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Local gene expression and immune responses of vaginal DNA vaccination using a needle-free injector

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ABSTRACT

The vaginal mucosa is the most common site of initiation of virus infections that are transmitted through heterosexual intercourse, including HIV and papillomavirus. Thus, in order to prevent or treat these infections, strong vaginal immunity is required as the first line of defense. In this study, to establish a less invasive, safe, convenient and effective immunization method, we examined the local (skin and vagina) gene transfection efficiency of a non-needle jet injector for daily insulin injection. In the skin experiment, the needle-free injector resulted in a marked increase in marker gene expression, compared to the conventional needle-syringe injection. In addition, intradermal DNA vaccination using the needle-free injector dramatically induced IFN-γ and antibody systemic responses in mice. Furthermore, we investigated the applicability of the needle-free injector as a vaginal vaccination tool in rabbits. Vaginal gene expression using the needle-free injector was significantly greater than that using needle-syringe injection. Moreover, intravaginal vaccination by the needle-free injector promoted vaginal IgA secretion and IFN-γ mRNA expression in the blood lymphocytes, to a degree significantly higher than that by needle-syringe injection. In conclusion, local vaginal DNA vaccination using a needle-free jet injector is a promising approach for the prevention and treatment of mucosal infectious diseases.

1. Introduction

The genital tracts, including the uterus, cervix and vagina, are the most common sites of initiation of virus infections that are transmitted through heterosexual intercourse, including human immunodeficiency virus (HIV), papillomavirus associated with cervical carcinoma, and herpes simplex virus. Thus, there is an urgent need for the development of safe, easy-to-use, effective, stable and inexpensive vaccines against these viral infections. In females and heterosexual males, these infections currently occur through heterosexual intercourse and transmission through the vaginal mucosa (De Schryver and Meheus, 1990; Quinn, 1996). Thus, to prevent or treat these infections, 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 defense in these infections. Mucosal vaccines have been administered by either oral or nasal route in order to induce immunity at multiple sites, including the female reproductive tract (McGhee and Kiyono, 1993). 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 (Jain et al., 1996), it was recently demonstrated for CD8+ CTLs (Belyakov et al., 2007). Previous studies suggest that the vaginal immunization route can be used effectively for inducing local immune responses in the female genital tract (Wassen et al., 1996; Hamajima et al., 2002).

In our previous study (Kanazawa et al., 2008, 2009), we found that our vaginal immunization using our optimized conditions with pCMV-OVA (pOVA) promoted local IgA production in the vaginal mucosa in mice to a greater extent than intradermal or nasal immunization. So, we believe that a strong vaginal immune response could be obtained by inducing strong gene expression of antigen-coding DNA vaccines in local vaginal tissue, and In order to improve transfection efficiency in antigen-presenting cells in vaginal subepithelial layer, it is important that the breakthrough the various barriers, such as vaginal epithelial layer, cellular and nuclear membrane. In our previous studies we used electroporation, which raises concerns regarding its safe use in humans, and thus a less invasive and more effective delivery into subepithelial layer method should be required.

We selected a needle-free jet injection which could inject a accurate amount of the solution easily without pain.

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Needle-free jet injection has been extensively investigated as a method to immunize laboratory animals, such as mice (Cui et al., 2003; Haensler et al., 1999), rabbits (Ren et al., 2002; Aquiar et al., 2002), pigs and dogs (Anwer et al., 1999) and monkeys through the transdermal route. In addition, jet injection has been tested subcutaneously in several human clinical trials (Jackson et al., 2001) and is already produced commercially for daily injection of insulin and h-growth hormone. The vast majority of studies in animals have demonstrated an enhancement in resulting immune responses with jet injection over conventional needle-syringe injection (Mumper and Cui, 2003). However, the majority of studies examined intadermal, intramuscular and subcutaneous injection, however, no needle-free jet injection into the vaginal mucosa has been previously reported.

