SHORT COMMUNICATIONS

Repeated epitope in the recombinant epitope-peptide could enhance ELDKWA-epitope-specific antibody response*

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Abstract Based on the hypothesis suggested by us that epitope-vaccine may be a new strategy against HIV mutation, we have studied several neutralizing epitopes on HIV envelope proteins. However we do not know whether a repeated epitope in a recombinant epitope-peptide can enhance epitope-specific antibody response or not. ELDKWA-epitope (aa669-674) on the C-domain of HIV-1 gp41 is a neutralizing epitope defined by the monoclonal antibody (mAb) 2F5 with broad neutralizing activity. In this study, we designed and prepared a series of the recombinant epitope-peptides bearing 1, 4 and 8 copies of ELDKWA-epitope respectively. In the comparison of the antisera induced by the three recombinant antigens, an obviously increased titre of ELDKWA-epitope-specific antibody was observed in the case of four and eight repeated epitopes. In flow cytometry analysis, the epitope-specific antibodies in both antisera showed stronger activity to bind the transfected CHO-WT cells that stably express HIV-1 envelope glycoprotein on the cell surfaces. These experimental results indicated that repeated epitope in the recombinant epitope-peptide could enhance ELDKWA-epitope-specific antibody response, which could contribute to designing an effective recombinant epitope-vaccine.

Keywords: neutralizing epitope, epitope vaccine, antibody response, repeated epitope, HIV-1.

In spite of extensive prevention programs, the HIV epidemic is spreading throughout the world in particular in sub-Saharan Africa where AIDS has become the leading cause of death[1]. It needs new strategy to develop an effective prophylactic vaccine to control the epidemic. When recombinant envelope proteins are used as subunits, the antibodies neutralize the homologous virus strain used to derive the vaccine, and some closely related strains, but fail to neutralize diverse HIV-1 strains[2—4]. It was confirmed that rgp120 subunit vaccine failed to prevent infection from HIV-1[5]. One major cause is related to the extensive genetic variability of the virus. Montefiori et al. suggested a strategy to increase the breadth of neutralization responses by a multivalent vaccine included a wide variety of HIV-1 primary isolate envelopes[6]. Besides, we suggested epitope-vaccine as a new strategy for developing an effective vaccine against HIV infection and mutation[7,8]. Previous studies demonstrated that epitope-vaccines could increase immunogenicity of predefined neutralizing epitopes[9—11]. Antigenicity and predefined specificities of the multi-epitope vaccine in candidate consisting of neutralizing epitope and mutated epitopes suggested a new way against HIV-1 mutation[12,13]. We have developed universal method for design and construction of recombinant epitope-peptide gene for epitope-vaccine strategy[14]. However, we do not know whether or not a repeated epitope in a recombinant epitope-peptide can obviously enhance epitope-specific antibody response.

ELDKWA-epitope (aa669-674) on the C-domain of HIV-1 gp41 is a relative conserved epitope and was identified as neutralizing epitope defined by the monoclonal antibody (mAb) 2F5 with broad neutralizing activity against African, Asian, American and European strains from clades A, B, and E, and 90% of the investigated viruses were neutralized[15,16]. In this study, we designed and prepared a series of the recombinant epitope-peptides bearing 1, 4 and 8 copies of ELDKWA-epitope respectively, and wanted to compare their potency to induce ELDKWA-epitope-specific antibody response.

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1 Materials and methods

1.1 Peptides, antibodies, cells and bacterial strains

The ELDKWA epitope-peptide P1 (CGELDKWASLWNWFNIT) was commercially synthesized in Genemed Synthesis Inc. (USA). Peroxidase-conjugated rabbit anti-mouse immunoglobulin and FITC-conjugated rabbit anti-mouse IgG were obtained from Dako (Denmark). CHO cells stably transfected with HIV-1HXB2 Env expressing vector pEE14 (CHO-WT) were cultured in glutamine-deficient minimal essential medium (GMEM-S) containing 400 μmol/L methionine sulfoximine (Gibco & Sigma, USA)[17]. The bacterial strains (BL21) transformed with pGEX-4T or pGEX-4T harboring one, four or eight copies of ELDKWA-epitope, which were named pGEX-4T-K, pGEX-4T-K_4 or pGEX-4T-K_8, were conserved in our laboratory[14]. Bacteria were cultured with LB medium with ampicillin (100 μg/mL).

