Enhanced mucosal and systemic immune responses to a vaginal vaccine coadministered with RANTES-expressing plasmid DNA using in situ-gelling mucoadhesive delivery system

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Abstract

To develop more potent and convenient mucosal vaccines, we investigated the effect of an in situ-gelling mucoadhesive vaginal vaccine delivery system and a genetic chemokine adjuvant on the local and systemic immune responses. The in situ-gelling mucoadhesive delivery system of hepatitis B surface antigen (HBsAg), composed of poloxamers and polycarbophil, showed the prolonged retention at the vaginal tissues. Following intravaginal administration to mice, HBsAg-specific IgA was induced in the vagina and saliva, and IgG was produced in the serum. RANTES-expressing plasmid (pRANTES) intravaginally coadministered with HBsAg showed the expression at the vaginal tissues, and more effectively induced the vaginal IgA and serum IgG immune responses than did cholera toxin (CT). The intramuscular coadministration of pRANTES with HBsAg also increased both serum IgG levels and mucosal IgA levels. Regardless of the adjuvants, the in situ-gelling mucoadhesive HBsAg delivery system enhanced the mucosal and systemic immune responses. At 42 days after the first immunization, the highest vaginal IgA levels were induced after intravaginal immunization of HBsAg plus pRANTES using the in situ-gelling mucoadhesive delivery system, showing 182- and 1035-fold higher titer compared to the groups receiving HBsAg alone in PBS by intravaginal and intramuscular routes, respectively. Our results indicate that the use of in situ-gelling mucoadhesive delivery systems with the genetic chemokine adjuvant pRANTES would be advantageous for more effective induction of mucosal and systemic immune responses to intravaginally administered vaccines.

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1. Introduction

Although the induction of a local immune response in the vagina may be particularly important in protection against viral and bacterial pathogens, the antigen-specific immune responses in genital secretions have been difficult to achieve [1]. Recently, the intravaginal route has been suggested to be promising for the induction of the vaginal antibody responses [2,3]. For the intravaginal immunization, phosphate-buffered saline (PBS) solution has been mostly used as a vehicle for the convenient and exact dosing of antigens. The immunogenicity of intravaginally administered vaccines in PBS, however, has been limited possibly due to the rapid leakage of the free-flowing solution type vaccines from the administered sites and to the unprotected degradation of antigens by enzymes in mucosal secretions [4]. For convenient immunization and prolonged retention of antigen at the administered sites, it would be desirable to develop a vaginal delivery system which may exist as a liquid during immunization but gel inside the vagina. The mucosadhesiveness of the vaginal vaccine delivery system might further increase the exposure of antigens to the mucosal tissues.

Another limitation in the development of vaginal vaccines has been the lack of safe and effective mucosal adjuvants. In experimental animals, cholera toxin (CT) has been used as a potent mucosal adjuvant [5]. High doses of CT have been used to induce mucosal immune responses after the nasal, rectal, and vaginal routes. However, given the severe toxicity of CT reported in mice and humans [6], there is a strong need to develop mucosal adjuvants which could safely and effectively enhance the immune responses.

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Chemokines, secreted 8–10 kDa low molecular weight proteins, act on leukocytes through selective G protein-coupled cell surface receptors and are now known to function in leukocyte maturation, trafficking, and homing of these cells [7]. RANTES, a CC chemokine, is a potent chemotactic molecule that may attract monocytes, memory T cells, and eosinophils which seem to play a critical role in the production of allergic inflammation. RANTES has also been found to have effects on the magnitude and cytokine polarity of T cell responses [8]. RANTES is produced by lymphoid and epithelial cells of the mucosa in response to various external stimuli, and has been shown to induce lymphocyte migration into the nasal mucosa of allergic patients [9]. Recently, RANTES has been reported to potentiate the antigen-specific mucosal immune responses [10]. Following nasal coadministration with an antigen, RANTES increased antigen-specific antibody titers in mucosal secretions and enhanced the numbers of antibody-forming cells in mucosal and systemic compartments. It has been suggested that RANTES might enhance mucosal and systemic antibody responses through help provided by Th1- and select Th2-type cytokines as well as through the induction of costimulatory molecule and cytokine receptor expression on T lymphocytes. RANTES in a protein form, however, might result in the high cost of vaccination. The plasmid DNA encoding RANTES has been shown to exhibit the adjuvant activity after intramuscular immunization [11]. Currently, the adjuvant activity of genetic RANTES following intravaginal coadministration with an antigen has not been known.

