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Optimization of multi-epitopic HIV-1 recombinant protein expression in prokaryote system and conjugation to mouse DEC-205 monoclonal antibody: implication for *in-vivo* targeted delivery of dendritic cells

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ABSTRACT

Objective(s): Multi-epitopic protein vaccines and direction of vaccine delivery to dendritic cells (DCs) are promising approaches for enhancing immune responses against mutable pathogens. *Escherichia coli* is current host for expression of recombinant proteins, and it is important to optimize expression condition. The aim of this study was the optimization of multi-epitopic HIV-1 $_{tat/pol/gag/env}$ recombinant protein (HIVtop4) expression by *E. coli* and conjugation of purified protein to anti DEC-205 monoclonal antibody as candidate vaccine.

Materials and Methods: In this study, expression was induced in *BL21 (DE3) E. coli* cells by optimization of induction condition, post induction incubation time, temperature and culture medium formula. Some culture mediums were used for cell culture, and isopropyl-beta-D-thiogalactopyranoside was used for induction of expression. Protein was purified by Ni-NTA column chromatography and confirmed against anti-His antibody in western-blotting. To exploit DCs properties for immunization purposes, recombinant protein chemically coupled to αDEC-205 monoclonal antibody and confirmed against anti-His antibody in western-blotting.

Results: The optimum condition for expression was 1 mM IPTG during 4 hr cultures in 2XYT medium, and final protein produced in soluble form. Conjugation of purified protein to αDEC-205 antibody resulted in smears of protein: antibodies conjugate in different molecular weights. .

Conclusion: The best cultivation condition for production of HIVtop4 protein is induction by 1 mM IPTG during 4 hr in 2XYT medium. The final concentration of purified protein was 500 µg/ml.

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Introduction

Human immunodeficiency virus (HIV) is a member of lentiviral family that causes acquired immunodeficiency syndrome (AIDS). This pathogen in AIDS condition damages immune system (1). There are lots of efforts to make a suitable vaccine against *HIV*. Among the various vaccines in this regard, epitope based vaccine strategy relied on multiple conserved and immunogens baring epitopes has advantages due to overcoming to rapid rate of mutations in HIV-1 (2-4). Identification of conserved and immunogenic epitopes of *HIV* genome has led to the development of vaccines incorporating only these critical epitopes in order to elicit the required immunologic response (5, 6). These epitope based vaccines have potential benefits like as biosafety, precise control over the immune system activation and ability of focus on conserved and highly immunogenic antigen regions (7). Among the HIV-1 antigens, Gag, Tat, Pol and Env have received considerable attention due to their critical roles in viral life cycle (8, 9), and have sites in the viral genome mapping to both T helper and T cytotoxic epitopes that are consistently less variable than those not mapping to any described T-cell epitopes (http://www.hiv. lanl.gov/content/index). Therefore, they are suitable candidates for vaccine development.

Other approach to produce efficient vaccine Against HIV is to deliver target antigens directly to

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DCs for eliciting enhanced cellular immune responses against this intracellular pathogen. There are multiple strategies for targeting of antigens to DCs, but current approach using incorporated antigens into specific monoclonal antibodies (mAbs) against particular DC surface molecules, significantly enhances CTL , helper T cells and antibody responses (10, 11). To achieve this, targeting of antigens to DEC-205 on CD8+ DCs is promising approach (12, 13). Due to the fact that CD8+ DC is major producer of IL-12p70, which is needed for CTL response development, targeting of DEC-205 on these DCs is very helpful in viral infection (14-16).

Furthermore, antigen targeting to DEC-205 induces the response of CD4+ T cells and indirectly improves antibody production (17-19).

E. coli is successfully used as vehicle for expression of foreign proteins (20), but it is needed to find suitable conditions for the best expression of each protein. Therefore, we focused on conditions to improve the productivity of *E. coli*-bacteriophage T7 RNA polymerase expression system. In this system, expression is under control of IPTG-inducible lac UV5 promoter that is commonly used in recombinant protein production (21). Apart from strong promoter, one of the most important parameters that affect on total protein production is the amount of IPTG (isopropyl-2-D-thio-galactopyranoside) using for induction of protein expression by host. Some studies have shown the impact of IPTG amount on the final status of expressed protein in *E. coli*-bacteriophage T7 RNA polymerase expression system (20, 22). Temperature is another important parameter that should be considered and, probably reduced temperature condition results in expression of the protein in soluble form. In most situations, especially if the target protein activity is important, soluble form of protein is desired (23). Other factors like the starting time for induction, post induction incubation time and culture media formulation are parameters that should be considered in recombinant protein expression technology in *E. coli*.

