The Role of recombinant fusion peptide HIV-1 in induce immunological response in BALB/c mice


Abstract— It is believed that vaccines that induce high level of cellular and humoral immunity reactive with a variety of epitopes derived from multi HIV antigens will be components of effective HIV vaccine Objective: In the present study we evaluated immunological response of recombinant fusion peptid gp41-p24 in BALB/c mice. Methods: The recombinant peptid gp41-p24 had been produced by cloning methods. This peptid was refolded with dialyzed for removal of urea 1 M with Hepese buffer 20 mM . There were one test group and one control group of Female BALB/c (H-2d) mice 6-8 weeks of age. Five mice in each group received 40µg of refolded peptid gp41-p24 and control group received PBS buffer. The immune response against this fusion peptid was evaluated using antibody titration and cytokines (IL-2 and IFN-γ) level with ELISA and Lymphocyte proliferation assays with LTT assay. Results: The maximum level of antibody secretion was observed after receiving the secondary booster in the test group. Cell proliferation and IL-2 were high after the first boost in test group. Level of IFN-γ secretion was significantly more than control group and there was not any significance between first booster and second booster (p<0.05). Conclusion: According to these data from the mouse model, we conclude that gp41-p24 fusion peptid can be used as immunogen for induction of Humoral and cellular responses against HIV-1.

Keywords— gp41-p24, IL-2, IFN-γ, LTT.

I. INTRODUCTION

It is believed that vaccines that induce high level of cellular and humoral immunity reactive with a variety of epitopes derived from multi HIV antigens will be components of effective HIV vaccine (1). The viral envelope glycopeptide (encoded by the env gene), including external glycopeptide gp120 and trans-membrane peptid gp41, plays a critical role in the viral entry processes that leads to infection (2). The basic genes of the virus consist of gag, pol and env; the trans-membrane peptid gp41 of HIV-1 is the most important antigen for sensitive and specific detection of anti-HIV antibody (3). Effective anti-retroviral therapy has attenuated the expansion of the epidemic in some industrialized countries. Promising studies of candidate vaccines in animal models have provided reasons to hope that developing a safe and effective AIDS vaccine will be possible (4). Development of a safe and effective vaccine against HIV-1 is the focus of worldwide efforts. Numerous vaccine modalities have been tested in animal models, including soluble recombinant peptid (5). The majority of vaccine candidates evaluated have included some from of the HIV-1 envelope glycopeptide (6, 7). Recombinant antigen comprising both gag and env-coded peptides able to elicit immune response in animals was evaluated. Animals have reported to develop strong humoral immune response to both p24 and gp41 parts of chimeric antigen (8). Recombinant peptid V3-gp41 gave the best antibody response (9). For eliciting immunity towards HIV faces major problems including the variability of incoming viruses and local immunomodulation induced by HIV infected cells so an effective HIV vaccine should contain as many HIV peptides as possible, including structural, regulatory peptides, in this situation protective mechanism seems to be strong and broad (10).

The aim of this study was evaluation of immune responses against recombinant fusion gp41-p24 peptide (HIV-1) consisting of the two immunologic part of HIV-1 gp41 and p24 sequences in mouse model

II. MATERIALS AND METHODS

A. Preparation of Recombinant fusion peptide gp41-p24

Preparation of Recombinant fusion peptide gp41-p24. Recombinant fusion peptide gp41-p24, as antigen, had been produced by cloning methods (3). This peptid was obtained from Pasture Institute (Tehran, Iran). To refold the recombinant fusion peptide gp41-p24, the dilution methods. An appropriate volume of Hepes buffer (pH 7.0) was added gradually to the peptid for reducing urea from 8 to 1 M.
Consequently, the peptide was dialyzed for removal of urea 1 M (3, 11).

B. Animals and immunization protocols

Female BALB/c (H-2d) mice 6-8 weeks of age were purchased from Pasteur Institute of Iran, Tehran, Iran, and were housed in the animal facility on standard rodent diet and allowed to acclimate for at least 1 week prior to this study. All experiments involving mice were done under strict compliance with the guidelines established by Pasteur institute of Iran. There were one Test group and one control groups of mice in this study, the number of mice was 30;15 controls and 15 for test groups, each one of the 15 groups in the three different times were analyzed, it means that at each days of 28, 42 and 56, five test mice, totally 15 test mice were studied. Also 15 mice were for control groups with similar timing program.

In the table 1 the number of studied mice at each group and the time of our study were included in the result.