In this study, to explore less invasive vaginal vaccination methods as alternatives to electroporation, we examined whether the needle-free jet injector originally developed for insulin and already used in humans could be an effective vaccine delivery device. First, gene expression in rats and induction of immune responses in mice by intradermal injection of pCMV-Luc (pLuc) or pCMV-OVA (pOVA) using this device were compared with those using conventional needle-syringe injection. Furthermore, in order to examine the application of a needle-free injector as an effective vaginal vaccine device, we determined local vaginal gene expression and OVA-specific IFN-γ secretion from blood-derived lymphocytes, and the OVA-specific antibody titer in serum and vaginal secretion in rabbits, following intravaginal pLuc or pOVA administration by developing using the needle-free jet injector having 45° angle nozzle designed in our laboratory.

2. Materials and methods

2.1. Materials

A spring-powered needle-free injector (ShimaJET®), a gas-powered needle-free injector (ShimaJET-GT) and a mucosal administration nozzle with an angle of injection of solution of 45° were kindly provided by Shimadzu Co. (Kyoto, Japan). The Luciferase Assay System (Promega Co. Ltd., Madison, WI, USA) was used for determining luciferase activity. The quantikine mouse IFN-γ assay kit (R & D Systems, Minneapolis, MN, USA) was used to determine IFN-γ production in immunized mice. In the immune response experiments, we used pDNA encoding ovalbumin (OVA), namely pCMV-OVA (pOVA, 7.7 kbp), as a model antigen-expressing pDNA. pOVA consists of an ovalbumin cDNA fragment from pAc-neo-OVA subcloned into pcDNA3.1 at the Hind III sites. In the gene expression experiments, we used pCMV-Luc, (pLuc, 7.6 kbp), which consists of a luciferase cDNA fragment subcloned into pcDNA3.1 at the Hind III and BamH1 sites. These pDNAs were amplified in *E. coli* (DH5α) and purified using an Endfree Plasmid Maxi kit (QIAGEN, USA) followed by ethanol precipitation and dilution in Tris/EDTA buffer (TE buffer). The DNA concentration was measured by UV absorption at 260 nm. All other chemicals were of the purest grade available.

2.2. Animals

Specific-pathogen-free female 6-week-old C57BL/6 mice, male 8-week-old Wistar rats and 9-week-old female Japanese White rabbits were obtained from SLC (Shizuoka, Japan). These animals 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 *ad libitum* 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.

2.3. Gene expression experiments

2.3.1. Rat skin

Eight-week-old male Wistar rats ($n = 5$) were used in this study. All rats were anesthetized by intraperitoneal injection of pentobarbital solution (50 mg/kg). After closely shaving the back of each rat using an electric clipper, naked pLuc (20 μg/40 μL) or PBS was injected into the skin using the needle-free injector (ShimaJET®) or a 27G needle-syringe with or without electroporation (200 V/cm, 12 pulses, 5 ms) on the injection spot after injection. At 18 h after injection of pLuc, the rats were sacrificed and 1 cm^2 of dermal tissue at the injection site was resected and the luciferase activity determined according to Section 2.3.3.

2.3.2. Rabbit vaginal mucosa

Nine-week-old female Japanese White rabbits ($n = 3$) were used in this study. All rabbits were anesthetized by intravenous injection of pentobarbital solution (30 mg/kg). Naked pLuc (100 μg/100 μL) or PBS was injected into the rabbit vagina using the needle-free injector (ShimaJET-GT) with the mucosal customized nozzle or a 27G needle-syringe. At 24 h after injection of pLuc, the rabbits were sacrificed and 0.7 cm^2 of vaginal tissue at the injection site was resected and the luciferase activity determined according to Section 2.3.3.

2.3.3. Determination of luciferase activity

Harvested tissues were homogenized in lysis buffer (0.05% Triton X, 2 mM EDTA, 0.1 M Tris) at a volume-to-weight ratio of 4 mL lysis buffer/g of each tissue. The homogenates were processed three times with freezing (−80 °C) and thawing and centrifuged at 14,000 rpm for 40 min. Luciferase activity in 10 μL of the supernatant was measured using a commercial luciferase assay kit and each measurement was carried out for 60 s using a luminometer (MicroLumat Plus LB96 V; Berthold, Germany). The protein concentration of each sample was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA), and the luciferase activity was normalized to each protein concentration of the sample and expressed as RLU/mg protein.