In our previous study, we used epitope selection and confirmation tools for designing and construction of a new DNA vaccine candidate encoding conserved and immunogenic epitopes from HIV-1 antigens of tat, pol, gag and env, and evaluated immune responses in balb/c mice (24). The aim of this study, therefore, was to optimize this multi-epitopic HIV-1 $tat_{1\text{-}20,\ 44\text{-}61}/pol_{150\text{-}190}/gag_{158\text{-}186}/env_{296\text{-}323,\ 577\text{-}610}$ recombinant protein (HIVtop4) expression by *E. coli BL21 (DE3)* and conjugation of purified protein to anti DEC-205 monoclonal antibody as candidate vaccine.

Materials and Methods

Media and cell culture

2XYT (Peptone 1.6%, Yeast 1%, Nacl 0.5%), LB (Luria Bertani) Miller broth (Peptone 1%, Yeast 0.5%, Nacl 1%), SOB (Super Optimal Broth) (Peptone 2%, Yeast 0.5%, NaCl 10 mM, KCL 2.5 mM, $MgCl_2$ 10 mM, $MgSO_4$ 10 mM), TB (Terrific Broth) (Peptone 1.2%, Yeast 2.4%, K_2HPO_4 72 mM, Glycerol 0.4%) and SB (Super Broth) (Peptone 3.2%, Yeast 2%, NaCl 0.5%) mediums (MERCK, Germany) were used for cell culture and optimization of protein expression. Cultures were performed in 200 ml medium containing 100 µg/ml Ampicilin (Sigma), using 1000 ml flask at 37˚C and 250 RPM, and induced by the addition of final concentration of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Fermentase) to express HIVtop4 protein.

Recombinant HIV-1 $_{tat/env/pol/gag}$ protein (HIVtop4) expression

The tat/pol/gag/env fragment was excised from pBMH-HIV-1tat/env/pol/gag with Eco-RI/XhoI and sub-cloned into the same enzymatic sites in pET-23a expression vector and the construct of PET23a-HIV-1tat/env/pol/gag which is controlled by T7 promoter and lac operator in *E. coli* was confirmed using double digestion by EcoR-I and Xho-I restriction enzymes (Fermentase, Germany) in accordance with the manufacturers' instructions and subsequently sequencing in Pasteur institute sequencing center. This construct was transformed in to competent *E. coli BL21 (DE3)* using heat-shock transformation method. Some colonies were selected, cultured and induced separately for selection of the best protein expressing colony at mid-log phase (OD_{600}=0.6-0.8) of 1, 2, 3 and 4 hr by addition of 0.1 to 1 mM IPTG.

Solubility of top4 protein was determined according to the QIAexpressionist™ protocol (Qiagen). Briefly, 10 ml LB medium containing 100 µg/ml ampicillin was inoculated in a 50 ml flask and grew overnight at 37˚C with vigorous shaking. 2.5 ml of the overnight culture was inoculated in 50 ml of pre-warmed media (with ampicillin) and grew at 37˚C with vigorous shaking (~300 rpm) until the OD_{600} of 0.5 to 0.8, and induced by adding IPTG to a final concentration of 1 mM for 4 hr and harvested by centrifugation at 4000 g for 20 min. Cell pellet was resuspended in 5 ml of lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole. pH 8) for native purification, and sonicated for 6 × 10 sec with 10 sec pauses on ice at 200 to 300 W (Hielscher ultrasound technology, Germany). Lysate was centrifuged at 10,000 g at 4˚C for 20 to 30 min, and supernatant saved on ice as soluble protein sample. The pellet was resuspended in 5 ml lysis buffer as insoluble matter. Soluble and insoluble samples were diluted to the same volume and analyzed by SDS-PAGE (Bio Rad, Germany).

