. Highly effective neutralizing scFvs against the tick born encephalitis virus, cytomegalovirus, avian influenza H5N1 and herpes simplex virus type1 are reported [14-18]. These antibodies were selected against a neutralizing epitope of the virus antigen. The aim of this study was to select and evaluate specific scFvs against a conserved neutralizing epitope of gp41 of HIV-1.

MATERIALS AND METHOD

Selection of specific scFv antibody against gp41

A library of phage transformed Ecoli of scFv which was developed previously [19] was used and phage rescue was performed to select specific clones against gp41 using panning proces. Briefly, immunotube (Nunc, Roskilde, Denmark) was coated with the gp41 peptide as the epitope at 4˚C overnight. The phage-rescued supernatant (10^{10} PFU/mL) diluted with skimmed milk 2% as blocking solution, added to the tube and incubated for 1h at room temperature. Following washing log phase E. coli TG1 cells were added and incubated at RT for 1hr with occasional shaking. The tube was centrifuged and the pellet was grown and rescued with helper phage M13KO7 (Amersham, Biosciences). Four rounds of panning were performed to remove nonspecific scFvs and select the specific and high affinity binders.

DNA Fingerprinting of the obtained clones after panning

The selected clones obtained after panning was tested by PCR to evaluate the existence of VH-Linker-VL inserts. DNA fingerprinting of the clones were determined by MvaI restriction enzyme (Roche Diagnostic GmbH, Mannheim, Germany) digestion followed by electrophoresis. The common patterns were revealed and two clones with the most frequent patterns were selected.

Determining the phage-antibody concentration

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

Phage ELISA: Reactivity of the selected scFvs against the epitope was assessed by phage ELISA. The 96 well ELISA plate was coated with the peptide (dilution: 100 µg/ml in PBS) at 4˚C overnight. An unrelated peptide was used as a negative control. The wells were blocked with 2% skimmed milk for 2 h at 37˚C. The plate was washed 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 coated wells as a negative antibody control. After incubation and washing, anti- fd bacteriophage antibody (Sigma, UK) was added and incubated for 1 hr at room temprature. Following washing, HRP-conjugated anti-rabbit IgG (Sigma, UK) was added and left at room temperature for 1 h. The plate was washed and 150µl of the substrate (1µl H2O2 with 0.5 mg/ml ABTS in citrate buffer) was added and the optical density of each well was detected at 405 nm by an ELISA reader (BP-800, Biohit, USA).

Statistical analysis

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

RESULTS

Selected scFvs against gp41

PCR and DNA-Fingerprinting of 20 panned clones against gp 41 peptide are shown in figures 1 and 2 respectively. The presence of VH-Linker-VL are shown by 950 bp PCR product. One common pattern with the frequency 65%, lanes 1, 3, 5, 6, 7, 10, 11, 12, 13, 15 16, 17, 19 and another common pattern with frequency 30%, lanes 2, 4, 8, 9, 18, 20 were obtained. One clone from each common pattern was used for further evaluation.

Reactivity determined by Phage ELISA

The reactivity of the selected scFv to the related peptide was determined by phage ELISA. The ODs obtained from reaction of the antibody (scFv1 and scFv2) with the corresponding epitope were significantly higher than the negative controls (Unrelated peptide, unrelated scFv, M13KO7 helper and no peptide well) and showed the specificity of the isolated scFv. OD= 0.39 versus OD= 0.15 (No peptide well OD), (P value<0.05) for scFv1 and OD= 0.41 versus OD=0.11 (no peptide well OD), (Pvalue< 0.05) for scFv2 were obtained (Fig 3).

Figure 1. PCR of selected clones after panning.