2.4. Localization of pLuc within rat dermal tissue using the needle-free jet injector

pLuc was labeled using the Label IT nucleic acid Cy3 labeling kit (Mirus Bio LLC, Milwaukee, WI, USA) following a protocol provided by the manufacturer, 5–50 μL of pLuc solution (1 mg/mL) and the same amount of Label IT reagent (Cy3) were mixed in 20 mM MOPS buffer (pH 7.5) and incubated at 37 °C for 2 h. Any unreacted labeling reagent was removed and pLuc was purified by ethanol precipitation.

To observe localization of pDNA within the skin, 10-μm frozen sections of the skin were prepared after administration of Cy3-labeled pLuc using a needle-syringe or needle-free injector (ShimaJET®). Sections were fixed in cold acetone and washed in PBS three times and then mounted with Fluoroment-G and observed by fluorescence microscopy.

2.5. Immunization in mice

2.5.1. Immunization schedule

Six-week-old female C57BL/6 mice ($n = 5$) were immunized three times with naked pOVA (30 μg/30 μL) through the skin using a needle-syringe or needle-free injector (ShimaJET-GT) at 2-week intervals. The mice were sacrificed 1 week following the final immunization and a serum sample and a spleen cell suspension then prepared for assay of serum IgG_{2a} and IFN-γ production.

2.5.2. Determination of mouse serum IgG_{2a} of anti-OVA antibodies by enzyme-linked immunosorbent assay (ELISA)

The serum samples were assayed for IgG_{2a} titers using an 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 of serially diluted serum samples then added. After overnight incubation at 4 °C, the wells were washed four times with PBS-T, and 100 μg/100 μL anti-IgG_{2a}-biotin conjugate (Southern Biotech, USA) added for overnight incubation at 4 °C. After washing six times with PBS-T, 100 μL of avidin-conjugated-peroxidase solution (Calbiochem, USA) diluted 1:1000 with B-PBS-T was added for 40 min at 37 °C, the wells were then washed eight times with PBS-T, and 50 μL TMB solution (Kirkegaard and Perry Laboratories, USA) added. After 10 min at room temperature, 50 μL 1 N H_2PO_4 solution was added and absorbance was measured at 450 nm. The concentrations of IgG_{2a} were calculated using the standard curve. A standard curve for IgG_{2a} was established using serially diluted mouse immunoglobulin reference serum (Bethyl Laboratories Inc., Montgomery, TX, USA).

2.5.3. Determination of IFN-γ cytokine secretion from mouse spleen cells

On day 35, 1 week after the final immunization, 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 protein in RPMI medium containing 10% FBS for 72 h, and then 100 μL of the supernatant culture medium was removed and the concentrations of IFN-γ measured using an ELISA kit (R & D Systems, Minneapolis, MN, USA).

2.6. Vaginal immunization in rabbits

2.6.1. Immunization schedule

Nine-week-old female Japanese White rabbits ($n = 3$–4) were immunized four times with naked pOVA (100 μg/100 μL) through the vaginal mucosa using a needle-syringe or needle-free injector (ShimaJET-GT) with the mucosal customized nozzle at 2-week intervals. Blood samples were collected 2 weeks after the last vaccination. A 500 μL blood sample per rabbit was collected from a vein, and allowed to clot into 1.5-mL Eppendorf tubes at 37 °C for 2 h. Serum was separated by centrifugation at 10,000 rpm and 4 °C for 10 min. A vaginal wash sample was collected 2 weeks after the third and fourth vaccinations by washing with 500 μL PBS. Serum and vaginal washes were stored at −20 °C until analysis.