His-tagged protein was further purified in native condition using nickel-nitrilotriacetic acid (Ni-NTA) affinity chromatography column (Qiagen, USA) according to the manufacturer's instructions. Briefly, expressing cells were resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole) by gentle vortexing, and sonicated by sonicator with a microtip on ice 6×10 sec bursts at 200 to 300 W

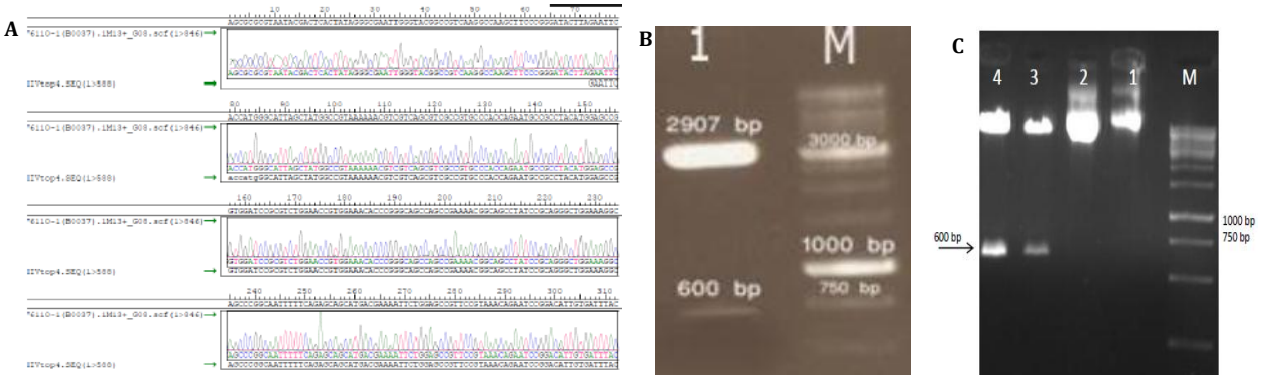

Figure 1. Double digestion of pBMH-HIV-1$_{tat/env/pol/gag}$ plasmid by EcoR-I and Xho-I restriction enzymes produced a segment of 600 bp, Lane M: DNA ladder, lane 1: Digested plasmid. (A). Result of HIV-1$_{tat/env/pol/gag}$ DNA sequencing (B). Confirmation of sub-cloning by double digestion (C), Lane M: DNA ladder, lane 1 and 2: Undigested plasmid, Lane 3 and 4: Digested plasmid

with a 10 sec cooling period between each burst, centrifuged for 20 to 30 min at 10000 g. Later, 1 ml 50% Ni-NTA resin was added to obtained supernatant and shaked gently at 200 RPM, 60 min, 4˚C. This mixture was loaded onto a column after washing steps with wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM Imidazole), and fractions of pure protein were eluted in elution buffer (50 mM NaH_2PO_4, 300 mM Nacl, 450 mM Imidazole). The purified protein was quantified with Bradford method, according to protocol (AppliChem) and stored at -70˚C until use.

Analysis of protein expression

Optical density of samples from each culture was measured at 600 nm (OD_{600}) with a UV-Vis spectrophotometer (Picodrop, UK). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and western blotting were performed to detect His-Tagged HIVtop4 peptide according to the standard protocol (25). Before electrophoresis, whole cell sample was mixed with sample buffer (0.5 M Tris–HCl (pH 6.8), 10% glycerol, 5% SDS, 5% β-mercaptoethanol (Bio-Rad, USA), and 0.25% bromophenol blue (Sigma) and incubated at 100˚C for 5 min for cell disruption. After briefly centrifuge, the samples were loaded onto a 12% slab gel. After electrophoresis, the gel was stained with coomassie blue (Bio-Rad). Monoclonal anti-His tag antibody (Invitrogen, USA) was used for western blot analysis. The purified protein was quantified with Bradford method, according to protocol (AppliChem).

