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Research Paper

Effective vaginal DNA delivery with high transfection efficiency is a good system for induction of higher local vaginal immune responses

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Abstract

Objectives To investigate the local vaginal and systemic immune responses of effective vaginal DNA delivery with high transfection efficiency, we determined the effects on Th1-dependent cytokine (interferon-γ) production in spleen and inguinal lymph node cells and antibody responses of vaginal pDNA immunization with a cell-penetrating peptide, and compared our vaginal immunization with intradermal and intranasal immunizations.

Methods Mice were immunized by vaginal, nasal or dermal administration of pCMV-OVA with or without peptide carriers, and serum, vaginal fluids, spleen and inguinal cells were harvested. The serum immunoglobulin (Ig)G_{2a} and vaginal IgA antibody responses were determined by sandwich enzyme-linked immunosorbent assay (ELISA). The interferon-γ production from spleen cells or inguinal lymph node cells was determined by an ELISA kit.

Key findings The direct vaginal immunization strongly induced IgA in the vaginal fluids and interferon-γ production in the local lymph node draining from the vagina. In addition, co-vaccination with the peptide carriers elevated these immune responses compared with vaccination with pCMV-OVA alone. Vaginal immunization with high transfection efficiency promoted vaginal IgA production to a significantly greater extent than intradermal or nasal immunization.

Conclusions These results suggested that direct vaginal DNA vaccines under high transfection conditions induced higher local vaginal antibody than that by intranasal or intradermal administration, and peptide carriers effectively elevated mucosal immune responses. Therefore, this vaginal DNA vaccination method may be expected to be useful in the prevention and treatment methods for vaginal infectious diseases such as HIV infection.

Keywords cell penetrating peptide; inguinal lymph node; mucosal infections; vaginal DNA vaccine; vaginal immunity

Introduction

There is an urgent need for the development of safe, effective, stable and inexpensive vaccines against severe viral infections, including human immunodeficiency virus (HIV), influenza virus and severe acute respiratory syndrome (SARS). The majority of HIV infections currently occur through heterosexual intercourse and transmission through the vaginal mucosa.[1,2] Thus, to prevent or treat HIV infection, strong vaginal immunity is required and needs to include cytotoxic T-lymphocytes (CTL) and immunoglobulin (Ig)A, which play an important role as the first line of defence against various infections.[3–7] We believe that a strong vaginal immune response can be obtained by inducing strong gene expression of antigen-coding DNA vaccines in antigen-presenting cells, including dendritic cells and macrophages, in vaginal tissue. In our previous study, to improve transfection efficiency in the vaginal mucosa, we examined the effects of electroporation, chemical absorption enhancers such as citric acid, cell penetrating peptides, and a nuclear localization signal on the gene expression of marker plasmid DNAs administered through the vaginal membrane in mice.[8] Electroporation with 15 pulses at 250 V/cm for 5 ms and pretreatment with 5% citric acid solution was found to promote gene expression in the vaginal mucosa at

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the stage of diestrus. Furthermore, a Tat analogue, a cell penetrating peptide, and a NF-κB analogue, a nuclear localizing signal, synthesized at our laboratory, significantly promoted the transfection of pDNA on the vaginal membrane.

Antigen presentation is known to be most reduced at the estrus stage of the estrous cycle, at which time estrogen levels are most elevated and ovulation takes place.[9] At the diestrus stage the number of antigen presenting cells have been found to be maximal in the vagina and the number of layers of epithelial cells lining the vagina of rodents decreases dramatically, which would be expected to enhance uptake of luminal antigen. Indeed, uptake of proteins and the ability of vaginal immunization to induce specific antigen responses in mice were optimal when preparations were administered during diestrus.[10] Thus, vaccine strategies for protection against sexually transmitted diseases must take into account that sex hormones affect immune responses.