On day 0, mice in the Test group (n=15) was primed by a single intramuscular (IM) injection with 40µg recombinant peptide gp41-p24 per mouse. On day 0, mice in control group (n=15) was primed by a single IM injection with PBS alone. Test and control groups were single boosted with the same dose given IM injection on day 14. Also, the Test group (n=5) and control group (n=5), were two boosted with the same doses given IM injection on day 28 (2, 11). In total, there were three groups of mice in the study (Table 1).

C. Determination of antibody responses after immunization

Sera of immunized mice were collected two weeks after last immunization. Micro plates (Fisher) were coated using coating buffer (PBS) to gather with 10 µg/ml of recombinant peptide gp41-p24 and incubated overnight at 4°C. The plates were washed with PBS buffer containing 0.05% Tween 20 (PBS-T) and blocked with 1% BAS in PBS buffer for 2 h at 37°C. sera of vaccinated mice and control group were diluted 1/100 in washings buffer and added to the wells (100µl per well) and the plates incubated 1h at 37°C. the wells were washed five times with washing buffer and Goat Anti-mouse IgG (Sigma) diluted to 1:10,000 in PBS buffer containing 0.5% BSA was added (100 µl per well), and incubated for 1h at 37°C. The wells washed with PBS-T and incubated with TMB reagent (Sigma) for 15 min at room temperature. The reaction was terminated with 2 N H2SO4 and the plates were measured for absorbance at 450 nm (12).

D. Lymphocyte proliferation assay

Under sterile conditions, spleens were removed and cell suspension was prepared. By passing tissues through a sterile mesh Cells so Red Blood Cells were osmotically lysed using ammonium chloride buffer (NH4CL 0.16 M, Tris 0.17 m). Cells suspension were washed twice with RPMI 1640 with centrifuge and counted and suspended in complete medium (RPMI 1640 supplemented with 10% fetal calf serum), (Gibco-BRL, Gaithersburg, MD), penicillin (100 U/ml), streptomycin (100 mg/ml), and 2 mM L-glutamine and 50 µM 2-mercaptoethanol. Splenocytes were cultured in triplicate (2×105 cells/well) in 96-well flat-bottom micro titer plates. Stimulated with purified recombinant peptide gp41-p24 (12µg/ml) or concanavalin A (1 µg/ml; Sigma), or medium alone (as negative control) and incubated at 37o C under 5% CO2 (13). After 5 days, the cultures were pulsed with 1µci of [3H] thymidine (5 ci/mm; Amersham, Arlington Heights, Ill). For 18h and harvested and cell proliferation was evaluated by liquid scintillation counting. Results are expressed as mean counts per minute (cpm) of stimulated less that of negative control (Δcpm), and stimulation index (SI, the ratio of the mean counts per minute of antigen-stimulated to that of negative control (13, 14).

E. Cytokine assay (IL-2 and IFN-γ) by ELISA method

Cytokine levels in culture supernatants were determined by ELISA assay. We used detection kit (R&D Systems, Minneapolis, MN,) for assaying cytokine levels IL-2 and IFN-γ (15). Briefly, 96-well flat-bottom plates were coated with 100 µl of anti-cytokine antibodies diluted in buffer, as specified by the manufacturer, and incubated overnight at 4°C. The wells were washed with PBS plus 0.05% Tween 20 (PBS-T) and blocked at 37o C for 2h with PBS 1% bovine serum albumin. Serial two fold dilution of supernatants appropriate dilutions of standard cytokines were added in duplicate and incubated at 37o C for 1 to 2 h. The wells were washed with PBS-T and incubated with the specific biotinylated anti-cytokine antibody diluted in PBS-T plus 1% BSA for 1 to 2h. After 3 or 4 washes, the wells were incubated with horseradish peroxides-conjugated streptavidin at 37°C for 15 min and developed with TMB reagent (Sigma). The reaction was terminated with 2N H2SO4 and the absorbance values were measured at 450 nm (9, 13).
**F. Statistical analysis**

Statistical analyses were performed with Sigma Stat, SPSS 12, 2005 and Prism 2, 2003. Standard deviations of the mean were calculated and statistically significant analysis (P<0.05) was considered significant by Student’s t-test.

![Statistical analysis](image)

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**III. RESULTS**

**A. Production of recombinant peptide gp41-p24**

After production and purification of recombinant peptide gp41-p24, refolding process with Hepese buffer 20 mM has been performed. SDS-PAGE analysis confirmed the presence of fusion peptide (figure1).