Conjugation of purified recombinant HIV-1$_{tat/env/pol/gag}$ protein to monoclonal antibodies

Purified HIVtop4 protein was conjugated to monovalent anti DEC-205 (Rat anti mouse DEC-205; IgG2a, clone NLDC145) or isotype matched control antibody (RTK2758) (Biolegend, USA) using Schiff base formation and reductive amination according to standard protocol (26). In brief, first anti DEC-205 or isotype matched control monoclonal antibodies were reduced using 100 mM 2-mercaptoethanesulfonic acid sodium salt (MESNA; Sigma-Aldrich) for 30 min at 37˚C and separated from the reducing agent over a desalting column. Then, these monovalent antibodies were oxidized at their carbohydrate residues using sodium periodate (sigma-aldrich) for production of aldehyde functional groups and separated from the oxidative agent over a desalting column. Purified HIVtop4 protein was added to the modified antibodies, and 5M sodium borohydride (sigma-aldrich) as reducing agent was used to facilitate schiff base intermediates formation and conjugation of protein to antibody. Antibody: HIVtop4 conjugates purified by dialysis using sterile Phosphate Buffered Saline (PBS) and passed over an Amicon centrifugal filter devices (cut off 50 kDa) (Millipore, Germany) to remove unconjugated protein, and then evaluated by spectrophotometry, SDS-PAGE, western blotting and ELISA.

Results

Confirmation of polytopic HIV-1$_{tat/env/pol/gag}$ gene segment

Synthesized pBMH-HIV-1$_{tat/env/pol/gag}$ plasmid with EcoR-I and Xho-I restriction sites was confirmed by sequencing (Figure 1A) and enzymatic digestion, which resulted a segment of 600 bp (Figure 1B). For expression in *E. coli*, polytopic HIV-1*tat/env/pol/gag* fragment was sub-cloned into PET23a expression vector, which has the same enzymatic sites, and final DNA construct was confirmed by mentioned enzymatic digestion (Figure 1C).

HIV-1$_{tat/env/pol/gag}$ protein expression

As shown in Figure 2 A, coomassie blue-stained SDS– PAGE analysis of the bacterial lysates indicated expression of a protein band with the expected molecular weight of 23 kDa corresponding to 6xHis-tagged HIVtop4. Accordingly, western blot analysis by anti His-tag antibody recognizing histidine amino acids also confirmed the proper expression of the protein within the crude bacterial lysate (Figure 2B).

Media culture optimization

For selection of best medium, we used 2XYT, LB Miller broth, SOB (Super Optimal Broth), TB (Terrific Broth) and SB (Super Broth) mediums for cell culture, and cultures were performed in 50 ml of

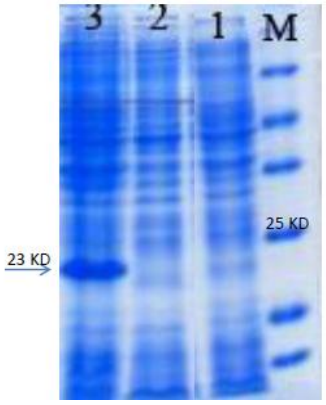

Figure 2A. SDS-PAGE analysis of expressed HIV $_{tat/env/pol/gag}$ in *Escherichia coli -BL21 (DE3)*. Lane 1: crude untransformed bacterial extract, Lane 2: crude bacterial extract before induction by 1 mM IPTG, Lane 3: crude bacterial lysate 4 hr after induction, Lane M: protein MW marker in kDa

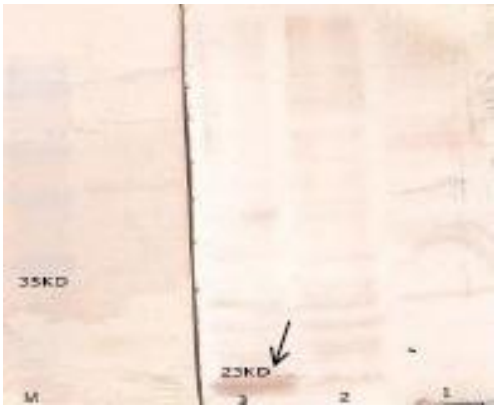

Figure 2B. Western blot analysis of expressed HIV $_{tat/env/pol/gag}$ in *Escherichia coli-BL21*. Lane 1: crude untransformed bacterial extract. Lane 2: crude bacterial extract before induction by 1 mM IPTG. Lane 3: crude bacterial lysate 4 hr after induction, Lane M: protein MW marker in kDa

each medium containing 100 µg/ml Ampicilin, using 250 ml flask at 37˚C and 250 RPM, and induced by the addition of final concentration of 1 mM IPTG to express HIVtop4 protein. SDS-PAGE results showed that the expression of this protein in 2XYT medium was better than others, and quantification by Bradford assay after purification resulted in ≈100 µg/ml TOP4 protein from culture in 2XYT medium and ≤70 µg/ml from other used media (Figure 3).