2.6.2. Determination of serum total IgG and vaginal IgA titer

The serum and vaginal wash samples were assayed for serum total IgG and vaginal IgA titers using an ELISA. Briefly, 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 of serially diluted serum or serially diluted vaginal wash sample was then added. Serum dilutions ranged from 1:1 to 1:1024 and vaginal wash dilutions ranged from 1:1 to 1:64. After overnight incubation at 4 °C, the wells were washed four times with PBS-T, and 10 ng/100 μL anti-rabbit-IgG-conjugated-horseradish peroxidase (HRP) (Calbiochem, Germany) or 0.267 ng/100 μL anti-rabbit-IgA-conjugated-HRP (Bethyl Laboratories Inc., TX, USA) added for overnight incubation at 4 °C. After washing six times with PBS-T, 50 μL TMB solution (Kirkegaard and Perry Laboratories, USA) was added. After 10 min at room temperature, 50 μL 1 N H_2PO_4 solution was added and absorbance was measured at 450 nm. The absorbance value was expressed as antibody titer. The antibody titer is expressed as geometric means ($\log_2$) of reciprocal dilutions of OVA-specific IgA in vaginal secretions.

Table 1
Primers sequences used for real-time RT-PCR.

Gene	Primers
IFN-γ	S: 5′-AAGCCTTGTCGGAGATGA-3′ A:5′-CTTTGTAGACGCCTTCCTC-3′
18s rRNA	S: 5′-TCCAGCTCCAATAGCGTAT-3′ A:5′-TAATCATGGCCTCAGTTCC-3′

IFN, interferon; 18s rRNA, 18s ribosomal RNA.

2.6.3. Determination of IFN-γ mRNA expression in lymphocytes from blood

Lymphocytes were prepared from serum as follows. Serum was diluted to 2:1 in RMPI1640 without FBS and 2 mL of diluted serum then carefully layered onto the surface of 2 mL Lympholate® Rabbit (Cedarlane Laboratories Ltd., Hornby, Canada), which is a lymphocyte separation reagent. After centrifugation at 800 × *g* for 30 min, 1 mL of a white cloud-like lymphocyte band was collected and washed twice with RPMI1640 medium without FBS. The resulting pellet was re-suspended and diluted to 2×10^6/mL with RPMI1640 medium with FBS, and added to a 24-well culture plate, at 1×10^6 cells/well in the presence of OVA protein in RPMI medium containing 10% FBS at 37 °C in an atmosphere of 5% CO_2. After a 24-h-incubation, total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The primers were designed according to a previous report (Yang et al., 2008) for use in PCR amplification of cDNA and IFN-γ quantification in real-time RT-PCR. The primer sequences used are listed in Table 1. 18s rRNA was used as the housekeeping gene for normalization of gene expression levels.

cDNA was synthesized using total RNA, 4 μL of RNase inhibitor and 4 μL of PrimeScriptTM RT reagent kit (Takara, Japan) at 42 °C for 15 min and then 85 °C for 5 s. Real-time PCR was performed with cDNA, SYBRGreen PCR Master Mix (Applied Biosystems, USA), RNase Free ddH_2O and primers. The reaction conditions were as follows: 1 cycle of enzyme inactivation for 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of amplification involving initial denaturation at 95 °C for 30 s, annealing at 60 °C for 35 s and elongation at 72 °C for 35 s. The expression level of 18s rRNA was determined as the internal control. According to the cycle threshold (C_t) value and standard curve equation, the relative content of mRNA was calculated and normalized as the mRNA expression of IFN-γ in each sample. The data are expressed as relative IFN-γ mRNA ratio versus the control value.

2.7. Statistical analysis

All values represent the mean ± S.E. Statistical analysis of the data was performed using an unpaired Student's *t*-test. Statistical significance was defined as $^{n.s.}P > 0.05$, $^{*}P < 0.05$ and $^{**}P < 0.01$.