In this study, recombinant hepatitis B surface antigen (HBsAg) as a model, we investigated the effect of vaginal vaccine delivery systems and the adjuvant activity of genetic RANTES following intravaginal immunization to mice. We report here that an in situ-gelling mucoadhesive vaginal delivery system could enhance the induction of mucosal and systemic immune responses, and that the vaginal coadministration of genetic RANTES could further potentiate the mucosal and systemic immune responses.

2. Materials and methods

2.1. Adjuvants

CT or plasmid DNA of RANTES was used as an adjuvant. CT, purified from Vibrio cholerae, was obtained from Sigma (MO, USA). The cDNA fragment of murine RANTES was cut from pBluescriptII SK(±) in pVAX1 vector containing cytomegalovirus early promoter/enhancer sequence and kanamycin resistance gene (Invitrogen, CA, USA). The resulting pVAX1 backbone was amplified using Escherichia coli DH5α and purified using the Qiagen plasmid gig kit (Qiagen, CA, USA). The purity of plasmid DNA was confirmed on a 1% agarose gel.

2.2. Preparation of in situ-gelling mucoadhesive formulations for intravaginal delivery

In situ-gelling mucoadhesive formulations were prepared using temperature-sensitive and mucoadhesive polymers. HBsAg (kindly provided by LG Chem Ltd., South Korea), and poloxamers (Pol, BASF, Ludwigshafen, Germany) were solubilized in distilled water. In some cases, CT or pRANTES was added to the Pol-based solution. The composition of the Pol mixtures (Pol 407/188, 12/20% (w/w)) was previously demonstrated to form gels at vaginal temperature conditions [12], and used in this study. The liquid form of Pol-based formulation was left at 4°C until a clear solution was obtained. Polycarbophil (PC, BF Goodrich, OH, USA) used as a mucoadhesive polymer was then slowly added to the solution with continuous agitation, resulting in Pol/PC solution.

2.3. Measurement of gelation temperature

Gelation temperature defined as the temperature at which the liquid phase makes a transition to gel, was determined as described previously [13]. In brief, a 10 ml transparent vial containing a magnetic bar and each formulation was placed in a water bath. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. The temperature-sensitive gelation of Pol/PC was tested by dropping 0.1 ml of PBS or Pol/PC solution containing 2% blue lake dye (FD&C blue #1) into 1 ml of PBS at different temperatures.

2.4. Animals

Female BALB/c mice were supplied from the Experimental Animal Center of Seoul National University (Seoul, South Korea). Mice were used at 6–8 weeks of age. Animals received food and water ad libitum and were kept under specific-pathogen-free conditions. SNU guidelines for the care and use of laboratory animals have been observed.

2.5. Retention of mucoadhesive delivery systems in the vaginal tissues

To test the retention of mucoadhesive delivery systems in the vaginal tissues, various formulations containing 2% blue lake dye (FD&C blue #1) were intravaginally administered into mice using a micropipette tip. At 1 h after administration, mice were sacrificed and the retention of mucoadhesive delivery systems at the administered sites was visualized by blue color of the dye.