To date, mucosal vaccines have been administered by either oral or nasal routes to induce immunity at multiple sites, including the female reproductive tract.[11] On the other hand, there is evidence that local exposure to antigen can result in a much stronger immune response at the region of exposure than at distant sites, as recently demonstrated for CD8+ CTLs.[12,13] Previous studies suggested that the vaginal immunization route could be used effectively for inducing local immune responses in the female genital tract.[14,15] However, there have only been a few studies examining vaginal administration of DNA vaccines.[16,17]

In this study, the local and systemic antibody-humoral and cell-mediated immunity responses in mice administered naked pCMV-OVA (pOVA) and peptide carrier/pOVA complexes into the vagina at the diestrus stage in the menstrual cycle were examined by electroporation. In addition, to investigate the utility of our optimized vaginal delivery protocol for induction of vaginal and systemic immune responses, we examined the serum and vaginal antibody responses, and interferon-γ (IFN-γ) production in spleen cells and inguinal lymph node cells regulated by the vaginal tract following vaginal immunization with pOVA during diestrus, and then compared these findings with intradermal and nasal immunization.

Materials and Methods

Materials and animals

The electroporator (Electro square porator T820: BTX Genetronics, San Diego, CA, USA) was kindly supplied by Hisamitsu Pharmaceutical Co., Inc. (Tokyo, Japan). Luciferase activity was determined using a luciferase assay system (Promega Co., Ltd, Madison, WI, USA). The Quantikine mouse IFN-γ assay kit (R & D Systems, Minneapolis, MN, USA) was used to determine IFN-γ production in cells in immunized mice. All other chemicals were of the purest grade available.

Specific-pathogen-free female six-week-old ICR and C57BL/6 mice were obtained from SLC (Shizuoka, Japan). The mice were housed under standard conditions of temperature (22–24°C), humidity (40–60%) and 12-h light/dark cycles with the light period starting at 08:00 h. Animals had free access to food and water. The animal experiments were carried out in accordance with a protocol approved by the Animal Care and Ethics Committee of Tokyo University of Pharmacy and Life Sciences.

The estrous stage of mice was determined through daily morning microscopic observation of vaginal smears taken with a swab. Cells stained with Giemsa solution were carefully examined and the estrous stage of each mouse identified as the diestrus, proestrus, estrus, or metestrus stage according to a previously established protocol.[18] Mice were utilized at the diestrus stage in all immunization experiments.

Plasmid preparations

In the immune response experiments, we used pCMV-OVA (pOVA, 7.7 kbp) as the model antigen-expressing pDNA encoding ovalbumin (OVA). pOVA consists of an OVA cDNA fragment from pAc-neo-OVA subcloned into pcDNA3.1 at the HindIII sites. These pDNAs were amplified in *Escherichia coli* (DH5α) and purified using an Endfree Plasmid Maxi kit (Qiagen, Valencia, CA, USA), followed by ethanol precipitation and dilution in Tris/EDTA buffer. The DNA concentration was measured by UV absorption at 260 nm.

Peptide carriers

The Tat analogue, which consists of Cys-Gly-NH_2 added to the N terminus of HIV-Tat (48–57), and the NF-κB analogue, which consists of Gly and Cys-Gly-NH_2 added to the C and N termini of NF-κB p50 (Table 1), were synthesized as CPP or NLS peptide gene vectors using the Fmoc-solid-phase peptide synthesis method with an ABI 433A peptide synthesizer (Applied Biosystems, Tokyo, Japan) as previously reported.[8] Both analogues were used after purification by reverse-phase HPLC. The molecular weight of each analogue was determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS): Tat analogue, 1396.1; NF-κB analogue, 1130.36. Peptide carriers/pDNA complexes were prepared by incubating pOVA with peptide carriers at 4°C for 30 min.

