Induction of mucosal and systemic immunity after intranasal immunization of HIV-1 gp120 and gp41 peptides with cholera toxin and neoadjuvant in a mouse model

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Abstract

Induction of mucosal and systemic immunity after intranasal immunization of HIV-1 gp120 and gp41 peptides with cholera toxin and neoadjuvant in a mouse model

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(Directed by Professor June Myung Kim)

Background: Mucosa is the main route for HIV to enter a human, therefore mucosal immunity is an important target for HIV vaccine development. Antibodies of the IgA, IgG, and IgM isotypes to the *env* protein of HIV are present in the cervix and cervicovaginal fluid of women who remain HIV-seronegative despite repeated exposure to the virus, further suggesting that mucosal immunity is protective against sexual transmission of HIV. To develop an effective mucosal vaccine, investigations should focus on an appropriate antigenic epitope, an effective and safe adjuvant, a vaccination schedule, route, and so forth. The aims of this study were to evaluate the immunogenicity of gp120 and gp41 peptides for inducing mucosal and systemic immunity and to prove the adjuvant effects of a mutant cholera toxin.

Materials and Methods: BALB/c mice were intranasally immunized with HIV-1 gp120 peptide (KQINMWQEVGKAMYACTRPNYNKRKRIHIGPGRAFYTTK) and HIV-1 gp41 peptide (EKNEQELLELDKWASLWC) with or without the mucosal adjuvant cholera toxin or nontoxic mutant cholera toxin. Blood was collected from the retro-orbital plexus using heparinized capillary tube, and vaginal wash samples were

collected by washing the vaginal cavity with 100 µL of sterile PBS. ELISA was used to determine the presence of anti-peptide antibodies in serum and vaginal wash samples. Ag-specific ELISPOT assay was performed using spleen cells to determine peptide specific immune responses.

Results: In all groups with gp120 or gp41 peptides, the levels of vaginal IgA significantly increased over the course of 4 weeks. In all groups, the changes of serum IgA levels were not significant. In gp120 group, the changes in serum IgG levels were not significant. In gp120+cholera toxin or mutant cholera toxin groups, the levels of serum IgG significantly increased. In the gp41 group, the changes in serum IgG levels were not significant. In the gp41+cholera toxin or mutant cholera toxin groups, the levels of serum IgG significantly increased. In the groups given gp120 peptide as antigen, the cytotoxic T lymphocyte (CTL) responses were significantly induced when compared to the PBS group. However, CTL responses were not induced in the groups given gp41 peptide. In the groups given gp120 peptide, cholera toxin and nontoxic mutant cholera toxin induced significant adjuvant effects in both humoral and cellular responses. However, in the groups given gp41 peptide, cholera toxin and nontoxic mutant cholera toxin did not induce a significant effect.

Conclusions: HIV-1 peptides, which consist of epitopes on gp120 or gp41, can induce mucosal and systemic immune responses by nasal immunization, and cholera toxin and nontoxic mutant cholera toxin are effective mucosal adjuvants for the induction of mucosal and systemic immunity in a mouse model. To develop proper mucosal vaccines for HIV, further studies are needed for defining epitopes to induce systemic and mucosal immunities, and developing effective and safe adjuvants.

Key Words: HIV, HIV infection, AIDS, mucosal immunity, gp120, gp41, cholera toxin

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I. Introduction

It is estimated that 40 million people worldwide are living with human immunodeficiency virus (HIV) infection[1]. Routes of HIV transmission include sexual contact, transfusion of blood and blood products, sharing of contaminated needles, vertical transmission, and occupational exposures[2]. The main mode of transmission varies according to region or ethnicity. However, the predominant route of HIV transmission is via sexual contact, where HIV is transmitted at the mucosal surface of the genitourinary tract or rectum[3]. Indeed, it is estimated that 70-85% of HIV infections are transmitted sexually[4-6]. HIV transmission may also occur after oral exposure to HIV[7]. Because the mucosa is the most common site of contact between the host and HIV, the induction of a mucosal immune response is likely to be an essential component for protection against HIV infection[8,9]. Antibodies of the IgA, IgG, and IgM isotypes to the *env* protein of HIV are present in the cervix and in the

cervicovaginal fluid of women who remain HIV-seronegative, despite repeated exposure to the virus, further suggesting that mucosal immunity is protective against sexual transmission[10-13].

There have been numerous attempts to produce a protective HIV vaccine which induces both mucosal and systemic immunities[8,18-32]. Although a variety of HIV vaccine immunization protocols have been shown to induce serum neutralizing antibody responses after systemic vaccine administration, immunization via a systemic route rarely induces a mucosal IgA immune response[14,15]. The development of HIV vaccines that induce secretory IgA responses is important for protection against sexual transmission of HIV, since secretory IgA exhibits unique effector functions that are well suited to protecting the mucosal surfaces. Mucosal immunization is known to be the proper method for inducing mucosal IgA responses[16]. Mucosal immunization at one anatomical location has also been shown to induce a detectable response at the site of immunization, as well as in distant mucosal effector tissues, an observation that has led to the term 'common mucosal immunologic system'[17].

Many obstacles have been encountered in designing mucosal vaccines. This includes the weakness in the human secretory antibody response elicited by vaccination at mucosal sites. The oral polio vaccine is the only available mucosal vaccine for human use[15,18,19]. To develop an effective mucosal vaccine, we should focus our investigation on finding the appropriate antigenic epitope, an effective and safe adjuvant, a vaccination schedule, route, and so forth.

A variety of approaches to produce a protective HIV vaccine have been investigated including: attenuated, recombinant bacterial vectors expressing antigenic epitopes from HIV, recombinant adenovirus vectors, recombinant vaccinia virus, hybrid hepatitis particles expressing a V3 loop peptide, DNA vaccines expressing gp120, and synthetic peptides containing T and B cell epitopes of HIV as immunogens[8,15,18-25]. Studies to develop a HIV mucosal vaccine have been conducted using recombinant

SIV proteins or peptides[8,18-22], live attenuated SIVs[23-25], SIV encoded virus or bacterial vectors[26-28], DNA vaccines[29,30], and a prime/boost regimen[31,32]. These studies point to the importance of a mucosal HIV vaccine for the prevention of HIV-1 infection.

HIV can exploit several cellular routes to penetrate the mucosal epithelial layers, including epithelial cells and dendritic cells[33-35]. HIV-1 can directly infect epithelial cells, or contact of HIV-1-infected cells with the apical surface of an epithelial cell can lead transcytosis of HIV-1 across the epithelial barrier[33,34]. And, recent studies support an important role for dendritic cells as the primary route of mucosal HIV-1 transmission[36-38].

The hybrid C4/V3 HIV peptide with T helper, cytotoxic T, and B cell epitopes from HIV-1 gp120 have been shown to elicit a specific neutralizing antibody response, as well as an HLA restricted CTL response, when administered systemically[39-42].

Mucosal secretions in exposed, uninfected women contain IgG antibodies to the ELDKWA sequence of gp41 that is recognized by the protective monoclonal antibody 2F5[43,44].

Nasal immunization is an effective approach for the induction of both mucosal and systemic immune responses[45]. Nasal immunization with peptide vaccines, together with mucosal adjuvant, more effectively induces mucosal immunity in the female reproductive tract than oral immunization does.