2.6. Reverse transcription-PCR (RT-PCR) analysis

Semiquantitative RT-PCR was used to determine the relative mRNA expression levels of vaginally delivered
pRANTES at the administered sites. Mice were sacrificed at 24 h after intravaginal coadministration of pRANTES (100 µg per mouse) with HBsAg (10 µg per mouse), and the vaginal tissues were removed. As controls, the vaginal tissues of untreated mice or mice treated with empty pVAX1 vector without encoding RANTES were used. Total RNA was extracted from the vaginal tissues using a TRizol® reagent (Gibco BRL, NY, USA). The cDNA was prepared using a First-Strand cDNA synthesis kit (Boehringer Mannheim, IN, USA). PCR amplification of the murine RANTES cDNA was performed as described previously [14]. The primer pair used to amplify the 300 bp segment of RANTES gene was 5′-TTGGCTACTCTCTCCAGGC-3′ for the sense and 5′-ATGCCGATTTTCCAGGA-CC-3′ for the antisense. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) house keeping gene was used as an endogenous reference. To amplify the 360 bp segment of GAPDH gene, 5′-ATCACCATCTCCAGGAGC-3′ oligonucleotide was used for the sense and 5′-AGAGGGCCA-TCCACAGTCTTC-3′ for the antisense. PCR products were analyzed by electrophoresis on a 2% agarose gel. The density of each band was measured using a gel-doc image analyzer (Vilber Lourmat, France).

2.7. Intramuscular and intravaginal immunization of mice against HBsAg

Female BALB/c mice received intramuscular or intravaginal immunization against HBsAg. For intramuscular immunization, mice were intramuscularly administered with 50 µl of sterile PBS containing HBsAg (1 or 10 µg). In some cases, pRANTES (100 µg) was intramuscularly coadministered with HBsAg. For intravaginal immunizations, HBsAg (10 µg) in PBS or Pol/PC was administered into the vagina of mice using a micropipette tip. In some cases, pRANTES (100 µg) or CT (10 µg) was intravaginally coadministered with HBsAg. Control mice received PBS or in situ-gelling mucoadhesive vehicle without HBsAg. All animals received two immunization doses at 3 weeks interval by the same routes.

2.8. Collection of sera and secretions

Preimmune samples of sera and secretions were obtained 1 day before immunization (day 0). Subsequent to immunization, collections were made at days 21 and 42. Serum samples were obtained by centrifugation of blood collected from the tail vein using a capillary tube with 1.1 mm diameter. Salivary samples were collected using a pipette fitted with a plastic tip after stimulation of salivary flow by intraperitoneal injection of pilocarpine hydrochloride (Sigma) at a dose of 1 µg/g mouse [15]. Vaginal secretions were collected by washing the tracts with 30 µl of sterile PBS [15]. The salivary and vaginal secretions were cleared by centrifugation at 10,000 × g to remove any tissue and cellular debris. All the samples were stored at −70 °C until analyzed.

2.9. Evaluation of antibody production

Amounts of HBsAg-specific antibodies were measured as described by Gursel et al. [16] with slight modification. ELISA plates were coated with HBsAg (2 µg/ml) and incubated at 4 °C overnight. The plates were washed and blocked with 2% (w/v) bovine serum albumin. Serially diluted mouse sera, salivary secretions, or vaginal washings (60 µl per well) were added, and incubated at room temperature for 2 h. Following washings, peroxidase-conjugated goat anti-mouse IgG antibody (1:10,000, ICN, OH, USA), or goat anti-mouse IgA antibody (1:2500, ICN) was added. After 1 hincubation and subsequent washing, 1-Step™ Turbo TMB-ELISA (3, 3′, 5′-tetramethyl benzidine substrate solution, Pierce, IL, USA) was added. Color development was determined spectrophotometrically at 450 nm. Results were expressed as reciprocal numbers of the final detectable dilution.