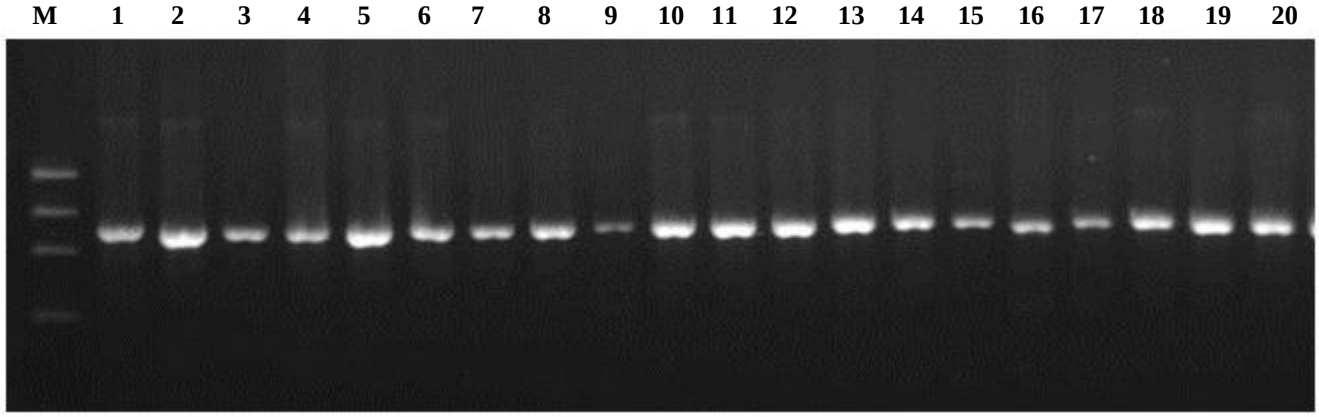


Figure 2. Fingerprinting patterns of the selected scFvs.

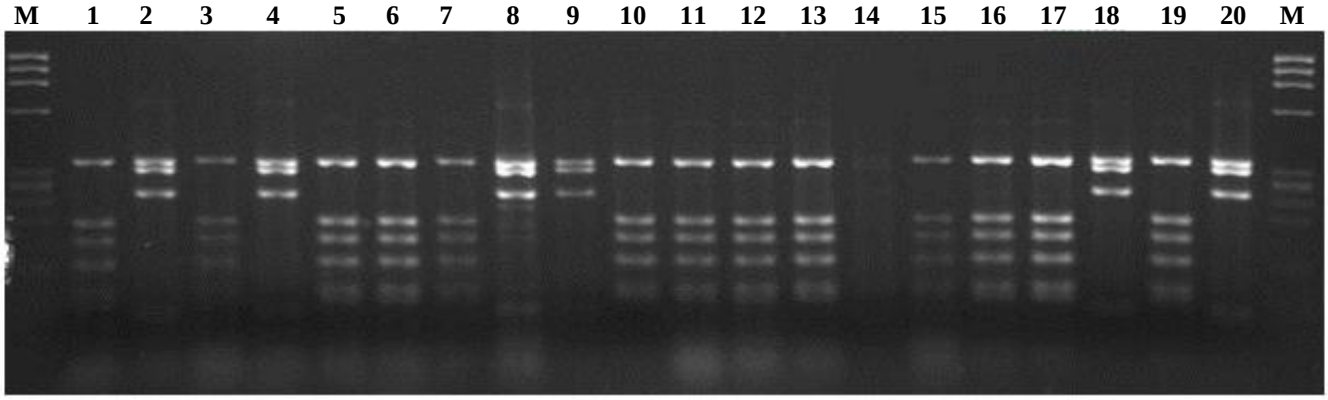
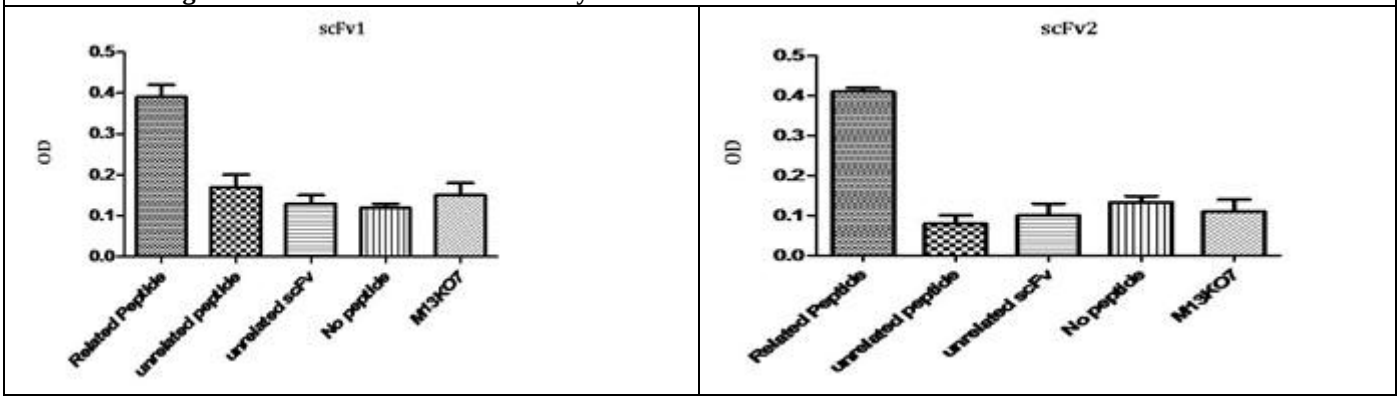