**B. Recombinant peptide gp41-p24 specific Antibody titration after immunization**

The average level of antibody production against recombinant peptide gp41-p24 was measured by INDIRECT ELISA method. The results showed that the level of antibody secretion was high after the first boost as well as the second boost but significantly (p<0.05) difference with those that were injected only once and there was not any significance between first booster and second booster (figure3).

![Cellular response toGp41-p24 fusion peptide in immunization in the different groups of BALB/c mice](image)

**C. Proliferation of cells after immunization.**

The cell proliferation assay was performed in all groups of BALB/c mice as mentioned in materials and methods. Our results the proliferation of total cells from spleen showed the highest in the test group that received recombinant fusion peptide gp41-p24. Also It was shown that the cell proliferation also is high after the first boost as well as the second boost but significantly (p<0.05) difference with those that were injected only once and there was not any significance between first booster and second booster (figure3).

![Proliferation of cells after immunization](image)

**D. Cytokines secretion after immunization (IL-2&IFN-γ).**

The average level of IL-2 secretion after injection with recombinant peptide gp41-p24 in different groups of BALB/c mice was calculated. The results showed that the maximum level of IL-2 secreted was in test group compared to control group (p<0.05) Also It was shown that the IL-2 level also is high after the first boost as well as the second boost significant compared to control group (p<0.05) with those that were injected only once and there was not any significance between first booster and second booster (figure4). The average level of IFN-γ secretion after immunization with recombinant peptide gp41-p24 in different groups of BALB/c mice was calculated. The results showed that the level of IFN-γ secretion in the test group (figure5). Was significantly than control group and there was not any significance between first booster and second booster. (p<0.05).

![Cytokines secretion after immunization](image)

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**Fig. 1.** The SDS-PAGE (12%) shows molecular weight of unfolded & refolded peptides. Lane 1 and lane 2 respectively. The molecular weight of these peptides are 32KD M w as comparison with peptide molecular weight marker(lane 3).

**Fig. 2** Gp41-p24 fusion peptide specific Antibody responses induced by vaccination of BALB/c mice

**Fig. 3** Cellular response toGp41-p24 fusion peptide in immunization in the different groups of BALB/c mice

**Fig. 4** IL-2 production in vaccinated mice and control group.
The HIV recombinant peptide can be used as an immunogen and inducer of humoral and cellular immune systems (6, 12, 16). In several studies, it has been described that HIV recombinant fusion peptides, specially ectodomain of gp41 from HIV-1 (11) in different doses and proper time using prime/boost method approved for use in mice and induced potent immune responses (both cellular and humoral) in animal models. The immunogenicity in mice of a recombinant fusion peptide gp41- HA consisting of the ectodomain of the HIV envelope glycopeptide gp41 fused to a fragment of the influenza virus HA2 hemagglutinin peptide (11). Another study they researched the ability of immune induction with peptide antigens env gp120 and p24 gag. All of the studies show that antibody response increased considerably after the second boosts.

(2). The result of clinical trials of candidate AIDS vaccines have confirmed that in order to ensure a broad immune response, an effective vaccine may have to express multiple HIV-1 peptides and induce both cellular and humoral responses with a large repertoire of specificities (17, 18, 19).

In this study we evaluated the humoral and cellular immune responses against Gp41-p24 fusion peptide. We observed that Gp41-p24 fusion peptide induced high level of humoral and cellular immunity compared to control group. This study has shown that Gp41-p24 fusion peptide can induce a significant increase in the lymphocyte proliferative response to fusion peptide compared to control group and because the lymphocyte proliferative response is generally related to the cell mediated immunity so Gp41-p24 fusion peptide could able stimulate cellular immunity (20, 21). The result of cytokine assay revealed that Gp41-p24 fusion peptide increase IFN-γ and IL-2 secretion significantly compared to control group. IFN-γ and IL-2 have critical role in induction of cellular (Th1 shifting) immunity and they are so important in prevention of AIDS progression (20, 22).

Antibody response represents humoral immunity to Gp41-p24 fusion peptide. The result of antibody titration showed that Gp41-p24 fusion peptide can increased specific IgG compared to control group. Humoral immunity in the balance of cellular immunity is so important in response to HIV infection. (22,23)

In conclusion, the results of study have shown that Gp41-p24 fusion peptide can stimulate humoral and cellular immunity. This was a pilot study and further studies using an adjuvant and combination it for enhance of induce immune responses in animal models.

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REFERENCES