3. Results

3.1. Gelation and prolonged residence of in situ-gelling mucoadhesive delivery system

Temperature-sensitive Pol-based formulations existed as liquid at room temperature but converted to a gel with temperature changes. The gelation temperatures of Pol-based formulations were lowered by mucoadhesive polymer PC, but not by HBsAg. In the absence of PC, the Pol-based solution (Pol 407/Pol 188, 12/20% (w/w)) gelled at 35 ± 0.4 °C. When 0.2% of PC was added to the Pol vehicle, the gelation temperatures slightly dropped down to 34.6 ± 0.3 °C. In the presence of HBsAg, the mixture of Pol 407 and Pol 188 (12/20% (w/w)) gelled at 35.4 ± 0.4 °C. When PC was added to the Pol vehicles containing HBsAg, the gelation temperature decreased to 33.8 ± 0.6 °C. The formulations containing mucoadhesive polymer PC higher than 0.2% were prepared and tested. However, they were hard to be administered from pipette tip due to higher viscosity, and not further used in this study. The Pol/PC solution containing a dye gelled in PBS at 37 °C whereas it homogenously mixed with 4 °C PBS (data not shown). PBS solution containing a dye, used as a control, did not show temperature-dependent change upon addition to PBS medium.

The Pol/PC-based formulation showed a prolonged retention at the vaginal tissues. At 1 h after intravaginal administration of mice using a dye-loaded PBS or Pol/PC, there were visible differences in the residual levels of dye between
the two groups (Fig. 1). The dye intravaginally delivered in PBS almost disappeared at 1 h post-dose (Fig. 1a), whereas the dye given in Pol/PC vehicle remained at the mucosal folds of the vaginal tissues (Fig. 1b).

3.2. Expression of intravaginally administered pRANTES

To test whether the pRANTES released from the Pol/PC vaginal delivery system is still functional and intact enough to be expressed, the mRNA expression of pRANTES at the vaginal tissues was studied. pRANTES released from the Pol or Pol/PC vehicles could exist as a functional form and be transcribed to mRNA at the vaginal tissues. Untreated mice and PBS-treated mice did not show significant differences in the relative mRNA expression levels of pRANTES normalized by GAPDH (Fig. 2). Mice intravaginally treated with empty pVAX1 vector in Pol/PC revealed mRNA levels of RANTES similar to the untreated mice. In contrast, the highest mRNA expression levels of pRANTES were observed after delivery in Pol/PC.

3.3. Vaginal antibody responses

The induction of HBsAg-specific vaginal IgA antibody was affected by the routes, antigen delivery vehicles, and mucosal adjuvants. Compared to the intramuscular immunization, the intravaginal route induced the higher levels of vaginal IgA to HBsAg given in PBS or Pol/PC over 42 days after the first immunization of HBsAg in PBS (Fig. 3). The in situ-gelling mucoadhesive vaginal delivery system, Pol/PC, showed the enhanced levels of vaginal IgA as compared to PBS vehicle. At 42 days after the first immunization, Pol/PC-mediated vaginal delivery of HBsAg showed vaginal IgA antibody titers four-fold higher than PBS-based vaginal delivery of HBsAg (Fig. 3b). Similarly, Pol/PC-mediated vaginal codelivery of HBsAg with pRANTES showed 13-fold higher vaginal IgA antibody titers as compared to PBS-based vaginal coadministration of HBsAg with pRANTES (Fig. 3b).

pRANTES showed more effective vaginal adjuvant activity than did CT, regardless of the vehicles used for intravaginal antigen delivery. pRANTES given in PBS revealed the three-fold higher levels of vaginal IgA antibody responses compared with CT in PBS at 42 days after the first immunization (Fig. 3b). When Pol/PC was used as a vaginal delivery system of HBsAg, the vaginal IgA titers were five times higher in pRANTES relative to CT (Fig. 3b). Since CT has not been used as a parenteral adjuvant due to the toxicity, the adjuvant effect of CT following intramuscular injection was not tested in this study.

Among various groups, the highest vaginal IgA was induced following intravaginal administration of HBsAg plus pRANTES via the in situ-gelling mucoadhesive delivery system. At 42 days after the first immunization,
the group treated with HBsAg plus pRANTES in Pol/PC vaginal delivery system showed 182- and 1035-fold higher vaginal IgA titers in comparison with the groups receiving HBsAg in PBS by intravaginal and intramuscular routes, respectively.