Optimal density600 of 0.7 was optimum starting time for Induction

In order to optimize the best starting time of induction, 2.5 ml of the overnight culture was inoculated in 50 ml of pre-warmed media (with ampicillin) and grew at 37˚C with vigorous shaking (~300 rpm) until the OD_{600} of 0.6, 0.7 and 0.8. In each mentioned OD, 10 ml of culture was induced by adding IPTG to a final concentration of 1 mM for 4 hr and harvested by centrifugation at 4000 g for 20 min for SDS-PAGE analysis. As shown in Figure 4, OD_{600} of 0.7 was the best starting time for induction by IPTG and quantification by Bradford test after purification confirmed by SDS-PAGE result.

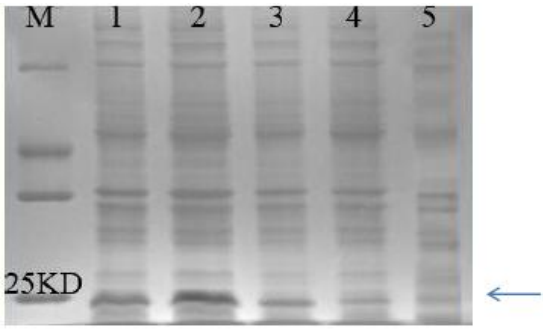

Figure 3. HIV $_{tat/env/pol/gag}$ protein expression in different culture mediums. Expression in SB medium (1), in 2XYT medium (2), in LB medium (3), in SOB medium (4), in TB medium (5), Molecular weight marker (M)

There were no differences in protein expression levels in different IPTG concentrations

For optimization of IPTG concentration in induction of protein expression, IPTG was used in concentrations of 0.2, 0.4, 0.6, 0.8 and 1 mM in cultures. After SDS-PAGE analysis, there were no significant differences between protein expressions in different concentrations of IPTG, but induction by 1 mM IPTG, was partially better than others concentrations (Figure 5A), and Bradford assay after purification also showed similar results. There was the best expression 4 hr after induction (200 µg/ml in 4 hr compared to 60 µg/ml in 5 hr after induction by IPTG) (Figure 5B). Also we did not observe any protein expression bands in temperatures of 28 and 32˚C (Data not shown).

Preparation of anti-DEC205 (or RTK2758): HIV $_{tat/env/pol/gag}$ conjugates

HIVtop4 protein purified through Ni^{++} affinity chromatography according protocol (Qiagen) and

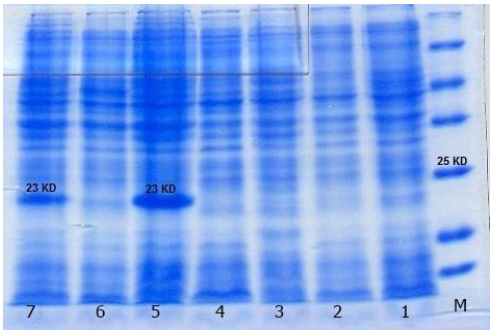

Figure 4. Impact of various starting time of induction on protein expression level. It was considered that induction in OD_{600} 0f 0.7 was the best of all. Lane 1: crude untransformed bacterial extract, Lane 2 and 3: crude transformed bacterial extract in OD_{600} of 0.6 before (2) and after induction by 1 mM IPTG (3), Lane 4 and 5: crude transformed bacterial extract in OD_{600} of 0.7 before (4) and after induction by 1 mM IPTG (5) and Lane 6 and 7: crude transformed bacterial extract in OD_{600} of 0.8 before (6) and after induction by 1 mM IPTG (7). Marker (M)