Although native cholera toxin is a potent mucosal adjuvant, it is not practical for use in humans because of its toxicity. Nasal application of cholera toxin B subunit or native cholera toxin results in its accumulation in the olfactory bulbs of the CNS through GM1 binding and in its subsequent retrograde axonal transport into the olfactory neurons[46]. Furthermore, native cholera toxin is known to induce high levels of total and antigen-specific IgE response due to the nature of IL-4 dependent adjuvanticity[47,48]. To overcome these potent pathological problems, a mutant of cholera toxin (mCT; E112K) that retains the adjuvant properties, minus the

toxicity-associated ADP-ribosyltransferase enzyme activity, has been used [49].

The aims of this study were to evaluate the immunogenicity of gp120 and gp41 peptides for inducing mucosal and systemic immunity, and to demonstrate the adjuvant effects of mutant cholera toxin.

II. Materials and Methods

1. Study animals

Female BALB/c mice, weighing 16-18 g, were procured from Samtaco Co., Seoul, Korea. All experimental groups consisted of six animals. All of the animals were provided food and water ad libitum. All experiments were conducted in accordance with the guidelines for the care and use of laboratory animals by the Yonsei University College of Medicine. All procedures for use and care of the mice were approved by Yonsei University's Institutional Animal Care and Use Committee.

2. Peptides and adjuvants used

The sequence of gp120 peptide is as follows: KQIINMWQEV GKAMYACTRPNYNKRKRIHIGPGRAFYTTK. The gp120 peptide contains, at the amino terminus, sequences from HIV gp120MN between amino acids 428-443 (KQIINMWQEVGKAMYA), previously shown to evoke CD4 T helper cell responses in mice[50,51]. This sequence has been linked to a carboxyl-terminal sequence derived from the third variable (V3) domain of HIV-1 gp120 (amino acids 320-324), which contains both a principal neutralizing determinant (amino acids 302-319) and a site designated as recognized by CD8+ cytotoxic T lymphocytes in BALB/c mice (amino acids 320-324;FYTTK)[52]. The sequence of gp41 peptide is as follows: EKNEQELLELDKWASLWC. This peptide corresponds to the 659-675 amino acid sequence of the gp41 protein of HIV-1 with the addition of a C-terminus-cysteine residue[43,44]. All peptides were commercially synthesized in Peptron Inc. (Daejeon, Korea). Peptides were solubilized in water with 25% acetic acid and dialyzed extensively against 25% acetic acid in distilled water. The peptide solution was then dialyzed twice against two liters of distilled water. The insoluble material was removed by filtration across a 0.45 μm filter, and the soluble material was then

lyophilized and stored at 4℃ until used.

Nontoxic mutant cholera toxin E112K was kindly provided by Dr. Hiroshi Kiyono of University of Tokyo, Japan.

3. Immunization

The mice were divided into seven groups. Group 1 mice were intranasally immunized with gp120 peptide and native cholera toxin, group 2 mice with gp120 peptide, group 3 with gp41 peptide and native cholera toxin, group 4 with gp41 peptide, group 5 with gp120 peptide and nontoxic mutant cholera toxin, group 6 with gp41 peptide and nontoxic mutant cholera toxin, and group 7 with PBS. The mice were intranasally immunized on day 0, 7, 14, and 21, with 50 μg of peptides, with or without 1 μg of native or nontoxic mutant cholera toxin. Antigen preparations were diluted to the appropriate concentration in sterile PBS, and 10 μL of the antigen mixture was introduced into each nostril (Figure 1). The mice were monitored until they regained consciousness.

Figure 1. Intranasal immunization of BALB/c mouse with HIV Ag and adjuvant.

4. Sample collection

Bloods and vaginal wash samples were collected on day 7, 14, 21, and 28 before nasal immunization. Blood was collected from the retro-orbital plexus using a heparinized Natelson capillary tube while the mice were under isoflurane anesthesia. Serum was obtained by standard methods and stored at -80℃ until use.

Vaginal samples were collected by washing the vaginal cavity with 50 μL of sterile PBS in and out of the vagina gently until a discrete clump of mucus was removed while the mice were under isoflurane anesthesia. This usually took four to eight

cycles of pipetting. A second vaginal wash with 50 µL of PBS was then done to ensure a more complete recovery of the vaginal secretion, and this material was combined with the first wash. Vaginal samples were centrifuged at 12,000 × g for 10 min shortly after collection in a microcentrifuge to separate the mucus from the PBS wash solution. The mucus and supernatant were then frozen separately at -80℃. The PBS wash solution contained a cocktail of proteinase inhibitors (153.8 nM aprotinin, 3.2 µM bestatin, and 10 µM leupeptin). In order to obtain complete recovery of IgA from the vaginal mucus, samples were thawed and extracted twice for 2 h each time in 100 µL of PBS per sample, with rotation at 20 rpm in a 12 mL polystyrene tube at 4℃. The two extracts and the original wash supernatant were pooled, up to 300 µL per sample, and stored at -80℃ until assayed for antibodies[53].

5. Cell isolation

Lymphocytes were removed from spleen. The spleen was teased apart using sterile forceps and passed through a sterile screen to obtain single-cell suspensions. The red blood cells were removed from the cell suspension using ammonium potassium chloride lysis buffer for 10 min.

6. Serological assays

The presence and titers of IgG and IgA antibodies, specific for the gp120 and gp41 peptides used in the immunization protocols, were evaluated in blood and vaginal wash samples obtained from the immunized mice by an enzyme-linked immunosorbent assay (ELISA). HIV-1 peptides were coated onto 96-well microtiter plates at 1 µg/mL in PBS. After an overnight incubation at 4℃, the contents of the wells were discarded, and nonspecific protein binding was blocked by incubation with 5% (wt/vol) dry milk in PBS for 1 h at room temperature. Diluted mouse sera or vaginal wash samples were added to the wells and incubated for 1 h at room

temperature. After three washes, wells were treated for 1 h at room temperature with peroxidase conjugated goat anti-mouse IgG or IgA. After three additional washes, the reaction was visualized with 0.075% 4-chloro-1-naphthol in 0.056% hydrogen peroxide and then stopped with 2N sulfuric acid. Absorbance was determined at 492 nm.

7. CTL assays

An enzyme linked immunospot (ELISPOT) assay was performed to establish the number of IFN-γ producing cells. Nitrocellulose membranes in 96-microwell polyvinylidene difluoride-backed plates were coated overnight at 4℃ with 50 μL of anti-mouse-IFN-γ monoclonal antibody. The antibody-coated plates were then washed four times with PBS and treated with 180 μL RPMI-1640 medium containing 10% heat inactivated fetal calf serum for 1 h at 37℃. The responder cells for this assay were spleen cells. A total of 2×10^5 of these spleen cells were incubated overnight, at 37℃ in 5% CO2, with HIV-1 peptides (10 μL/mL) in nitrocellulose membrane 96-well plates. The plates were washed four times with PBS containing 1% BSA and 2 μL/mL of the secondary antibody (biotin-conjugated anti-mouse-IFN-γ monoclonal antibody) were added to each well; the plates were then incubated for 2 h at 37℃ in CO2. The plates were washed four times with PBS containing 1% BSA and then treated with avidin-bound, biotinylated horseradish peroxidase H for 1 h at room temperature. The plates were then washed an additional three times with PBS containing 1% BSA and three times with PBS alone, followed by a 5 min incubation with 100 μL of 3-amino-9-ethylcarbazole per well. The reaction was stopped with running tap water. Red-brown spots, representing single cell producing IFN-γ, were counted using a dissecting microscope. Spleen cells stimulated with phytohemagglutinin were used as a positive control. The number of antigen-specific spleen cells producing IFN-γ was calculated by subtracting the number of

spot-forming cells in the medium control from the peptide-stimulated cells.