3.4. Salivary antibody responses

Similar to HBsAg-specific vaginal IgA antibody, HBsAg-specific salivary IgA antibodies were more effectively induced by the coadministration of HBsAg with pRANTES (Fig. 4). In comparison with the group treated with HBsAg (10 μg) in PBS, the group given the same dose of HBsAg plus pRANTES in PBS showed three-fold higher values of salivary IgA titers at 21 days (Fig. 4b) after the first intramuscular administration. Following intravaginal administration, the group treated with pRANTES and HBsAg in PBS showed four times higher salivary IgA titers than the group received HBsAg alone in the same vehicle, Pol/PC (Fig. 4b). The coadministration HBsAg and pRANTES in Pol/PC showed the five-fold increased salivary IgA titers compared to HBsAg alone in the same vehicle, Pol/PC. Unlike the vaginal IgA antibody responses, the salivary IgA responses were the highest following the intravaginal coadministration HBsAg with CT in Pol/PC.
**Fig. 5.** Systemic immune responses after intravaginal and intramuscular administration. Female BALB/c mice were immunized twice at 3 weeks intervals. HBsAg-specific serum IgG titers were measured at 3 weeks (a) and 6 weeks (b) after the first vaccination. Vehicles indicate the groups which received PBS or Pol/PC vehicle alone without HBsAg via various administration routes. The results are expressed as the mean ± S.E. (n = 5); (∗): significantly different from intravaginally administered HBsAg (ANOVA and Duncan’s multiple range test, P < 0.05); (‡): significantly different from intravaginally administered HBsAg + pRANTES (ANOVA and Duncan’s multiple range test, P < 0.05).

3.5. Serum antibody responses

The HBsAg-specific serum IgG antibody responses could be induced by intravaginal immunization route, and further improved by the in situ-gelling mucoadhesive vaginal delivery system and the genetic chemokine adjuvant pRANTES. At 21 days after the first immunization with 10 μg of HBsAg, the intramuscular route revealed the higher levels of serum IgG antibodies than did the intravaginal route (Fig. 5a). Similarly, at 42 days after the first immunization, the serum IgG antibody titers were higher in mice treated via the intramuscular route relative to the intravaginal route (Fig. 5b). However, when mice were immunized with 10 μg of HBsAg and pRANTES in Pol/PC via the intravaginal route, the serum IgG antibody titers were comparable to those obtained after intramuscular administration with 1 μg of HBsAg and pRANTES (Fig. 5b). Pol/PC-mediated vaginal delivery of HBsAg plus CT revealed the increased serum IgG titers compared to PBS-based vaginal immunization of HBsAg with CT.

After the coadministration of HBsAg with pRANTES, the increased serum IgG levels were observed regardless of immunization routes and delivery vehicles. At 21 days after the first administration, HBsAg-specific serum IgG responses were four-fold increased following intramuscular immunization with HBsAg (10 μg) plus pRANTES compared to the same dose of HBsAg alone (Fig. 5a). Similarly, after intravaginal immunization in PBS, the two-fold higher serum IgG responses were obtained with HBsAg plus pRANTES than with HBsAg alone. When Pol/PC was used as a vaginal delivery system, the group treated with HBsAg and pRANTES revealed serum IgG antibody levels six-fold higher than HBsAg in the same vehicle at 42 days after the first administration (Fig. 5b). Systemic adjuvant activity was more potent in pRANTES than in CT. The intravaginal delivery of HBsAg (10 μg) plus pRANTES in Pol/PC revealed three-fold higher levels of serum IgG antibodies than did the intravaginal coadministration of HBsAg (10 μg) with CT in the same vehicle.

4. Discussion

In this study, we demonstrated that the use of in situ-gelling mucoadhesive vaginal delivery system and the genetic chemokine adjuvant significantly enhanced the mucosal and systemic immune responses to the recombinant HBsAg.