3. Results

3.1. Gene expression and immune responses intradermally injected by needle-syringe injection or a needle-free jet injector

The luciferase activity in rat skin injected by conventional needle-syringe injection or a needle-free injector is shown in Fig. 1(a). The luciferase activity in the rat skin inoculated with the needle-free jet injector was 300-fold greater than that by needle-syringe injection. Next, the distribution of pDNA in rat skin injected by needle-syringe injection or a needle-free injector was deter-

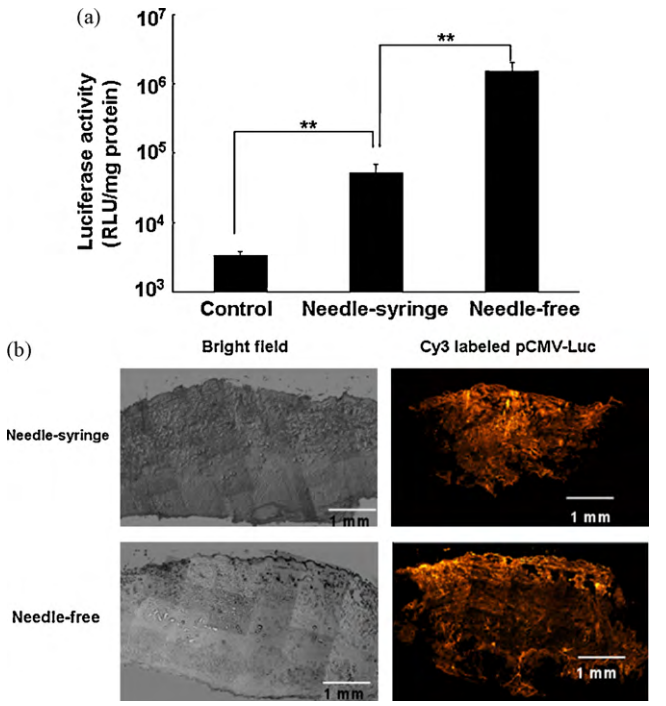

Fig. 1. Luciferase activity and distribution of Cy3-pLuc in rat skin after intradermal injection of pLuc solution by conventional needle-syringe injection and a needle-free injector. (a) Luciferase activity in the rat skin was determined after intradermal injection of pLuc (20 μg) or PBS by conventional needle-syringe injection or a needle-free injector. Each bar represents the mean ± S.E. ($n = 5$). $^{**}P < 0.01$. (b) Distribution of Cye3-pLuc in rat skin tissue was observed after administration of Cy3-pLuc (20 μg) by needle-syringe injection (upper) and the needle-free injector (lower). Skin tissue was collected and 10-μm frozen sections examined by fluorescence microscopy.

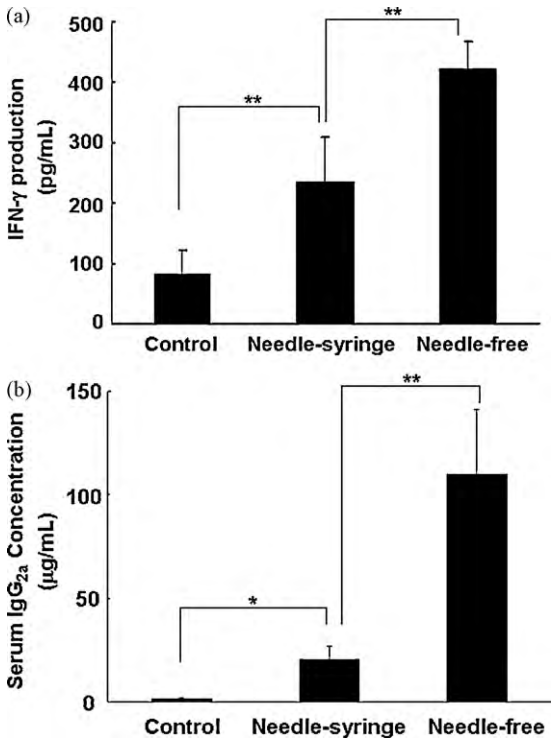

Fig. 3. OVA-specific immune responses in mice after intradermal injection of pDNA by conventional needle-syringe injection or a needle-free injector. Mice were intradermally immunized three times by needle-syringe injection or a needle-free injector injection of pOVA solution at 2-week intervals. Mice were sacrificed 1 week after final immunization. Spleen cells (5×10^5 cells/mL) were prepared and co-cultured with OVA (2 mg/mL) for 72 h before ELISA. IFN-γ in the conditioned-medium (a) and serum IgG_{2a} (b) concentration were analyzed by ELISA. Each bar represents the mean ± S.E. ($n = 5$). $^{*}P < 0.05$, $^{**}P < 0.01$.

mined. As shown in Fig. 1(b), Cy3-pLuc solution administered via needle-syringe injection typically forms a sphere of fluid at the injection spot of the tissue, whereas Cy3-pLuc solution administered through the needle-free jet injector disperses more widely into the dermal tissue.