8. Statistical analysis

SPSS 11.0 software package was used for statistical analysis. The Mann-Whitney *U* test, Kruskal-Wallis test, and Friedman test were used to determine the significance of differences between groups. All *p* values were two tailed and a *p* value of <0.05 or less was considered significant.

III. Results

1. Vaginal immunoglobulin

In the gp120 group, the levels of vaginal IgA significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 190, 200, 200, and 250 ng/mL, respectively, p=0.001, Figure 2). In gp120+cholera toxin group, the levels of vaginal IgA significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 115, 170, 235, and 610 ng/mL, respectively, p=0.001). In gp120+mutant cholera toxin, the level of vaginal IgA significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 135, 252, 335, and 728 ng/mL, respectively, p=0.041).

In the gp41 group, the levels of vaginal IgA significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 200, 329, 603, and 715 ng/mL, respectively, p=0.007). In gp41+cholera toxin group, the levels of vaginal IgA significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 135, 242, 410, and 635 ng/mL, respectively, p=0.007). In gp41+mutant cholera toxin, the level of vaginal IgA also increased significantly (median values at 1, 2, 3, and 4 weeks after immunization; 87, 224, 600, and 712 ng/mL, respectively, p=0.011, Figure 2).

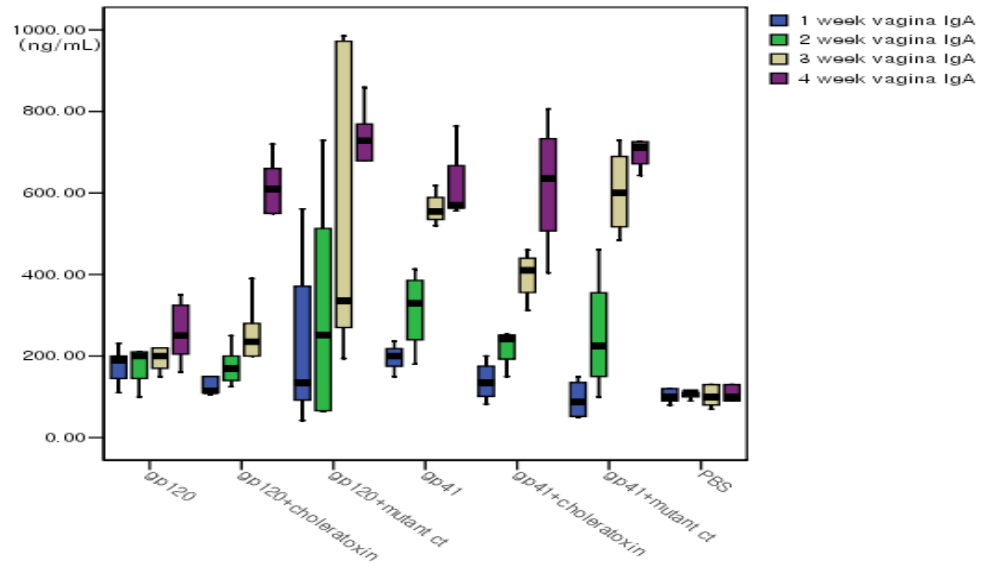

Figure 2. Changes of specific IgA levels in vaginal washing fluids.

2. Serum immunoglobulin

In all groups, the changes of serum IgA levels were not significant (p>0.05, Figure 3).

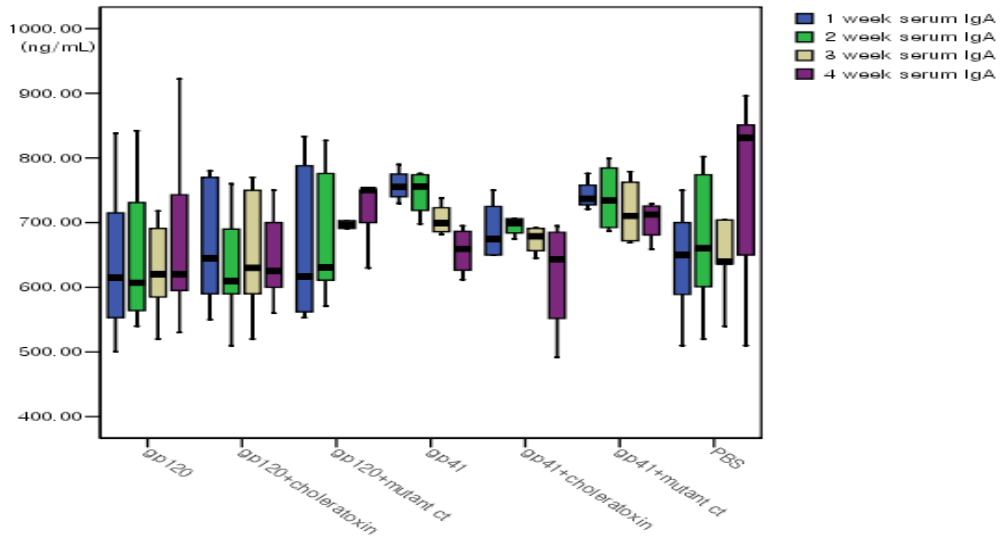

Figure 3. Changes of specific IgA levels in serum.

In the gp120 group, the changes in serum IgG levels were not significant (median values at 1, 2, 3, and 4 weeks, 324, 330, 340, and 355 ng/mL, respectively, $p>0.05$, Figure 4). In the gp120+cholera toxin group, the levels of serum IgG significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 310, 317, 372, and 411 ng/mL, respectively, $p=0.001$). In the gp120+mutant cholera toxin, the levels of serum IgG significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 321, 331, 356, and 405 ng/mL, respectively, $p=0.004$).

In the gp41 group, the changes in serum IgG levels were not significant (median values at 1, 2, 3, and 4 weeks after immunization; 394, 400, 406, and 414 ng/mL, respectively, $p=0.552$). In the gp41+cholera toxin group, the levels of serum IgG significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 385, 396, 411, and 437 ng/mL, respectively, $p=0.029$). In the gp41+mutant cholera toxin, the level of serum IgG significantly increased (median values at 1, 2, 3, and 4 weeks after immunization; 393, 404, 427, and 455 ng/mL, respectively, $p=0.011$, Figure 4).