The in situ-gelling mucoadhesive delivery system, Pol/PC, existed as a liquid at room temperature but formed a gel when added to 37 °C medium. Such temperature-sensitive gelation might have several advantages over conventional PBS-based vaginal vaccination. The temperature inside human vagina is known to be 37.2 °C [17]. The gelation of Pol/PC at 37 °C indicates that HBsAg-containing Pol/PC might convert to a gel inside vaginal cavity, preventing the substantial leakage of HBsAg from the administered sites. The liquid form at room temperature might be convenient when administering the exact amount of antigens and adjuvants to vaccine recipients. Moreover, the liquid type of Pol/PC vaginal delivery system may minimize the foreign feeling during vaginal delivery.

Of intravaginal immunization groups, the in situ-gelling mucoadhesive formulation containing HBsAg resulted in the higher mucosal and serum antibody responses than PBS-mediated delivery groups. The higher immunogenicity of HBsAg intravaginally given in Pol/PC might be partially attributed by the reduced leakage and retention of antigen from the administered sites via gelation in the vagina.
The mucosal immunogenicity exerted by PC may lead to the prolonged retention and increased interaction of vaginally delivered antigen with the mucosal epithelium [18]. Recently, the enhanced mucosal immunity has been reported in mucocutaneous polymer microspheres as delivery systems [19]. Hemagglutinin-containing mucocutaneous microspheres have shown the higher IgA and serum IgG responses than hemagglutinin vaccine alone [20]. These findings support that mucocutaneous polymer might be one of useful components in mucosal vaccine carriers.

Of mucosal vaccines, intranasal vaccines have been studied for induction of vaginal IgA antibody responses. Intranasal immunization with recombinant HBsAg associated with cationic particles has been reported to induce vaginal IgA antibodies [21]. After nasal immunization, the induction of anti-human immunodeficiency virus peptide IgA antibodies was observed in vaginal secretions [22]. Recombinant Norwalk virus-like particles administered by intranasal route was reported to induce vaginal IgA antibody responses [23]. Intranasal vaccines might be advantageous over intravaginal vaccines in that the nasal route has been considered as a more convenient alternative to infections in pharmaceutical fields [24]. Intranasal vaccines, however, have been reported to exert limited and lower potency in the induction of vaginal IgA antibodies as compared to vaginal vaccines. Vaginal immunizations using attenuated herpes simplex virus type-2 induced the greater immunity against reinfection of the vaginal epithelium than did nasal immunizations [25]. Higher magnitude of genital tract antibody responses was generated by vaginal immunization of a cholera vaccine rather than nasal vaccine dosages [26]. Given these findings, the choice of immunization routes needs to be based on the balance between the convenience and the potency. The in situ-gelling vaginal delivery systems might improve both the convenience and the potency of immunization.

Our results indicate that vaginally delivered pRANTES might be a feasible mucosal or systemic adjuvant. The chemokine RANTES has been known to exert an adjuvant function by attracting monocytes, immature dendritic cells and T- and B-cells [27]. RANTES, a member of the CC chemokine family, induces leukocyte migration by binding to specific receptors such as CCR1, CCR3, CCRA, and CCR5. RANTES also functions as a physiological ligand for the HIV-1 co-receptors CCR3 and CCR5 [28], indicating the therapeutic implication of RANTES in the treatment and prevention of HIV infection [27,29]. Chemokine RANTES in a protein form has been reported to potentiate antigen-specific mucosal immune responses after nasal coadministration with ovalbumin antigen [10]. The expression plasmid DNA encoding RANTES was shown to induce antigen-specific cell-mediated immunity after intramuscular and intranasal immunizations [11]. Though the mechanisms by which RANTES exerts mucosal and systemic adjuvant activity need to be further studied, local production or delivery of RANTES has been shown to cause chemotaxis of immune cells in vivo. Local production of RANTES could induce lymphocyte migration into the nasal mucosa of allergic patients [9]. Cutaneous injection of RANTES accelerated the recruitment of eosinophils [30]. Moreover, it has been suggested that RANTES could initiate and enhance antigen-specific mucosal and systemic humoral antibody responses through help provided by cytokines and through the induction of costimulatory molecule and activation of T lymphocytes. The potent vaginal immune response-inducing effect of pRANTES indicates that pRANTES might be used as a mucosal adjuvant rather than toxoid CT. Although the safety of mucosally administered pRANTES still needs to be evaluated, repeated administration of the DNA constructs encoding chemokines such as MCP-1, MIP-1 alpha, or RANTES has been shown to inhibit the development and progression of polyadjuvant-induced arthritis [31].