In addition, to improve the transfection efficiency of pLuc in the skin, we assessed the efficacy of electroporation after administration by needle-syringe injection or a needle-free injector (Fig. 2). The luciferase activity following administration by needle-syringe injection with electroporation was higher than that by needle-syringe alone injection. In contrast, the luciferase activity following

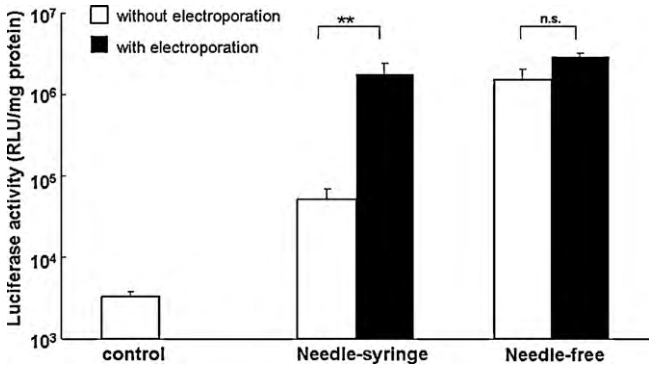

Fig. 2. Luciferase activity in rat skin after intradermal injection of pLuc solution by conventional needle-syringe injection or the needle-free injector with electroporation. Luciferase activity in rat skin was determined after intradermal administration of pLuc (20 μg) by conventional needle-syringe injection or needle-free injector, both with electroporation (200 V/cm, 5 ms, 8 pulses). Each bar represents the mean ± S.E. ($n = 5$). $^{**}P < 0.01$, $^{n.s.}P > 0.05$.

administration by a needle-free injector with electroporation did not differ from that by the needle-free injector alone.

Next, in order to investigate the Th1-dependent immune responses and antibody production in mice by intradermal pOVA vaccination using needle-syringe injection or the needle-free injector, OVA-specific IFN-γ production in spleen cells in intradermal pOVA-immunized mice was assessed by ELISA. Mice were immunized three times with naked pOVA through the skin using needle-syringe injection or the needle-free injector at 2-week intervals. Serum and a spleen cell suspension were prepared for assay 1 week after the final immunization. As shown in Fig. 3(a), OVA-specific IFN-γ production in spleen cells in mice immunized by both needle-syringe injection and the needle-free injector were significantly greater compared with non-immunized mice. Furthermore, OVA-specific IFN-γ production from spleen cells in mice immunized by the needle-free injector was significantly greater than that by conventional needle-syringe injection. Next, serum OVA-specific IgG_{2a} levels were also determined. As shown in Fig. 3(b), serum OVA-specific IgG_{2a} levels in immunized mice were significantly greater than in the controls. Moreover, serum OVA-specific IgG_{2a} in immunized mice using the needle-free injector was markedly higher than that in immunized mice using needle-syringe injection, as was OVA-specific IFN-γ production.

3.2. Vaginal gene expression and immune responses in rabbit immunized intravaginally by needle-syringe injection or the needle-free injector

To investigate whether the needle-free jet injector is an effective local mucosal vaccination device, local gene expression and

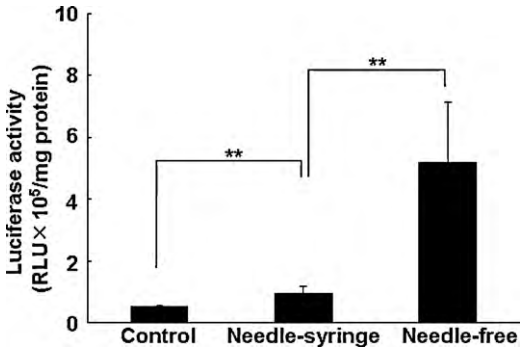

Fig. 4. Luciferase activity in rabbits vagina after intravaginal injection of pLuc solution by conventional needle-syringe injection or a needle-free injector. Luciferase activity in rabbit vagina was determined after intravaginal administration of pLuc (100 μg) by conventional needle-syringe injection or a needle-free injector. Each bar represents the mean ± S.E. ($n = 3$). $^{**}P < 0.01$.