Vaginal immunization

Female ICR mice at the diestrus stage had a cotton ball soaked in 5% citric acid aqueous solution (approximately 100 μl) inserted into the vaginal tract. The cotton ball was removed 2 h later and phosphate-buffered saline (PBS), naked pOVA (20 μg) or the Tat and NF-κB analogues/pOVA complex (200 μg Tat and 200 μg NF-κB/20 μg

Table 1 Structure of synthesized Tat and NF-κB analogues

Peptides	Sequence
Tat analogue	Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Cys-Gly-NH_2
NF-κB analogue	Gly-Gln-Arg-Lys-Arg-Gln-Lys-Cys-Gly-NH_2

Underlined sequences were modified from the natural sequence of each peptide.

pOVA) was then administered into the vaginal tract using a micropipette (40 μl Tris/EDTA buffer).

The vaginal membrane was electroporated at the surface immediately after vaccine administration of PBS or one of the pOVAs, using an electroporator. A custom-designed needle electrode, consisting of two parallel needles (anode and cathode) 5 mm in length and 5 mm apart, each consisting of three platinum needles 1 mm in diameter, was used to apply 15 pulses of electricity at 250 V/cm for 5 ms. These electroporation parameters were established as optimal conditions for greatest gene transfection efficacy with minimal vaginal irritation in our previous study.[8] Vaginal immunization was carried out three times at one-week intervals, and the mice were killed one week following the final immunization to determine the immune response. Untreated mice were utilized as control.

The vaginal tract was washed by flushing with 150 μl PBS before the mice were killed and the harvested wash solution used to determine local vaginal immunity. Blood was collected by cardiac puncture under anaesthesia induced with intraperitoneal injection of pentobarbital (50 mg/kg) and the serum was isolated by centrifugation and then frozen at −20°C. After blood collection, two inguinal lymph nodes and the spleen were removed and pooled from five mice per experimental plasmid/complex group. Cell suspensions of inguinal lymph node and spleen were prepared by pushing the tissue through a nylon mesh.

Immunization using various inoculation routes

Five female C57BL/6 mice were immunized three times by intradermal injection, nasal immunization or vaginal immunization with 30 μg naked pOVA (in 30 μl Tris/EDTA buffer) on days 0, 14 and 28. For nasal immunization, pOVA (in 15 μl) was administered into both the left and right nostril of mice under anaesthesia induced by intraperitoneal injection of pentobarbital (50 mg/kg). The method of vaginal immunization was as outlined above. On day 35, serum, vaginal wash solution and a cell suspension of the spleen were collected.

Determination of serum IgG_{2a} and vaginal IgA of anti-ovalbumin antibodies by enzyme-linked immunosorbent assay

The serum (diluted 1 : 10) and vaginal wash samples were assayed for IgG or IgA titres using an enzyme-linked immunosorbent assay (ELISA). OVA (2 mg/ml) in PBS was distributed into each well of 96-well flat-bottom polystyrene plates (50 μl/well). Following overnight incubation at 4°C, wells were blocked with 3% BSA containing Tween 20 PBS (B-PBS-T) for 2 h at 37°C, washed twice with Tween 20 PBS (PBS-T), and 50 μl serially diluted serum samples then added. After overnight incubation at 4°C, the wells were washed four times with PBS-T, followed by overnight incubation of 100 μg/100 μl anti-IgG_{2a}–biotin conjugate (Southern Biotech, Birmingham, AL, USA) or anti-IgA–biotin conjugate (Southern Biotech) at 4°C. After washing six times with PBS-T, 100 μl avidin-conjugated-peroxidase solution (Calbiochem, San Diego, CA, USA) diluted 1 : 1000 with B-PBS-T was added. The solution was incubated for 40 min at 37°C, the wells then washed eight times with PBS-T, and 50 μl TMB solution (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) was added. After 10 min at room temperature, 50 μl 1 M H_2PO_4 solution was added and absorbance was measured at 450 nm. The concentrations of IgG_{2a} or IgA were calculated using the standard curve. A standard curve for IgG_{2a} or IgA was established using serially diluted mouse immunoglobulin reference serum (Bethyl Laboratories, Inc, Montgomery, TX, USA).