Our observation that intravaginal immunization of HBsAg was more effective in inducing the vaginal IgA than systemic immunization, indicates the importance of immunization routes for vaginal immune responses. Consistent with our results, intravaginal immunization of attenuated herpes simplex virus type-2 induced the greatest immunity against reinfection of the vaginal epithelium [25]. Among oral, rectal, and intravaginal immunizations, only the vaginal route increased IgA antibodies in genital tract secretions in women [2]. Vaginal immunization with human papillomavirus 6bL1 DNA could induce long-lasting vaginal IgA responses whereas no vaginal immune response was detected after intramuscular or intrarectal immunization [3]. Such increases in vaginal immune responses would be of utmost clinical importance as vaginal defense lines play a major role in preventing the sexually transmitted infection. However, a previous report showed that the intravaginal immunization with CT produced no vaginal antibodies whereas the rectal immunization induced vaginal IgA antibodies [32]. It is speculated that the type of antigen, the immunization dose, and the frequency of boosting might attribute to the discrepancy of our finding and theirs. Moreover, we observed that intravaginal immunization of HBsAg was effective in inducing the vaginal and salivary IgA responses. The local and distal mucosal immune responses after intravaginal immunization are still rather controversial. Similar to our finding, vaginal immunization of human immunodeficiency virus (HIV) peptide vaccine mixed with CT induced antigen-specific IgA antibody in mouse fecal extract and vaginal wash [33]. However, vaginal immunization of cholera toxin B was reported to induce vaginal IgA but no rectal antibody in women [2]. RANTES, which gave higher vaginal titers than CT, showed lower salivary IgA titers. Currently, the mechanisms by which RANTES and CT showed difference in the induction of salivary IgA levels remain unclear. However, the possibility exists that the difference might be correlated with the different mechanisms of adjuvant actions between pRANTES and CT. RANTES, given in a plasmid DNA form in this study, was observed to be expressed at the vaginal tissues. The chemotactic activity and concentration gradients of RANTES derived from pRANTES might be
higher at the administered sites rather than at the relatively distant nasal tissues. Meanwhile, cholera toxin (CT) nasally coadministered with a diphtheria toxoid antigen has been shown to exert adjuvant activity for the induction of IgA antibodies in nasal and vaginal secretions [34]. Though the mechanisms of CT adjuvanticity need to be further elucidated, CT has been reported to promote the priming of antigen-specific T cells and to induce both mucosal and systemic immune responses via a Th2 cell-dependent pathway [35]. Recently, there is emerging evidence that dendritic cells are one of the principal cell types that mediate mucosal and systemic adjuvant effects of CT in vivo [36,37]. Systemic immune responses were induced following intranasal immunization with HBsAg and influenced by vaginal vaccine delivery systems. Consistent with our results, systemic and mucosal antigen-specific immune responses were induced after vaginal immunization of vaccine [38]. Moreover, we observed that systemic immune responses were potentiated by in situ-gelling mucoadhesive delivery systems. A previous report showed that plasmid DNA in a gel formulation could be protected from degradation by DNase I [39]. Our previous study showed the systemic absorption of nasally administered plasmid DNA could be increased after delivery in Pol/PC formulation [40]. Given these previous studies, the possibility exists that in situ-gelling mucoadhesive Pol/PC system might have stabilized and enhanced the systemic absorption of pRANTES and HBsAg, partially contributing to the potentiated systemic immune responses.

In conclusion, the thermosensitive and mucoadhesive polymer-based vaginal vaccine delivery systems might be useful in enhancing the mucosal and systemic immune responses. Moreover, the mucosal and systemic adjuvant activity of genetic chemokine pRANTES could be applied to other mucosal vaccines.

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