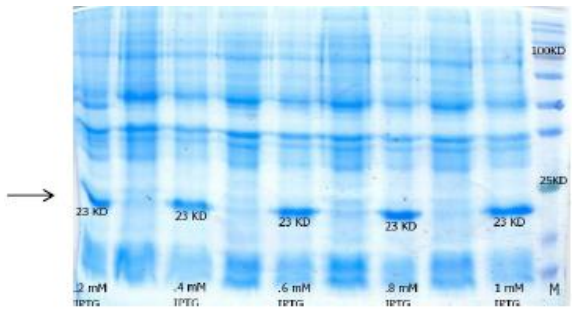

Figure 5A. Impact of various concentrations of IPTG on protein expression level. It was considered that induction by 1 mM IPTG was the best of all. Marker (M)

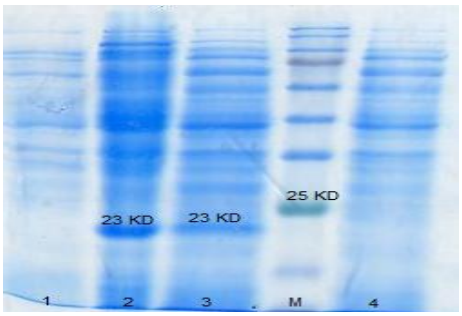

Figure 5B. Impact of various incubation times after induction on protein expression level. Lane 1: Three hrs after incubation, Lane 2: Four hrs after incubation, Lane 3: five hrs after incubation and Lane 4: crude untransformed bacterial extract. Marker (M)

confirmed against HIS-Tag in western-blotting. As shown in Figure 6A and 2B, the results indicated expression of a protein band with the expected molecular weight of 23 kDa. Quantification of the endotoxin levels indicated below 8 EU/ml of the purified protein, which was proper for the final aim of the immunization (Lonza, Germany). Monovalent anti DEC-205 or RTK2758 monoclonal antibodies were oxidized at their carbohydrate residues using sodium periodate (sigma-aldrich) for production of aldehyde functional groups, and separated from the oxidative agent over a desalting column. LPS free HIVtop4 protein was added to antibodies to form Schiff base intermediates, and 5M sodium cyanoborohydride (sigma-aldrich) as reducing agent used for facility of conjugation. Antibody:HIVtop4 conjugates were purified by dialysis using sterile PBS buffer (pH 7.2-7.4) and passed over an Amicon centrifugal filter devices (cut off 50 kDa) (Millipore, Germany) to remove unconjugated protein. Then they were evaluated by spectrophotometry and SDS-PAGE and confirmed against HIS-Tag in western-blotting (Figure 6A, B and C) and ELISA by HRP conjugated α-Rat IgG and α-His-Tag antibody (Figure 6D and 6E). Based on the observed conjugation efficiency and the 1:6 mass ratio of IgGs to HIVtop4 protein, conjugation resulted in smears of protein: that included antibodies of above 180 kDa. There were a few conjugated products in 100 kDa also.

Discussion

A potent and effective vaccine against HIV-*I* infection still remains elusive. For this purpose, construction of the vaccine composed of various epitopes derived *HIV-I* proteins could be valuable in eliciting a protective cellular and humoral immune responses (27). In our previous study, we designed a polyepitope candidate vaccine for HIV-1 which is based on proteins coded by *pol, env, tat* and *gag* genes that are conserved and can bind to a range of human and mouse MHCs and also to T-cell, B-cell, T-helper and T-cytotoxic receptors (24). In this study, we optimized this multi-epitopic HIV-1 $tat_{1\text{-}20,\ 44\text{-}61}/pol_{150\text{-}190}/gag_{158\text{-}186}/env_{296\text{-}323,\ 577\text{-}610}$ recombinant protein (HIVtop4) expression by using *E. coli BL21 (DE3)* as expression system.

In biotechnology, *E. coli* is a favorable host for production of recombinant proteins and a strong promoter like T7 promoter is critical when a large amount of protein is needed (28). Culture medium formula, temperature, induction condition like amount of IPTG, starting time of induction and incubation time after induction, are parameters that affect on total protein production. Due to these facts, in this study "pET23a" which is controlled by T7 promoter and lac operator was used as vector and construct of pET23a-HIV-1 $tat_{1\text{-}20,\ 44\text{-}61}/pol_{150\text{-}190}/gag_{158\text{-}186}/env_{296\text{-}323,\ 577\text{-}610}$ after confirmation by enzymatic digestion with EcoR-I and Xho-I as restriction enzymes and sequencing (Figures 1A,B), transformed to competent *E. coli BL21 (DE3)* for protein expression by using IPTG as inducing agent.