Determination of IFN-γ cytokine secretion from inguinal lymph node and spleen cells

On day 35, one week after the final immunization, the inguinal lymph nodes or spleen cells were removed from the immunized mice. Each cell sample (5×10^5 cells/well) was placed in 96-well plates and incubated at 37°C in the presence of OVA in RPMI medium containing 10% fetal bovine serum for 72 h, and then 100 μl the supernatant culture medium was removed and the concentrations of IFN-γ measured using an ELISA kit.

Statistical analysis

All values were expressed as the mean ± standard error (SE) of four determinations. Statistical analysis of the data was performed using an unpaired Student's *t*-test for two groups or analysis of variance with the Dunnett's test for multiple comparisons between more than three groups. Statistical significance was defined as $^{*}P < 0.05$ and $^{**}P < 0.01$.

Results

Anti-OVA serum IgG_{2a} and vaginal IgA production after vaginal immunization

Figure 1 shows the serum IgG_{2a} levels after vaginal immunization. No increase in IgG_{2a} response was observed in control, PBS or naked pOVA alone. The serum IgG_{2a}

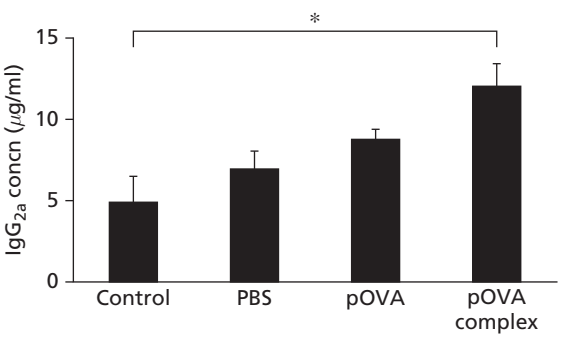

Figure 1 Ovalbumin-specific serum immunoglobulin G_{2a} production after immunization with pCMV-OVA through the vaginal mucosa using electroporation. Mice were immunized three times at one-week intervals through the vaginal mucosa at diestrus with phosphate-buffered saline (PBS), naked pCMV-OVA (pOVA), Tat and NF-κB analogues/pOVA complexes (denoted pOVA complex) using electroporation (250 V/cm, 5 ms, 15 pulses) with 5% citric acid as an absorption enhancer. Control mice were not treated. Mice were killed one week after the final immunization and serum immunoglobulin G_{2a} (IgG_{2a}) responses were analysed by ELISA. Each bar represents the mean ± SE (n = 5). $^{*}P < 0.05$.

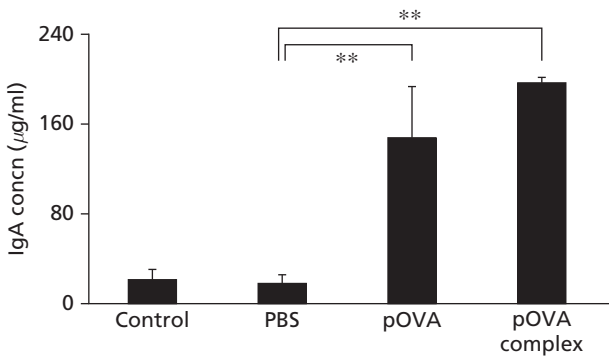

Figure 2 Ovalbumin-specific vaginal immunoglobulin A production after immunization with pCMV-OVA through vaginal mucosa using electroporation. Mice were immunized three times at one-week intervals through the vaginal mucosa at diestrus with phosphate-buffered saline (PBS), naked pCMV-OVA (pOVA), Tat and NF-κB analogues/pOVA complexes (denoted pOVA complex) using electroporation (250 V/cm, 5 ms, 15 pulses) with 5% citric acid as an absorption enhancer. Control mice were not treated. Mice were killed one week after the final immunization and immunoglobulin A (IgA) responses in rinsed vaginal fluid were analysed by ELISA. Each bar represents the mean ± S.E. ($n = 5$). $^{**}P < 0.01$.

concentration in mice immunized with naked pOVA increased only a little as gene expression in the vagina was not sufficiently strong to induce systemic antibodies under these immunization conditions. On the other hand, an increased level of anti-OVA IgG_{2a} was obtained in mice immunized with the Tat and NF-κB analogues/pOVA.