Table 2
The number of OVA-specific serum total IgG and vaginal IgA responders in rabbits.

Groups	Number of responders/total number	
	Serum IgG	Vaginal IgA
No treatment	0/3	0/3
Needle-syringe	0/4	2/4
Needle-free injector	1/3	3/3

immune responses were measured in rabbits injected with pDNA intravaginally using conventional needle-syringe injection or the needle-free injector.

The local vaginal luciferase activities in the rabbits are shown in Fig. 4. We intravaginally injected naked pLuc solution (100 μg/100 μL) using needle-syringe injection or the needle-free injector. The local luciferase activity in the pLuc administration groups (needle-syringe injection and the needle-free injector) was significantly greater than that in the non-treated group. Furthermore, the luciferase activity in rabbit vagina was clearly higher with the needle-free injector than with needle-syringe injection.

We also determined serum total IgG and local vaginal IgA secretion in rabbits given three or four vaginal vaccinations with pOVA using needle-syringe injection or the needle-free injector. As shown in Table 2, a serum total IgG response in the untreated and needle-syringe groups did not appear, whereas the IgG response in one rabbit of three immunized using the needle-free injector strongly increased. A local vaginal IgA response in rabbits immunized four times by either needle-syringe injection or the needle-free injector appeared in almost all rabbits (Table 2). The number of local antibody responders was two of four rabbits immunized by needle-syringe injection and all rabbits immunized by the needle-free injector. The secretory vaginal IgA titer in rabbits immunized four times was clearly higher than that in rabbits immunized three times (Fig. 5). Furthermore, the secretory vaginal IgA titer in rabbits immunized using the needle-free injector was significantly higher than that of rabbits immunized using needle-syringe injection. The IFN-γ mRNA expression in lymphocytes from rabbits that underwent four vaginal vaccinations with pOVA was also determined. As shown in Table 3, the relative IFN-γ mRNA ratio in rabbits immunized of pOVA through the vaginal mucosa using needle-syringe injection was 2.66 ± 1.32, and three of the four immunized rabbits responded. In the case of the needle-free injector, the mean relative IFN-γ mRNA ratio was 1439.4 ± 295.07, and all immunized rabbits responded.

Table 3
The relative IFN-γ mRNA ratio in rabbit blood lymphocytes.

Groups	Relative IFN-γ mRNA ratio	Responders/total
No treatment	1.00 ± 0.44	
Needle-syringe	2.66 ± 1.32**	3/4
Needle-free injector	1439.40 ± 295.07**	3/3

Relative IFN-γ mRNA ratios represent the mean ± S.E., $n = 3$–4.
** $P < 0.01$.

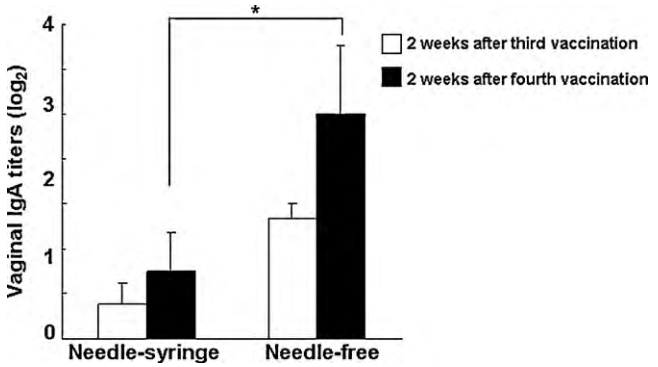

Fig. 5. OVA-specific vaginal IgA titer in rabbits after intravaginal injection of pOVA by conventional needle-syringe injection or a needle-free injector. Rabbits were intravaginally immunized three or four times by needle-syringe injection or needle-free injector injection of pOVA solution at 2-week intervals. Vaginal washes were collected 2 weeks after the third or fourth vaccinations and vaginal IgA titers were analyzed by ELISA. Data are expressed as geometric means ($\log_2$) of reciprocal dilutions of OVA-specific IgA in vaginal secretions. Each bar represents the mean ± S.E. ($n = 3$–4). $^{*}P < 0.05$.