Figure 3. ELISA results of the selected scFv against gp41 peptide, unrelated peptide, unrelated scFv, no peptide and M13kO7 as negative controls showed no reactivity.



DISCUSSION

Targeted therapy using specific antibodies has been used to design novel antiviral therapy. Recombinant antibodies, the most ideal form of immunotherapy against infection diseases, have emerged as effective therapeutic agents for an increasing number of human infections. In the last decade they have become one of the largest groups of new agents approved for viral immunotherapy. Recombinant DNA technology paved the way for production of smaller recombinant antibody fragments (rAb) as a substitution for mAbs as rAb maintains its target specificity and can be produced more economically. These antibodies contain a wide spectrum of formats that the most common of them appears to be the scFv antibody [20- 22]. In this study a phage antibody display library of scFv was used to select specific scFvs against a conserved neutralizing epitope of gp41 of HIV. After four rounds of panning against the derived peptide, 20 clones were selected and Mva-I fingerprinting was performed to select the common patterns. The results demonstrated successful panning process and selection of two specific scFvs against

the epitope. Panning against a conserved sequence of gp41 (CELDKWAGELDKWA), demonstrated two dominant common patterns with the frequencies 65% and 30% which represented the selection of two specific scFvs against gp41. Monoclonal antibodies against the ELDKWA-epitope on the C-domain of gp41 have shown broad neutralization of many HIV strains [23-25]. This sequence is a part of the peptide which was used in the current study. This peptide was applied for production of monoclonal antibodies which inhibited HIV env-mediated syncytium formation [10]. Many studies have used panning procedure to achieve specific scFv antibodies against different pathogens causing infectious disease. Lillo *et al.,* [26] could select scFv antibodies against a capsular protein (F1 antigen) of Yersinia pestis by using panning procedure. Krishnaswamy *et al.,* [27] were able to identify a scFv antibody by panning against Aspergillus fumigates membrane fraction (AMF) as antigen. Introducing the conserved sequences of viral glycoproteins the possibility for producing highly effective antibodies is provided. Lee *et al.,* [28] cloned human scFvs specific for melanoma and breast cancer cells. He could select three scFv-phage clones against cell-surface glycosphingolipid G. The selected scFvs were reactive in ELISA test against the corresponding epitope. A significant higher OD for reaction of the scFvs with the corresponding epitope in comparison with the no peptide wells were obtained. Phage ELISA has been previously used by a number of researchers to evaluate the specificity of antibodies for the targeted epitopes or antigens. Wu *et al.,* [29] developed antibodies specific for large macromolecular complexes from a phage display library. Phage ELISA was performed to confirm the specificity of selected clones. The results showed the usefulness of panning protocol in enriching phage particles specifically bind to signal recognizing peptide and its receptors.

Various immunotherapy formats have been evaluated in HIV-infected patients over the last 20 years [30, 31]. Applying scFvs as small and effective antibodies are new strategy in this regard. Isolation of specific anti-gp41 scFvs which recognize a conserved neutralizing epitope could suggest further evaluation of the scFvs for their use as effective antibodies in passive immunization against many HIV strains in high risk individuals.

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