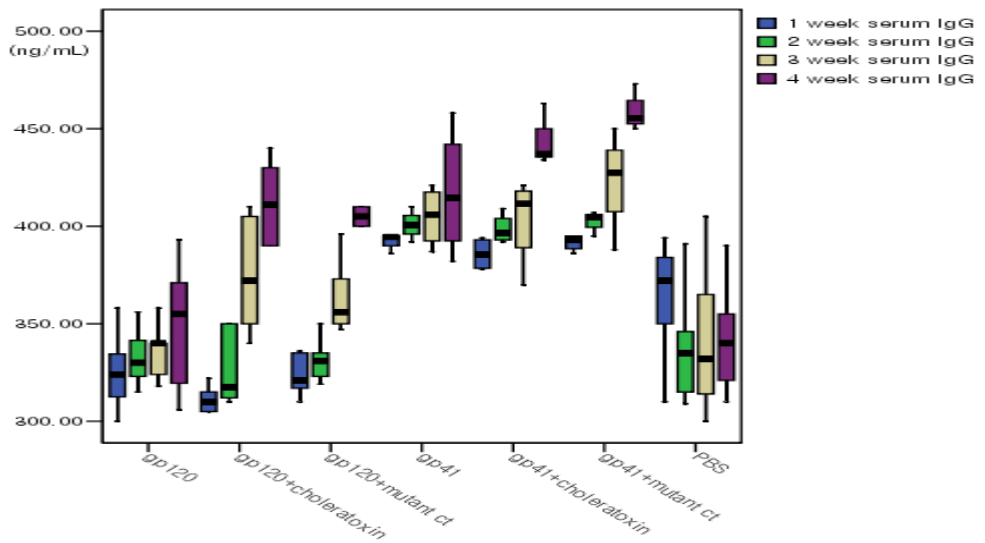

Figure 4. Changes of specific IgG levels in serum.

3. Cytotoxic T lymphocyte responses

In the groups using gp120 peptide as antigen, the cytotoxic T lymphocyte (CTL) responses were significantly induced compared to the PBS group (p<0.05). However, CTL responses were not induced in the groups using gp41 peptide (Figure 5).

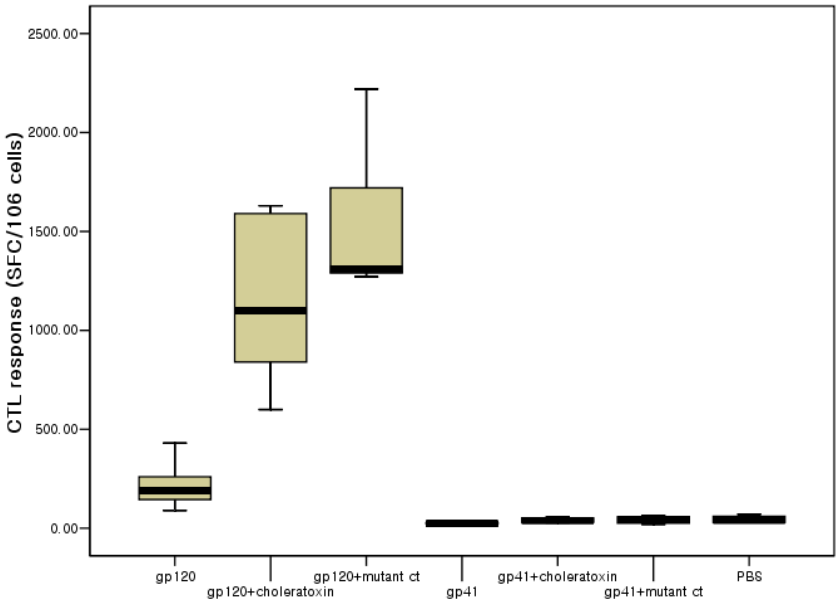

Figure 5. Cytotoxic T lymphocyte responses in spleen cells.

4. Effects of adjuvants

In the groups using gp120 peptide, both native and mutant cholera toxin induced a significant increase in vaginal IgA and serum IgG (Table 1). Also, both forms of the cholera toxin induced significant CTL responses.

However, in the groups using gp41 peptide, neither form of the cholera toxin induced significant adjuvant effects.

Table 1. Differences in humoral and cellular immune responses according to the use of adjuvants

	without adjuvant	with cholera toxin	with nontoxic mutant cholera toxin	*p* value
gp120				
vaginal IgA at 4 weeks (ng/mL)	250	610	728	0.002
serum IgA at 4 weeks (ng/mL)	620	625	750	0.222
serum IgG at 4 weeks (ng/mL)	355	411	405	0.005
CTL (SFC/10^6cells)	190	1,100	1,310	0.001
gp41				
vaginal IgA at 4 weeks (ng/mL)	570	635	712	0.436
serum IgA at 4 weeks (ng/mL)	659	643	712	0.114
serum IgG at 4 weeks (ng/mL)	414	437	455	0.145
CTL (SFC/10^6cells)	24	39	42	0.166

IV. Discussion

This study provides evidence that HIV-1 peptides, which consist of epitopes in gp120 or gp41, can induce mucosal and systemic immune responses by nasal immunization. Also, this study indicates both cholera toxin and nontoxic mutant cholera toxin are effective mucosal adjuvants for the induction of mucosal and systemic immunity in gp120 group. The 40-amino acid gp120 synthetic peptide contains a T helper, a neutralizing B cell, as well as CTL epitope from gp120 of HIV-1[39-41,52]. It has been previously shown that the hybrid C4/V3 HIV peptide containing T helper, CTL, and neutralizing B cell epitopes from HIV-1 gp120 induced type-specific neutralizing antibody response as well as class-I restricted CTL responses when administered systemically[39-42]. ELDKWA on HIV-1 gp41 is a conserved epitope recognized by one broadly neutralizing monoclonal antibody 2F5, which is a promising candidate target for vaccine design[43,44]. The gp41 peptide, corresponding to the 659-675 amino acid sequence with the addition of a C terminus-cysteine residue, contains the ELDKWA epitope. These peptides are selected among candidates which induces immune responses in a mouse models. The antibody reactivity to ELDKWA-epitope on the C-domain of gp41 is associated with disease progression in children perinatally infected with HIV-1, and at least 80% of children have not detectable antibody reactivity to this epitope, indicating that the ELDKWA determinant could be an important component in the formulation of a vaccine[54]. Sequence analysis of primary isolates suggests that the major determinant of monoclonal antibody 2F5 binding corresponds to the amino-acid sequence LDKW. Naturally occurring and in vitro selected neutralization-resistant viruses contained changes in the D and K positions of the ELDKWA motif, and the amino-acid changes from D to N, D to E, and K to N caused abrogation of 2F5-binding to the ELDKWA epitope. The restricted antigenic variability of the ELDKWA-epitope enables ELDKWA-epitope to be developed as an effective epitope/peptide-vaccine.

Vaccination at a single site would provide both humoral and cell-mediated protection, not only at the relevant mucosal surface, but also throughout the body. In this regard, nasal vaccination has shown particular potential. In mice, monkeys and humans, nasal administration of vaccines has induced specific mucosal IgA antibody responses in the salivary glands, upper and lower respiratory tracts, male and female genital tracts, and the small and large intestines[55]. The nasal route can also induce CTLs in distant mucosal tissues including the female genital tract[16,17,55]. In addition, nasal immunization studies in humans and mice produced greater systemic antibody responses than other mucosal immunization routes[55], presumably because antigens or antigen-presenting cells were readily trafficked to draining lymph nodes from this site. In mice and monkeys, nasal immunization with certain live viral vectors generated systemic antiviral CTLs and IgG at concentrations that were comparable to those induced by parenteral vaccination routes[55,56]. Although nasal immunization might be particularly effective for protection against respiratory pathogens, optimal protection of the gastrointestinal tract, the rectum and female genital tract might still require oral, rectal or vaginal vaccines.