4. Discussion

The vaginal mucosa is the most common site of initiation of virus infections that are transmitted through heterosexual intercourse, including HIV and papillomavirus (De Schryver and Meheus, 1990; Quinn, 1996). Thus, in order to prevent or treat these infections, strong vaginal immunity is required as the first line of defense. We previous reported that our vaginal pDNA immunization protocol with high gene expression using electroporation promoted local IgA production in the vaginal mucosa to a greater extent than intradermal or nasal immunization in mice.

In this study, to determine a more noninvasive, convenient and effective method than electroporation, we examined the local (skin and vagina) gene transfection efficiency of a non-needle jet injector that is already used as a jet injector for daily insulin injection. We found here that the luciferase activity in rat skin inoculated with the needle-free jet injector was 300-fold greater than that by needle-syringe injection (Fig. 1(a)). As shown in Fig. 1(b), the pLuc solution via the needle-syringe typically forms a sphere of fluid at the injection spot of the tissue, whereas the pLuc solution through the needle-free jet injector disperses more widely into the dermal tissue, likely due to the high pressure of the fluid stream. This wide distribution by the needle-free injector possibly achieves markedly higher luciferase activity in rat skin. In addition, the luciferase activity following injection by needle-syringe injection with electroporation was higher than that by needle-syringe injection alone (Fig. 2), whereas the luciferase activity following administration by the needle-free injector with electroporation did not differ from that by the needle-free injector alone. Thus, needle-free injection provides a similarly wide and effective delivery of pDNA into local tissue cells to electroporation. These results indicate that the needle-free injector can deliver pDNA widely in dermal tissue and might deliver to a number of antigen-presenting cells (APCs), which induce immune responses. Next, OVA-specific IFN-γ production as well as OVA-specific IgG_{2a}

production levels in mice immunized by the needle-free injector was also significantly greater than those by conventional needle-syringe injection (Fig. 3). These results were caused by a wider distribution of pDNA solution in the dermal tissue injected through the needle-free jet injector, resulting in a higher contact incidence between the pOVA and APCs, such as antigen-presenting cells and lymphocytes found in dermal tissue. These results indicate that needle-free injection will be a fairly useful method of mucosal gene vaccination.

Furthermore, we briefly investigated the utility of the needle-free injector as a vaginal vaccination tool using rabbits. This injection device, which hasan injector angle of 45° was designed for use in the human vagina from the entrance to inject into the middle site of the tract. Gene expression in the vagina using the needle-free injector was significant greater than that using needle-syringe injection, similar to the intradermal experiment (Fig. 4). Moreover, intravaginal vaccination using the needle-free injector significantly promoted vaginal IgA secretion and IFN-γ mRNA expression in lymphocytes compared to conventional needle-syringe injection. These results demonstrate that the needle-free injector can be used not only as an intradermal vaccination device but also as a mucosal vaccination device. pDNA delivery by a needle-free injector into mucosa, including vagina, has not been previously reported. This study has demonstrated for the first time that a needle-free injector can be used for effective local mucosal vaccination. Furthermore, we used naked pDNA without a gene vector in this study. In our previous study, a cell penetrating peptide effectively elevated vaginal mucosal gene expression and immune responses (Kanazawa et al., 2008). Thus, the combination of a needle-free injector and effective gene carriers may greatly promote the effects of vaginal vaccination. Moreover, needle-free vaccine delivery could be expected to avoid the risk of transmission of infectious disease between patients or between patients and healthcare providers (Giudice and Campbell, 2006). In conclusion, local vaginal DNA vaccination using a needle-free jet injector is potentially a useful, safe, easy and potent method for the prevention and treatment of mucosal infectious diseases.

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