The secretory IgA levels against OVA in the vaginal secretions suggested that vaginally local mucosal immune responses were elicited. As shown in Figure 2, a negligible IgA response was observed in the untreated control or PBS administered groups. However, the IgA responses in mice administered naked pOVA and Tat and NF-κB analogues/pOVA complexes were found to be significantly increased compared with the control or PBS group.

Th1-dependent cytokines in inguinal lymph node and spleen cells after vaginal immunization

To clarify the effect of vaginal immunization on local and systemic Th1 immune responses, we determined the OVA-specific IFN-γ (Th1-type cytokine) production from inguinal lymph node cells (local) and spleen cells (systemic) following co-culture with OVA protein (Figure 3). The levels of IFN-γ in mice immunized with naked pOVA were higher than in control or PBS-administered mice. Furthermore, the peptide carriers Tat and NF-κB significantly enhanced these cellular immune activities following vaginal mucosal administration. Although the levels of IFN-γ in the spleen cells were higher than those in the inguinal lymph node cells, the local cellular immunity regulating the vaginal tract could be obviously enhanced by vaginal administration.

Vaginal and systemic immune responses by various inoculation routes

To determine whether vaginal DNA vaccination by our optimized delivery protocols was useful for induction of

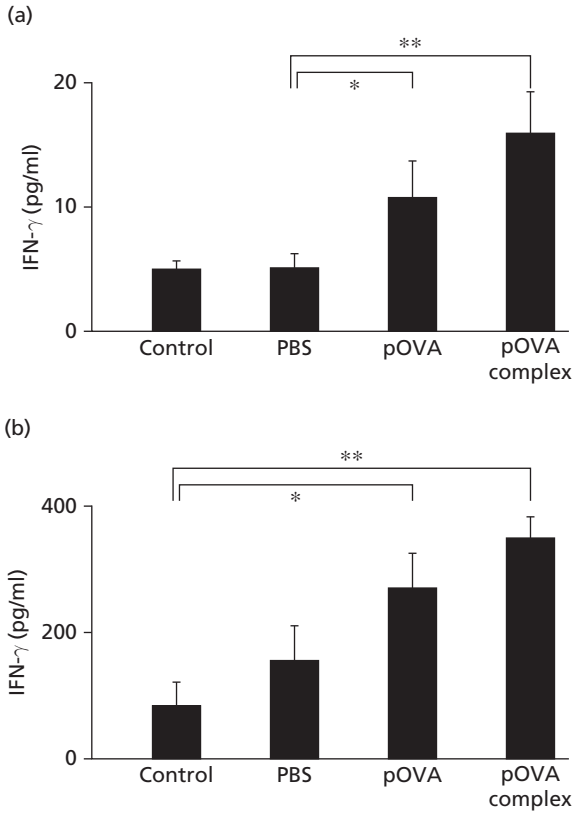

Figure 3 Ovalbumin-specific interferon-γ production from inguinal lymph node and spleen cells after immunization with pCMV-OVA through vaginal mucosa using electroporation. Mice were immunized three times at one-week intervals through the vaginal mucosa at diestrus with phosphate-buffered saline (PBS), naked pCMV-OVA (pOVA), Tat and NF-κB analogues/pOVA complexes (denoted pOVA complex) using electroporation (250 V/cm, 5 ms, 15 pulses) with 5% citric acid as an absorption enhancer. Control mice were not treated. Mice were killed one week after final immunization. The spleen (a) and inguinal lymph node (b) cells (5×10^5 cells/ml) were prepared and co-cultured with ovalbumin (OVA) (2 mg/ml) for 72 h. Interferon-γ (IFN-γ) in the conditioned-medium was analysed by ELISA. Each bar represents the mean ± SE ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$.