The growth behavior of recombinant *E. coli BL21 (DE3)* containing the pET23a-HIVtop4 plasmid was investigated at 37°C. It was observed that approximately 3.5 to 3.45 hr after culture, the OD_{600} of cultured transformed bacteria was 0.6 and during the 4 to 4:20 hr from inoculation time, mentioned OD reached 0.7 to 0.8 in presence of 100 µg/ml of ampicillin.

IPTG which is an inducer of the lactose operon, binds to the lac repressor and releases the tetrameric repressor from the lac operator (29). It is used in protein expression in the concentration range of 100 µM to 1.5 mM depending on the strength of induction required, the genotype of cells or plasmid used (30). There are some studies showing that optimization of the starting time of induction is important because induction at different phases of recombinant bacterial growth affect on yield of protein production (or activity) (22). Therefore, we cultured the transformed bacteria until the OD_{600} reached to 0.6, 0.7 and 0.8, and induced in each mentioned ODs by adding 1mM IPTG. As shown in Figure 4, OD_{600} of 0.7 was the best starting time and there was not any protein production at OD_{600} of 0.6. On the other hand, production of this protein decreased at OD_{600} of 0.8. As well, the decreased concentration of IPTG

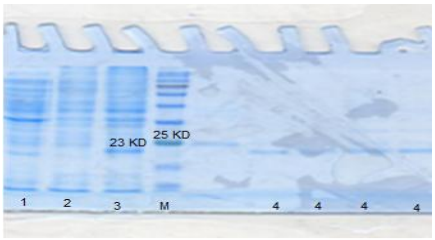

Figure 6A. HIVtop4 protein purification by Ni-NTA affinity chromatography. Lane 1: crude untransformed bacterial extract, Lane 2: crude bacterial extract before induction by IPTG, Lane 3: crude bacterial extract after induction, Lane 4: HIVtop4 protein after purification, M: molecular weight marker

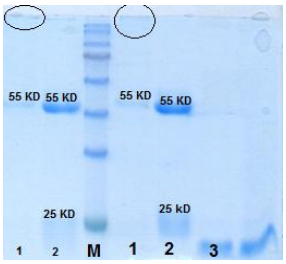

Figure 6B. SDS-PAGE result of conjugation of HIVtop4 protein to monovalent αDEC monoclonal antibody and RTK2758 isotype control. Lanes 1: uncojugated αDEC monoclonal antibody (NLDC145) and RTK2758 isotype control antibody, Lanes 2: conjugated antibodies, Lane 3: Purified HIVtop4 protein, M: Molecular weight marker. Rings in top of lanes indicate conjugated products

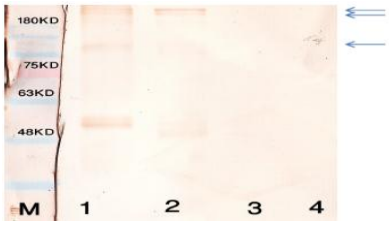

Figure 6C. HIVtop4 protein conjugations to monovalent αDEC monoclonal antibody and RTK2758 isotype control. Lane 1: HIV TOP4: αDEC monoclonal antibody (NLDC145) conjugated product, Lane 2: HIVtop4: RTK2758 isotype control antibody conjugated product, Lane 3: uncojugated αDEC monoclonal antibody (NLDC145), Lane 4: unconjugated RTK2758 isotype control antibody. Arrows indicate conjugated products