HIV might be considered a mucosal pathogen because transmission occurs mainly through exposure of mucosal surfaces to HIV and HIV-infected cells. Mucosal transmission of simian immunodeficiency virus in non-human primates, and presumably of HIV in humans, can occur without epithelial cell damage to the oral, rectal, and genital mucosa[56,57].

Epithelial cells are not productively infected by HIV, but they serve as gateways for delivering infectious HIV to antigen-presenting dendritic cells and macrophages[58,59]. As mucosal antigen presenting cells interact with local CD4+ T cells, they infect and disable the very cells needed to mount an effective immune response. Infection of local target cells can occur rapidly after deposition of virus on mucosal surfaces[60]. However, dissemination of the virus to regional lymph nodes and other tissues can be

delayed for up to several days, providing a window of opportunity for local control of the infection by mucosal immune effectors[57,61]. In any case, whether transmitted mucosally or injected, HIV and SIV replicate preferentially in mucosal tissues, such as the intestinal mucosa, that are rich in CD4+ T cells[62,63]. Therefore, the ultimate goals of HIV vaccines should be to first interrupt mucosal transmission at its earliest stages, before the virus has crossed the epithelial barrier and infected its first target cell, and then to prevent the establishment of viral reservoirs in mucosal tissues.

To achieve these goals, HIV-specific vaccines must generate multiple immune effectors, including HIV envelope-specific antibodies in mucosal secretions and CTLs and neutralizing HIV envelope-specific antibodies in the mucosa and circulation. Given what we know about the induction of mucosal immune responses, it is unlikely that injected HIV vaccines alone will induce the mucosal responses that are required. Although correlates of mucosal protection are not yet established, there is evidence from highly exposed, uninfected human subjects that mucosal HIV-specific CTLs and IgA antibodies in secretions are associated with resistance to sexually transmitted HIV infection[10,64]. The challenge is to identify the required key effectors and then design a vaccination strategy to induce them.

Although HIV-1 peptides have been shown to induce neutralizing antibodies, as well as CTL responses when administered systemically, mucosal immune responses are rarely induced with this method. To induce mucosal immune responses to HIV, mice were immunized with the HIV peptides and the mucosal adjuvants by an intranasal route. Intranasal immunization with HIV-1 synthetic peptides plus adjuvants induced both high serum IgG responses as well as vaginal IgA responses. Therefore, intranasal immunization with HIV peptides is as effective at inducing serum IgG responses as systemic immunization, but has the advantage of inducing vaginal IgA responses. Another advantage of intranasal immunization is that this route is noninvasive and thus eliminates the use of needles.

The use of cholera toxin induced high titered serum IgG and CTL responses as well as vaginal IgA responses. The mechanism by which cholera toxin acts as a mucosal adjuvant is not clear. Several reports suggest that the adjuvant action of cholera toxin may be related to its ability to prime T cells to the coadministered antigen, increase permeability to luminal antigens, and stimulate production of the costimulatory cytokines IL-1 and IL-6 by epithelial cells[65-68]. Due to its side effects, the use of cholera toxin as an adjuvant in humans seems unlikely. Thus, extensive effort has been expended on creating genetically manipulated, nontoxic mutants of cholera toxin that would retain adjuvanticity, but not toxicity[69]. In the current study, we sought to examine the mucosal adjuvanticity of mutant cholera toxin E112K as a nasal adjuvant when coadministered to mice with HIV-1 gp120 or gp41 peptides. In this study, the nasal application of mutant cholera toxin as a mucosal adjuvant effectively induced HIV-1 gp120 peptide-specific antibody responses in both mucosal and systemic immunity, and cellular immune responses of spleen cells. The differences of adjuvant effects between peptides were not clearly understood, but the specific relationships between peptide and adjuvant could be presumed. Further studies are needed for exam the differences of adjuvant effects between peptides.

This study provides evidence that nontoxic mutant cholera toxin E112K is an effective mucosal adjuvant for the induction of HIV-1-specific immunity in the mouse model. Nontoxic mutant cholera toxin E112K retain adjuvant properties despite lacking the ADP-ribosyltransferase enzyme activity associated with toxicity[49,69]. These findings reveal the efficacy of mutant cholera toxin E112K as a mucosal adjuvant and suggest its potential for use in trial vaccines.

However, native cholera toxin and even its nontoxic mutant forms pose additional dangers when administered via the nasal route. Nasal vaccines using either native cholera toxin or its nontoxic mutant as adjuvant risk entering the CNS because of the proximity of the olfactory nerves/epithelium and olfactory bulbs to the brain. This

potential for neurotoxicity has been a major obstacle for the use of enterotoxin-based mucosal adjuvants in humans intranasally. Neuronal association of cholera toxin B through GM1 ganglioside binding appears to preclude efficient clearing of these enterotoxin-based mucosal adjuvants and causes extended accumulation in neuronal tissues associated with the olfactory tract[70]. These results show that nasally administered cholera toxin derivatives retain some toxicity and are targeted to the CNS, posing a serious obstacle to human use. However, in the previous studies, nontoxic mutant cholera toxin E112K did not elicit any increase in NGF-β expression in the olfactory tissues of non human primates[71]. Only minimal NGF-β1 synthesis was detected in the olfactory CNS tissues of rhesus macaques given mutant cholera toxin E112K as nasal adjuvant.

Although intranasal immunization with HIV-1 peptides and adjuvant induced high serum IgG titers, serum IgA was not induced. Since mice were immunized via a mucosal route, we anticipated the induction of serum IgA responses. Induction of serum IgA responses to intranasally administered antigen varies with the immunizing antigens[72-74]. However, anti-gp120 peptide IgA spot-forming cells were detected in the lymph nodes and spleen of the mouse after intranasal immunization with HIV-1 gp120 peptides with cholera toxin, even though serum anti-peptide IgA responses were not detected[16]. It is possible that high serum IgG responses competed for antigens in the ELISA and inhibited the ability to detect marginal serum IgA responses. Alternatively, the anti-peptide IgA spot-forming cells detected in the lymph nodes may have been transiently migrating through the lymph nodes to mucosal effector sites. This latter hypothesis seems more likely, since anti-HIV-1 peptide IgA responses were detected in vaginal secretions in the absence of serum IgA responses.

In the groups given gp120 peptide as antigen, CTL responses were significantly induced when compared to the PBS group. However, CTL responses were not induced in the groups given gp41 peptide. This results were caused by the differences of

epitopes between peptides. The gp120 peptide contained CTL epitope, but gp41 peptide did not contain any CTL epitopes.

This study has provided evidence the intranasal immunization with HIV-1 gp120 peptide plus a cholera toxin adjuvant is able to induce antigen-specific serum IgG, vaginal IgA, and systemic CTL responses. Intranasal immunization with HIV-1 gp41 peptide can induce antigen-specific serum IgG as well as vaginal IgA responses. It also shows that mCT E112K is an effective mucosal adjuvant when administered with HIV-1 gp120 peptide intranasally. Further studies should be undertaken upon the effective and safe mucosal vaccines for the protection of HIV infections.