1.2 Expression and purification of recombinant antigens

Overnight cultures of the recombinant bacteria having plasmid pGEX-4T, pGEX-4T-K, pGEX-4T-K_4 or pGEX-4T-K_8 were diluted 1∶50 in 200 mL of LB mediums containing 100 μg/mL ampicillin and grown at 37 ℃ for about two hours. Protein expression was then induced by addition of 1 mmol/L isopropylthiogalactoside (IPTG) to the culture medium. After 4 more hours incubation under vigorous agitation, *E. coli* cells were pelleted and resuspended in 20 mL of ice-cold PBS and sonicated twice for 2 min with 10 min rest on ice. After centrifugation at 25000 r/min, the supernatant was filtered through a 0.45 μm membrane and loaded onto a Glutathione-Sepharose 4B (Pharmacia) column. Being monitored by FPLC OS/2 system (Pharmacia), GSH-Sepharose column was eluted with 10 mmol/L GSH in 50 mmol/L Tris-HCl (pH 8.0) as the direction of manufacturer.

1.3 Immunization of experimental animals

After purified with Glutathione-Sepharose 4B column, GST and three GST fusion proteins bearing 1, 4 and 8 copies of ELDKWA-epiotpe respectively, were used to immunize mice in Group 1, 2, 3 and 4 (Fig. 1). Each of four Balb/c mice in one group was immunized with 50 μg of purified antigens after mixed with complete Freund's adjuvant. Boosters were given in incomplete Freund's adjuvant on days 14, 28 and 42. Antisera were separated on the 5th day after the fourth booster. Pre-immune sera (normal sera, NS) were collected before immunization. After vaccination course, the sera from the immunized mice of Group 1, 2, 3 and 4 were collected and named AS1, AS2, AS3 and AS4.

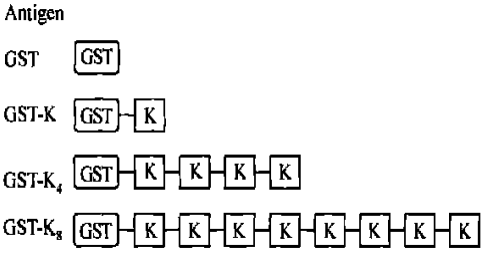

Fig. 1. Construct of recombinant antigens for immunization. K in the figure represents an ELDKWA-epitope of HIV gp41.

1.4 Detection of epitope-specific antibodies by ELISA

The peptides were coated overnight on a microtiter plate at 4 ℃. Nonspecific binding was blocked by incubation with 0.3% gelatin in PBS. After washing three times with PBS-Tween 20 (0.1% Tween 20), mouse sera were added and incubated for 1 hour at room temperature. After washing, peroxidase-conjugated rabbit anti-mouse immunoglobulin was added. After further washing, freshly prepared 2, 2′-azino-di-(3-ethylbenzthiazoline sulfonate)-peroxide solution was added and the optical density was measured.

1.5 Detection of epitope-specific antibodies in flow cytometry analysis

HIV-Env^+ CHO-WT cells (2×10^5)[17], were first incubated with antisera or normal sera (pre-immune sera) (1∶100 dilution in PBS) for 1 hour at 4 ℃, washed twice with PBS, and then incubated with FITC-conjugated rabbit anti-mouse IgG (1∶50 dilution in PBS) for 1 hour at 4 ℃, washed and then analyzed on a FACSCalibur (Becton-Dickinson, USA).