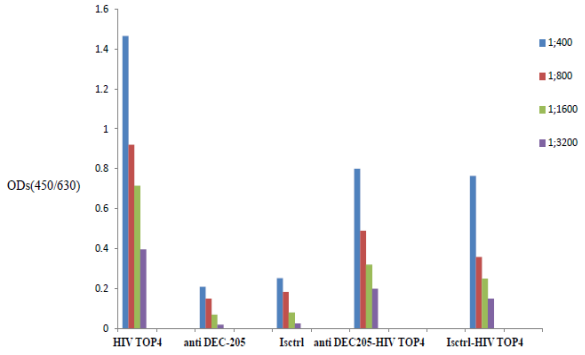

Figure 6D. ELISA assay for confirmation of presence of αDEC or RTK2758 isotype control antibodies in conjugated products. One micro gram of each antibodies and unconjugated or conjugated products and as well as 3 µg serum of rat (as positive control) were coated in ELISA plates. Dilutions of HRP conjugated α-Rat IgG antibody was used in this assay for final detection. Isctrl: iso type control antibody

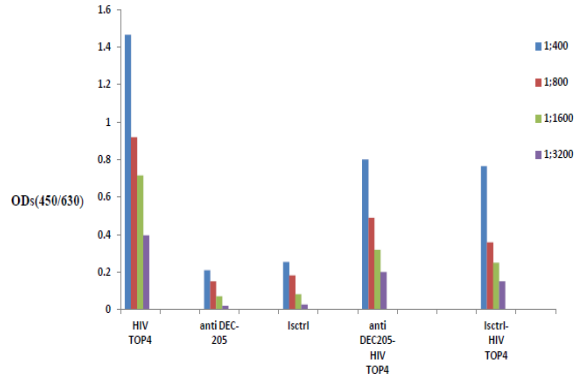

Figure 6E. ELISA assay for confirmation of presence of His Tagged HIVtop4 protein in conjugated products. One micro gram of each antibodies and unconjugated or conjugated products were coated in ELISA plates. Dilutions of HRP conjugated α-His Tag antibody was used in this assay for final detection. Isctrl: isotype control antibody

in low temperatures and incubation time after induction are important cultivation parameters which influence on protein production yield (21). For this reason, we used different concentration of IPTG in 37˚C (15 and 28˚C, unpublished data), and it was considered that induction by 1 mM IPTG in 37˚C was better (Figure 5A). As showed in Figure 5B, during 3 hr after incubation there was not any protein production, but the maximum amount of protein production was observed in the fourth hr after induction, and in further incubation the rate of protein expression was decreased.

In our ongoing study, at the first step and for production of monovalent antibodies, we used mild reducing agent MESNA (mild 2-mercaptoethane sodium sulfonate) which cleaves inter heavy chain disulfide bonds (31). At second step, for antigens directly to DEC-205+ dendritic cells in our future study, we conjugated modified rat anti mouse DEC-205 monoclonal antibody or RTK2758 as isotype matched control antibody to purified HIVtop4 protein using schiff base formation and reductive amination protocol. In this protocol, carbohydrate groups in FC portion of antibodies were involved in the procedure, and therefore Fab portion was not affected (26). The SDS-PAGE analysis and western-blot results confirmed conjugated products (Figures 6B and C) in smears of protein:antibodies conjugates especially in molecular weight of above 180 kDa. The αDEC-205: HIVtop4 conjugates, as well as conjugates produced with isotype matched nonreactive antibody were subjected to ELISA assay alongside known quantities of HIVtop4 protein (or α-DEC205/isotype control antibodies) to quantify the amount of HIVtop4 in the conjugates and confirmation of conjugation. Based on the observed conjugation efficiency and the 1:6 mass ratio of IgGs to HIVtop4 protein, we estimated generally ≥50% of the conjugated products consisted of HIVtop4 protein.

After doing verification tests on functional integrity of mAbs, we will use these conjugated

products in our incoming study for *in vivo* delivery of HIVtop4 protein to dendritic cells as a vaccine candidate to evaluate targeted immunization strategy in animal model.

Conclusion

We concluded that in order to increase the HIVtop4 protein production with molecular weight of 23 kDa, a 2XYT culture medium supplemented with 1mM IPTG for induction of expression together with 4hr incubation would be more efficient than utilizing any other cultivation conditions that used in this study. The final concentration of purified HIV-1 tat/pol/gag/env protein in this condition was 500 µg/ml. SDS-PAGE analysis, western-blot and ELISA results confirmed conjugated products in smears of protein:antibodies conjugates in molecular weight of above 180 kDa.

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