V. Conclusion

HIV-1 peptides, which consist of epitopes in gp120 or gp41, can induce mucosal and systemic immune responses by nasal immunization. Cholera toxin and nontoxic mutant cholera toxin are effective mucosal adjuvants for the induction of mucosal and systemic immunity in mouse model. To develop proper mucosal vaccines for HIV, further studies are needed for defining epitopes to induce systemic and mucosal immunities, and developing effective and safe adjuvants.

References

1. UNAIDS and WHO. AIDS Epidemic Update: December 2003. Geneva: UNAIDS, 2002
2. UNAIDS and WHO. Report on the Global HIV/AIDS Epidemic 2002, Geneva UNAIDS, 2002
3. Milman G, Sharma O. Mechanism of HIV/SIV mucosal transmission. AIDS Res Hum Retroviruses 1994;10:1305-1312
4. Kozlowski PA, Neutra MR. The role of mucosal immunity in prevention of HIV transmission Curr Mol Med 2003;3:217-228
5. De Schryver A, Meheus A. Epidemiology of sexually transmitted diseases: the global picture. Bull WHO 1990;68:639-654
6. Hospedales J. Heterosexual spread of HIV infection. Rev Infect Dis 1989;1:663-665
7. Baba TW, Koch J, Mittler ES, Greene M, Wyand M, Penninck D, et al. Mucosal infection of neonatal rhesus monkeys with cell free SIV. AIDS Res Hum Retroviruses 1994;10:351-357
8. Lehner T, Bergmeier LA, Panagiotidi C, Tao L, Brookes R, Klavinskis LS, et al. Induction of mucosal and systemic immunity to a recombinant simian immunodeficiency viral protein. Science 1992;258:1365-1369
9. Lehner T. Innate and adaptive mucosal immunity in protection against HIV infection. Vaccine 2003;21:S2/68-S2/76
10. Mazzoli S, Trabattoni D, Lo Caputo S, Piconi S, Ble C, Meacci F, et al. HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. Nature Med 1997;3:1250-1257
11. Kaul R, Trabattoni D, Bwayo JJ, Arienti D, Zagliani A, Mwangi FM, et al. HIV-1-specific mucosal IgA in a cohort of HIV-1-resistnat Kenyan sex workers. AIDS 1999;13:23-29

12. Devito C, Hinkula J, Kaul R, Lopalco L, Bwayo JJ, Plummer F, et al. Mucosal and plasma IgA from HIV-exposed seronegative individuals neutralize a primary HIV-1 isolate. AIDS 2000;14:1917-1920
13. Kaul R, Plummer F, Clerici M, Bomsel M, Lopalco L, Broliden K. Mucosal IgA in exposed, uninfected subjects: evidence for a role in protection against HIV infection. AIDS 2001;15:431-432
14. Lue C, van den Wall Bake AW, Prince SJ, Julian BA, Tseng ML, Radl J, et al. Intraperitoneal immunization of human subjects with tetanus toxoid induces specific antibody-secreting cells in the peritoneal cavity and in the circulation, but fails to elicit secretory IgA responses. Clin Exp Immunol 1994;96:356-363
15. Lehner T, Tao L, Panagiotidi C, Klavinskis LS, Brookes R, Hussain L, et al. Mucosal model of genital immunization in male rhesus macaques with a recombinant simian immunodeficiency virus p27 antigen. J Virol 1994;68:1624-1632
16. Staats HF, Nichols WG, Palder TJ. Mucosal immunity to HIV-1: Systemic and vaginal antibody responses after intranasal immunization with the HIV-1 C4/V3 peptide T1SP10 MN(A). J Immunol 1996;157:462-472
17. McDermott MR, Bienenstock J. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. J Immunol 1979;122:1892-1898
18. Lehner T, Brookes R, Panagiotidi C, Tao L, Klavinskis LS, Walker J, et al. T- and B-cell functions and epitope expression in nonhuman primates immunized with simian immunodeficiency irus antigen by the rectal rout. Proc Natl Acad Sci USA 1993;90:8638-8642
19. Lehner T, Wang Y, Cranage M, Bergmeier LA, Mitchell E, Tao L, et al. Protective mucosal immunity elicited by targeted iliac lymph node immunization with a subunit SIV envelope and core vaccine in macaques. Nat Med

1996;2:767-775

20. Kubota M, Miller CJ, Imaoka K, Kawabata S, Fujihashi K, McGhee JR, et al. Oral immunization with simian immunodeficiency virus $p55^{gag}$ and cholera toxin elicits both mucosal IgA and systemicIgG immune responses in nonhuman primates. J Immunol 1997;158:5321-5329
21. Imaoka K, Miller CJ, Kubota M, McChesney MB, Lohman B, Yamamoto M, et al. Nasal immunization of nonhuman primates with simian immunodeficiency virus $p55^{gag}$and cholera toxin adjuvant induces Th1/Th2 help for virus-specific immune responses in reproductive tissues. J Immunol 1998;161:5952-5958
22. Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, et al. Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques. Nat Med 2001;7:1320-1326
23. Lohman BL, McChesney MB, Miller CJ, Otsyula M, Berardi CJ, Marthas ML. Mucosal immunization with a live, virulence-attenuated simian immunodeficiency virus (SIV)vaccine elicits antiviral cytotoxic T lymphocytes and antibodies in rhesus macaques. J Med Primatol 1994;23:95-101
24. Quesada-Rolander M, Makitalo B, Thorstensson R, Zhang YJ, Castanos-Velez E, Binerfeld G, et al. Protection against mucosal SIVsm challengein macaques infected with a chimeric SIV that expresses HIV type 1 envelope. AIDS Res Hum Retroviruses 1996;12:993-999
25. Nilsson C, Makitalo B, Thorstensson R, Norley S, Binninger-Schinzel D, Cranage M, et al. Live attenuated simian immunodeficiency virus (SIV)mac in macaques can induce protection against mucosal infection with SIVsm. AIDS 1998;12:2261-2270
26. Buge SL, Richardson E, Alipanah S, Markham P, Cheng S, Nalyan N, et al. An adenovirus-simian immunodeficiency virus *env* vaccine elicits humoral, cellular, and mucosal immune responses in rhesus macaques and decreases viral burden

following vaginal challenge. J Virol 1997;71:8531-8541

27. Benson J, Chougnet C, Robert-Guroff M, Montefiori D, Markham P, Shearer G, et al. Recombinant vaccine-induced protection against the highly pathogenic simian immunodeficiency virus SIV(mac251): dependence on route of challenge exposure. J Virol 1998;72:4170-4182

28. Izumi Y, Ami Y, Matsuo K, Someya K, Sata T, Yamamoto N, et al. Intravenous inoculation of replication-deficient recombinant vaccinia virus DIs expressing simian immunodeficiency virus *gag* controls highly pathogenic simian-human immunodeficiency virus in monkeys. J Virol 2003;77:13248-13256