2 Results and discussion

To compare the potency of three fusion proteins to induce ELDKWA-epitope-specific antibody response, the ELDKWA-epitope-specific antibodies induced in mice were detected in ELISA-assay. The antibodies in antisera AS2, AS3 and AS4 could bind to

gp41 peptide P1 bearing the ELDKWA-epitope, while the control AS1 to GST (excluding ELDKWA-epitope) and normal sera (NS) did not, which indicates that all of three fusion proteins bearing ELDKWA-epitope could induce ELDKWA-epitope-specific antibodies (Fig. 2). Interestingly, the intensity of the reaction was obviously associated with the ELDKWA-epitope number on a fusion protein. This effect was clearly observed in four dilutions of these three antisera (dilution up to 1∶6400) (Fig. 2).

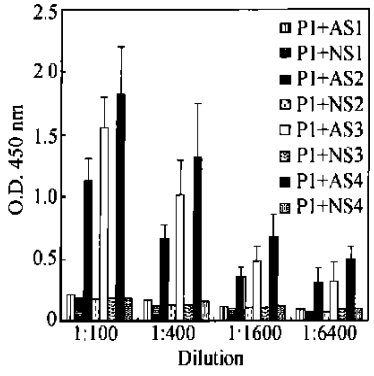

Fig. 2. Detection of epitope-specific antibodies in ELISA-assay. Peptide P1 (CGELDKWASLWNWFNIT) was coated on a microtiter plate. The specific binding of epitope-specific antibodies was detected by peroxidase-conjugated rabbit immunoglobulins to mouse IgG. The antisera AS2, AS3 and AS4 were induced by three fusion proteins bearing 1, 4 and 8 copies of ELDKWA-epitope respectively, while AS1 to GST (excluding ELDKWA-epitope) served as control. The pre-immune sera NS1, NS2, NS3 and NS4 served as normal sera control.

To confirm the effect of the repeated epitope, we examined the binding of the ELDKWA-specific antibodies in antisera AS2, AS3 and AS4 to the transfected CHO-WT cells that stably express HIV-1 envelope glycoprotein on the cell surfaces. Flow cytometry analysis demonstrated that the antibodies in antisera AS2, AS3 and AS4 could bind to the HIV-Env$^+$ CHO-WT cells, indicating that these antibodies induced by three fusion proteins could recognize the native HIV-1 envelope proteins. As expected, the intensity of the antibody binding to these cells was obviously associated with the ELDKWA-epitope number on a fusion protein molecule. The enhancement of antibody response by repeated epitope was clearly observed in the three antisera (Fig. 3). These experimental results indicated that repeated epitope in the recombinant epitope-peptide could enhance ELDKWA-epitope-specific antibody response, which could contribute to designing an effective recombinant epitope-vaccine.

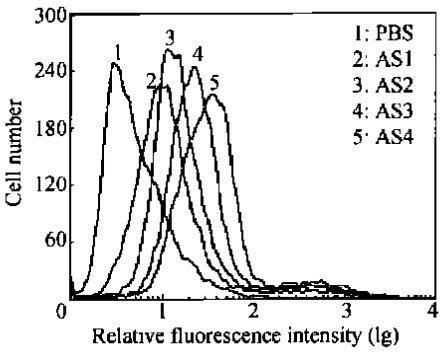

Fig. 3. Detection of epitope-specific antibodies binding to HIV-1 envelope glycoprotein expressed on CHO-WT cells in flow cytometry analysis. The antisera AS2, AS3 and AS4 were induced by three fusion proteins bearing 1, 4 and 8 copies of ELDKWA-epitope respectively, while AS1 to GST (excluding ELDKWA-epitope) and PBS served as control. The antisera diluted in PBS (1∶100) were incubated with HIV-Env$^+$ CHO-WT cells and the epitope-specific antibodies were detected by FITC-conjugated rabbit anti-mouse IgG (1∶50 dilution in PBS) on a FACSCalibur. Curve 1, PBS instead of the antiserum was incubated with the HIV-Env$^+$ CHO-wt cells at first, and then incubated with FITC-conjugated rabbit anti-mouse IgG.

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