local vaginal and systemic immune activity, we compared the vaginal local and systemic immune responses in mice after DNA immunization by the intradermal, nasal and vaginal routes. Figure 4a shows that the IFN-γ levels from spleen cells from mice immunized via all inoculation routes were strongly elevated and were all similar. Figure 4b shows the serum IgG_{2a} levels in mice immunized by intradermal, nasal or vaginal inoculation of pOVA. Intradermal administration of pOVA resulted in the highest induction of IgG_{2a}. However, in the local vaginal immune response, vaginal administration of pOVA clearly induced the highest vaginal OVA-specific IgA production among the inoculation routes (Figure 5).

Discussion

We have investigated the immune responses induced by intravaginal pOVA immunization by electroporation, with or

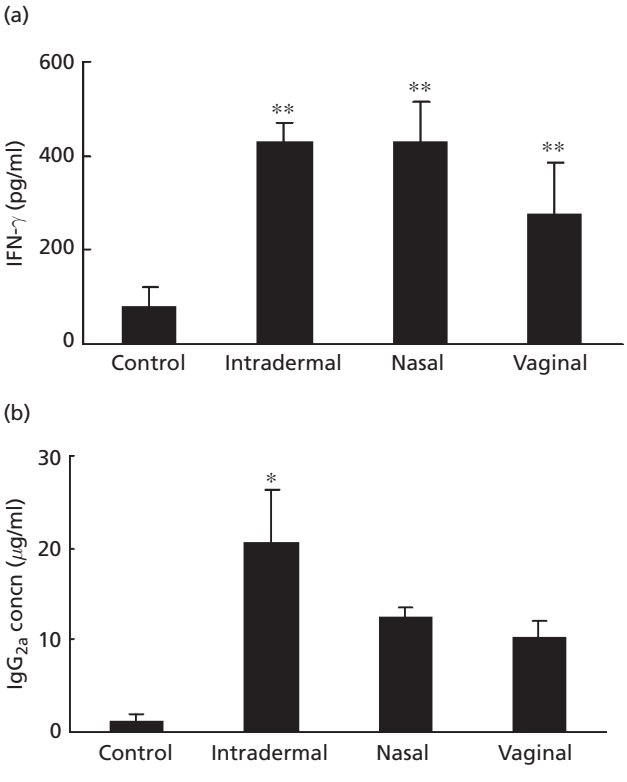

Figure 4 Systemic ovalbumin-specific immune responses after immunization by various routes. Mice were immunized with pOVA three times via the intradermal, nasal or vaginal routes at two-week intervals. Control mice were not treated. Mice were killed one week after final immunization. The spleen cells (5×10^5 cells/ml) were prepared and co-cultured with ovalbumin (OVA) (2 mg/ml) for 72 h. (a) Interferon-γ (IFN-γ) in the conditioned-medium was analysed by ELISA. (b) Serum immunoglobulin G_{2a} (IgG_{2a}) levels were analysed by ELISA. Each bar represents the mean ± SE ($n = 5$). (a) $^{**}P < 0.01$ compared with control. (b) $^{*}P < 0.05$ compared with control, intradermal or nasal immunization.