29. Wang SW, Kozlowski PA, Schmelz G, Manson K, Wyand MS, Glickman R, et al. Effective induction of simian immunodeficiency virus-specific systemic and mucosal immune responses in primates by vaccination with proviral DNA producing intact but noninfectious virions. J Virol 2000;74:10514-10522

30. Fulller DH, Rajakumar PA, Wilson LA, Trichel AM, Fuller JT, Shipley T, et al. Induction of mucosal protection against primary, heterologous simian immunodeficiency virus by a DNA vaccine. J Virol 2002;76:3309-3317

31. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MYA vaccine. Science 2001;292:69-74

32. Dale CJ, Liu XS, De Rose R, Purcell DF, Anderson J, Xu Y, Leggatt GR, et al. Chimeric human papilloma virus-simian/human immunodeficiency virus virus-like-particle vaccines: immunogenicity and protective efficacy in macaques. Virology 2002;301:176-187

33. Moore JS, Rahemtulla F, Kent LW, Hall SD, Ikizler MR, Wright PF, et al. Oral epithelial cells are susceptible to cell-free and cell-associated HIV-1 infection *in vitro.* Virology 2003;313:343-353

34. Meng G, Wei X, Wu X, Sellers MT, Decker JM, Moldoveanu Z, et al. Primary

intestinal epithelial cells selectively transfer R5 HIV-1 to CCR5+ cells. Nat Med 2002;8:150-156

35. Cameron PU, Freudenthal PS, Barker JM, Gezelter S, Inaba K, Steinman RM. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science 1992;257:383-387
36. Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel, et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell;2000:587-597
37. Turville SG, Santos JJ, Frank I, Cameron PU, Wilkinson J, Miranda-Saksena M, et al. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. Blood 2004;103:2170-2179
38. Hu Q, Frank I, Williams V, Santos JJ, Watts P, Griffin GE, et al. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. J Exp Med 2004;199:1065-1075
39. Palker TJ, Matthews TJ, Langlois A, Tanner ME, Martin ME, Scearce RM, et al. Polyvalent human immunodeficiency virus synthetic immunogen comprised of envelope gp120 T helper cell sites and B cell neutralization epitopes. J Immunol 1989;142:3612-3619
40. Hart MK, Palker TJ, Matthews TJ, Langlois AJ, Lerche NW, Martin ME, et al. Synthetic peptides containing T and B cell epitopes from human immunodeficiency virus envelope gp120 induce anti-HIV proliferative responses and high titers of neutralizing antibodies in rhesus monkeys. J Immunol 1990;145;2677-2685
41. Haynes BF, Torres JV, Langlois AJ, Bolognesi DP, Gardner MB, Palker TJ, et al. Induction of HIVMN neutralizing antibodies in primates using a prime-boost regimen of hybrid synthetic gp120 envelope peptides. J Immunol 1993;151:1646-1653

42. Hart MK, Weinhold KJ, Scearce RM, Washburn EM, Clark CA, Palker TJ, et al. Priming of anti-human immunodeficiency virus CD8+ cytotoxic T cells in vivo by carrier-free HIV synthetic peptides. Proc Natl Acad Sci USA 1991;88:9448-9452

43. Muster T, Steindl F, Purtscher M, Trkola A, Klima A, Himmler G, et al. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type I. J Virol 1993;62:6642-6647

44. Conley AJ, Kessler JA II, Boots LJ, McKenna PM, Schleif WA, Emini EA, et al. The consequence of passive administration of an anti-human immunodeficiency virus type I neutralizing monoclonal antibody before challenge of chimpanzees with a primary virus isolate. J Virol 1996;70:6751-6758

45. McGhee JR, Kiyono H. The mucosal immune system. In Fundamental Immunology, 1998 4th Ed. Paul WE, ed. Academic Press, San Diego p909

46. Van Ginkel FW, Jackson RJ, Yuki Y, McGhee JR. The mucosal adjuvant cholera toxin redirects vaccine proteins into olfactory tissues. J Immunol 2000;165:4778-4782

47. Xu-Amano J, Kiyono H, Jackson RJ, Staats HF, Fujihashi K, Burrows PD, et al. Helper T cell subsets for immunoglobulin A responses: oral immunization with tetanus toxoid and cholera toxin ad adjuvant selectively induces Th2 cells in mucosa associated tissues. J Exp Med 1993;178:1309-1320

48. Okahashi N, Yamamoto M, Vancott JL, Chatfield SN, Roberts M, Bluethmann H, et al. Oral immuunization of interleukin-4 (IL-4) knockout mice with a recombinant *Salmonella* strain or cholera toxin reveals that CD4+ Th2 cells producing IL-6 and IL-10 are associated with mucosal immunoglobulin A responses. Infect Immun 1996;64:1516-1525

49. Yamamoto S, Takeda Y, Yamamoto M, Kurazono H, Imaoka K, Yamamoto M,

et al. Mutants in the ADP-ribosyltransferase left of choleta toxin lack diarrheagenicity but retian adjuvanicity. J Exp Med 1997;185:1203-1210

50. Berzofsky JA, Pendleton CD, Clerici M, Alhers J, Lucey DR, Putney SD, et al. Construction of peptides encompassing multideterminant clusters of human immunodeficiency virus envelope to induce in vitro T cell responses in mice and humans of multiple MHC types. J Clin Invest 1991;88:876-884

51. Ahers JD, Pendleton CD, Dunlop N, Minassian A, Nara PL, Berzofsky JA. Construction of an HIV-1 peptide vaccine containing a multi-determinant helper peptide linked to a V3 loop peptide 18 inducing strong neutralizing antibody responses in mice of multiple MHC haplotypes after two immunization. J Immunol 1993;150:5647-5665

52. Palker TJ, Clark ME, Langlois AJ, Matthews TJ, Weinhold KJ, Randall RR, et al. Type-specific neutralization of the human immunodeficiency virus with antibodies to *env*-encoded synthetic peptides. Proc Natl Acad Sci USA 1988;85:1932-1936

53. Parr EL, Parr MB. Immunoglobulin G, plasma cells, and lymphocytes in the murine vagina after vaginal or parenteral immunization with attenuated herpes simplex virus type 2. J Virol 1998;72:5137-5145

54. Liao M, Lu Y, Xiao Y, Dierich MP, Chen YH. Induction of high level of specific antibody response to the neutralizing epitope ELDKWA on HIV-1 gp41 by peptide-vaccine. Peptides 2000;21:463-468

55. Neutra MR, Kozlowski PA. Mucosal vaccines:the promise and the challenge. Nature Immunol 2006;6:148-158

56. Kozlowski PA, Neutra MR. The role of mucosal immunity in prevention of HIV transmission. Curr Mol Med 2003;3:217-228

57. Zhang ZQ, Schuler T, Zupancic M, Wietgrefe S, Staskus KA, Reimann KA, et al. Sexual transmission and propagation of SIV and HIV in resting and activated

CD4+ T cells Science 1999;286:1353-1357

58. Amerongen HM, Weltzin R, Farnet CM, Michetti P, Haseltine WA, Neutra MR. Transepithelial transport of HIV-1 by intestinal M cells: a mechanism for transmission of AIDS. J Acquir Immune Defic Syndr 1991;4:760-765

59. Hu J, Gardner MB, Miller CJ. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoclation and infects intraepithelial dendritic cells. J Virol 2000;74:6087-6095

60. Hu J, Gardner MB, Miller CJ. Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoclation and infects intraepithelial dendritic cells. J Virol 2000;74:6087-6095

61. Spira AI, Marx PA, Patterson BK, Mohoney J, Koup RA, Wolinsky SM, et al. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 1996;183:215-225

62. Veazey RS, Lackner AA. Getting to the guts of HIV pathogenesis. J Exp Med 2004;200:697-700

63. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depleting during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004;200:749-759

64. Kaul R, Rowland-Jones SL, Kimani J, Fowke K, Dong T, Kiama P, et al. New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers. Immunol Lett 2001;79:3-13

65. Clarke CJ, Wilson AD, Williams NA, Stokes CR. Mucosal priming of T-lymphocyte responses to fed protein antigens using cholera toxin as an adjuvant. Immunology 1991;72:323-328

66. Hornquist E, Lycke N. Cholera toxin adjuvant greatly promotes antigen priming of T cells. Eur J Immunol 1993;23:2136-2143

67. Lycke N, Karlsson U, Sjolander A, Magnusson KE. The adjuvant action of cholera toxin is associated with an increased intestinal permeability for luminal antigens. Scand J Immunol 1991;33:691-698

68. Bromander AK, Kjerrulf M, Holmgren J, Lycke N. Cholera toxin enhances alloantigen presentation by cultured intestinal epithelial cells. Scand J Immunol 1993;37:452-458

69. Yamamoto S, Kiyono H, Yamamoto M, Imaoka K, Fujihashi K, van Ginkel FW, et al. A nontoxic mutant of cholera toxin elicits Th2-type responses for enhanced mucosal immunity. Proc Natl Acad Sci USA 1997;94:5267-5272

70. van Ginkel FW, Jackson RJ, Yuki Y, McGhee JR. The mucosal adjuvant cholera toxin redirects vaccine protein into olfactory tissues. J Immunol 2000;165:4778-4782

71. Yoshino N, Lu FXS, Fujihashi K, Hagiwara Y, Kataoka K, Lu D, et al. A novel adjuvant for mucosal immunity to HIV-1 gp120 in nonhuman primates. J Immunol 2004;173:6850-6857

72. Hirabayashi Y, Kurata H, Funato H, Nagamine T, Aizawa C, Tamura S, et al. Comparison of intranasal inoculation of influenza HA vaccine combined with cholera toxin B subunit with oral or parenteral vaccination. Vaccine 1990;8:243-248

73. Katz J, Harmon CC, Buckner GP, Richardson GJ, Russell MW, Michalek SM. Protective salivary immunoglobulin A responses against *Streptococcus mutans* infection after intranasal immunization with S. mutans antigen I/II coupled to the B subunit of cholera toxin. Infect Immun 1993;61:1964-1971

74. Shahin RD, Amsbaugh DF, Leef MF. Mucosal immunization with filamentous hemagglutinin protects against *Bordetella pertussis* respiratory infection. Infect Immun 1992:60:1482-1488

Abstract (In Korean)

마우스 모델에서 HIV gp120, gp41 펩타이드와 cholera 독소, 비독성 변이 cholera 독소를 이용한 비강 면역화의 점막 및 전신 면역 증진 효과

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배경: 점막은 HIV가 인체로 들어가는 주요 경로가 되며, 점막 면역은 HIV 백신 개발의 주요 표적이다. HIV에 반복적으로 노출됨에도 불구하고 혈청학적 HIV 음성이 유지되는 여성들을 대상으로 한 연구에 의하면 질, 자궁의 점막에 HIV *env* 단백에 대한 IgA, IgG, IgM 항체가 다량 존재하였는데, 이러한 결과는 점막 면역이 성접촉을 통한 HIV 감염의 예방에 중요한 역할을 할 수 있다는 것을 시사한다. 효과적인 점막 백신을 개발하기 위해서는 적절한 항원 결정기, 효과적이고 안전한 adjuvant, 백신 일정과 경로 등이 규명되어야 한다. 본 연구의 목적은 gp120과 gp41의 특정 항원 결정기를 포함한 펩타이드의 점막 면역 및 전신 면역 증진 효과와 비독성 변이 cholera 독소의 adjuvant 효과를 규명하는 것이다.

재료 및 방법: BALB/c 마우스에 HIV-1 gp120 펩타이드 (KQINMWQEVGKAMYACTRPNYNKRKRIHIGPGRAFYTTK)나 gp41 펩타이드 (EKNEQELLELDKWASLWC)를 점막 adjuvant인 cholera 독소나 비독성 변이 cholera 독소와 함께 0, 7, 14, 21 일에 비강 주입하였다. 혈액은 모세관 튜브를 이용하여 후안와 혈관망에서 채취하였고, 질세척액은 100 μL의 PBS로 질을 세척하여 채취하였다. 혈액 및 질세척액 내의 항체 농도를 ELISA를 이용하여 측정하였고, 항원 특이 세포면역기능을 측정하기 위하여 비장세포로 ELISPOT assay를 수행하였다.

결과: gp120 펩타이드나 gp41 펩타이드를 이용한 모든 군에서 질세척액 내

HIV 특이 IgA 농도는 4주 동안 유의하게 증가하였다. 그러나 혈액 내 IgA 농도는 유의하게 변화하지 않았다. gp120 펩타이드 군에서 혈액 내 IgG 농도는 유의하게 변화하지 않았으나, gp120 펩타이드와 cholera 독소나 비독성 변이 cholera 독소를 사용한 군에서는 혈액 내 IgG 농도가 유의하게 증가하였다. gp41 펩타이드를 사용한 군에서는 혈액 내 IgG 농도가 유의하게 변화하지 않았으나, gp41 펩타이드와 cholera 독소나 비독성 변이 cholera 독소를 사용한 군에서는 혈액 내 IgG 농도가 유의하게 증가하였다. gp120 펩타이드를 사용한 군들에서는 PBS 군에 비하여 세포독성 T 림프구 반응이 유의하게 상승되었으나, gp41을 사용한 군들에서는 세포독성 T 림프구 반응이 유의하게 유도되지 않았다. gp120 펩타이드를 사용한 군들에서 cholera 독소나 비독성 변이 cholera 독소가 체액성 및 세포성 면역을 증진시키는 유의한 adjuvant 효과를 나타냈다. 그러나, gp41 펩타이드를 사용한 군들에서는 cholera 독소나 비독성 변이 cholera 독소가 유의한 adjuvant 효과를 나타내지 않았다.

결론: 마우스 모델에서 HIV-1 gp120 펩타이드와 gp41 펩타이드는 비강 주입을 통해 유의한 점막 면역 및 전신 면역 유도 효과를 나타내었으며, cholera 독소와 비독성 변이 cholera 독소는 점막 및 전신 면역을 증진시키는 유의한 adjuvant 효과를 나타냈다. 효과적인 HIV 점막 백신 개발을 위해서는, 전신 및 점막 면역을 유도하는 항원결정기를 규명하고, 효과적이고 안전한 adjuvant를 개발하기 위한 계속적인 연구가 필요하리라 생각된다.

핵심되는 말: HIV, HIV 감염, 에이즈, 점막 면역, gp120, gp41, cholera 독소