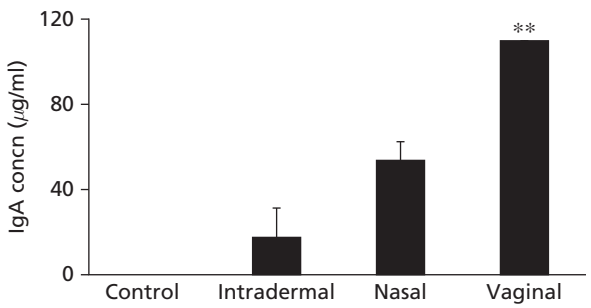

Figure 5 Vaginal mucosal ovalbumin-specific immunoglobulin A secretion in mice after immunization by various routes. Mice were immunized with pOVA three times via the intradermal, nasal or vaginal routes at two-week intervals. Control mice were not treated. Mice were killed one week after final immunization. Vaginal immunoglobulin A (IgA) levels in rinsed vaginal fluids were analysed by ELISA. Each bar represents the mean ± SE ($n = 5$). $^{**}P < 0.01$ compared with control, intradermal or nasal immunization.

without our synthesized CPP and NLS peptides, Tat/NF-κB analogues, in mice at diestrus. This stage was chosen based on a previous study in which luciferase gene expression was higher at diestrus than at the other menstrual stages, due to the very thin and porous epithelial cell layers present compared with the other menstrual stages.[8] In addition, immune-associated cells in the vaginal submucosal membrane increase at diestrus, and thus antigen-specific immune responses following vaginal DNA vaccination at the diestrus stage should be expected to induce both CTL induction and antibody production.[19] To examine the antigen-specific systemic and vaginal mucosal humoral immune responses, we measured the OVA-specific serum IgG_{2a}, a marker of systemic humoral immunity, and vaginal mucosal IgA as a marker of local vaginal humoral immunity in the immunized mice.

Although the female genital tract contains diffuse mucosa-associated lymphoid tissue typical of an immune effector site, it differs from the intestinal mucosa in that a larger fraction of the total Ig in associated secretions is of the IgG isotype.[20] The serum IgG_{2a} concentration in mice immunized with naked pOVA increased only a little as gene expression in the vagina was not sufficiently strong to induce systemic antibodies under these immunization conditions (Figure 1). On the other hand, an increased level of anti-OVA IgG_{2a} was obtained in mice immunized with the Tat and NF-κB analogues/pOVA. In our previous study, the Tat and NF-κB analogues significantly promoted vaginal transfection of the pDNA of the marker gene, pLuc.[8] Those results suggested that the elevation of antigen-specific systemic antibodies in mice immunized with the Tat and NF-κB analogues/pOVA complex was probably caused by strong expression of antigen (OVA) in the vaginal mucosa, including antigen presenting cells, such as dendritic cells.

As shown in Figure 2, the IgA responses in mice following vaginally administered naked pOVA and Tat and NF-κB analogues/pOVA complexes were found to be significantly increased compared with the control or PBS group. These results indicated that vaginal DNA immunization under conditions of high transfection efficiency of pDNA could strongly induce local vaginal immune responses. It is important that a direct vaginal immunization protocol having strong vaginal gene expression could induce significantly higher production of vaginal local IgA than systemic serum IgG_{2a}. This data was consistent with previous studies that demonstrated that the greatest levels of antigen-specific IgA may have been generated in regions that were closest to the site of antigen exposure.[21] The present results confirmed that this phenomenon was also true in DNA immunization. Furthermore, these findings suggested that vaginal DNA vaccination by our optimized vaginal delivery protocol, which had high gene expression efficiency in the vagina, could induce the production of both systemic and local antibodies, in particular marked induction of local IgA antibody secretion in the vaginal tract.

To clarify the effect of vaginal immunization on local and systemic Th1 immune responses (cellular immunity), we determined the OVA-specific IFN-γ (Th1-type cytokine) production from inguinal lymph node (local) and spleen (systemic) cells following co-culture with OVA protein. The levels of IFN-γ from inguinal lymph node cells (Figure 3a) can

be used to indicate induction of vaginally local Th1-dependent cellular immunity, because vaginal mucosal immune tissue belongs to the inguinal lymph nodes. The levels of IFN-γ in the inguinal lymph node and spleen cells in mice immunized with pOVA complexed with the peptide carriers Tat and NF-κB showed a similarly significantly enhanced cellular immune activity (Figure 3). These findings suggested that local lymph node immunity regulating the vaginal tract and the systemic Th1-dependent immune responses could be promoted by intravaginal direct immunization with a pDNA vector coupled with peptide carriers such as CPP and NLS. A successful HIV vaccine would require a strong local CTL activity at the site of viral entry, the vaginal mucosa and draining lymph node, as well as systemically. DNA vaccination using direct vaginal immunization with functional peptide carriers may be effective in HIV vaccination.

Mucosal immunization is known to be a good method for vaccine delivery and to date has been commonly and successfully used in vaccination programmes. The mucosal surfaces in our body are efficient immune producing organs, performing constant surveillance of foreign antigens. Several mucosal routes and surfaces, including oral, nasal, lung, rectal and vaginal mucosa, have been considered as potential sites.[22]

To determine whether vaginal DNA vaccination by our optimized delivery protocols would be useful for induction of local vaginal and systemic immune activity, we compared the systemic and vaginally local immune responses in mice after DNA immunization by the intradermal, nasal and vaginal routes. The IFN-γ levels from spleen cells in mice immunized via all inoculation routes (Figure 4a) significantly increased. This indicated that these local mucosal immunization routes were as effective for inducing systemic cellular immunity as intradermal immunization. Intradermal administration of pOVA resulted in the highest induction of IgG_{2a} (Figure 4b), possibly because the intradermal environment is particularly primed for producing immune responses due to the presence of large numbers of dendritic cells. Dermal dendritic cells are readily transfected *in vivo* by cutaneous immunization with foreign genes, and then migrate to the lymph nodes, where stimulation of T and B cells occurs.[23] Although the nasal and vaginal inoculation routes of pOVA also exhibited considerably higher induction in cellular immunity in the spleen cells, the systemic humoral immunity was relatively low compared with that by intradermal administration.

In the local vaginal immune response, vaginal administration of pOVA clearly induced the highest vaginal IgA production among the inoculation routes (Figure 5). This result was consistent with previous studies which have demonstrated that the greatest levels of antigen-specific IgA antibodies may be generated in regions that are closest to the site of antigen exposure such as the oral, rectal and vaginal sites.[12,24,25] The current findings clearly indicated that the vaginal mucosa may be the most suitable site for administration of a mucosal DNA vaccine against HIV.

Conclusions

We believe that a successful HIV vaccine will need to induce strong local immunity at the site of viral entry, the vaginal mucosa and the draining lymph node, as well as systemically. Even the most promising vaccine formulations may fail to establish protective immunity if the route of vaccine administration is not optimal for induction of local immune responses in the vagina. We feel that a strong vaginal immune response will be obtained as a result of strong gene expression of an antigen-coding DNA vaccine in antigen-presenting cells, including dendritic cells and macrophages, in vaginal tissue.

In this study, we have evaluated the vaginal and systemic immune responses by vaginal pDNA immunization using the optimized delivery protocol developed in our previous study. This protocol strongly induced not only local antibodies in vaginal fluids and serum, but also significantly increased the levels of the Th1-dependent cytokine as a marker of cellular immunity in the inguinal lymph node draining from the vagina and spleen cells. Thereafter, we compared our vaginal immunization system with intradermal and nasal immunization, which have been utilized previously as effective inoculation sites. We found that our vaginal immunization protocol promoted local IgA production in the vaginal mucosa to a greater extent than intradermal or nasal immunization. The system will be improved by the development of more noninvasive methods of delivery, such as needle-free or microneedle injection, as the safety of electroporation in humans has not been established.

These findings suggest that vaginal DNA vaccines may be useful in the prevention and treatment methods for HIV infection. The number of IFN-γ producing cells (ELISPOT assay) and the CTL activity of our vaginal immunization protocol need to be determined. Our results have advanced our knowledge into the use of vaginal pDNA vaccination against bacterial and viral infection.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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