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Aerosol delivery of Virus-like Particles to the genital tract induces local and systemic antibody responses

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Abstract

The induction of mucosal immune responses in the genital tract may be important for increasing the effectiveness of vaccines for sexually transmitted infections (STIs). In this study, we asked whether direct immunization of the mouse genital tract with a non-replicating virus-like particle (VLP)-based vaccine could induce local mucosal as well as systemic antibody responses. Using VLPs derived from two bacteriophages, Q β and PP7, and from a mammalian virus that normally infects the genital tract, human papillomavirus (HPV), we show that intravaginal aerosol administration of VLPs can induce high titer IgG and IgA antibodies in the female genital tract as well as IgG in the sera. Using a mouse model for HPV infection, we show that intravaginal immunization with either HPV type 16 VLPs or with PP7 bacteriophage VLPs displaying a peptide derived from the HPV minor capsid protein L2 could protect mice from genital infection with an HPV16 pseudovirus. These results provide a general method for inducing genital mucosal and systemic antibody responses using VLP-based immunogens.

Keywords

VLP; HPV; aerosol; mucosal immunity

1. Introduction

Many, if not most, pathogens gain entry into a host through mucosal surfaces. Mucosal tissues, such as in the gastrointestinal, respiratory, and genital tracts, in turn, are colonized by resident lymphocytes and antigen presenting cells that can initiate responses to antigen. Because the environment of each of these mucosal compartments is distinct, it has been suggested that directly immunizing the mucosal compartment targeted by a specific pathogen could result in the induction of protective immune responses at the site of infection [1]. For example, the inhaled FluMist vaccine and the oral poliovirus vaccine target the respiratory and gastrointestinal tracts and provide mucosal and systemic protection against influenza and poliovirus. However, the genital tract has remained largely under-utilized as a site for possible vaccine delivery.

The genital mucosa in particular is vulnerable to infection by a host of bacterial and viral pathogens. For example, sexually transmitted human papillomavirus (HPV), HIV, and

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herpes simplex virus infections occur in the genital tract, and are associated with cervical cancer, AIDS, and genital herpes, respectively. Although in the case of HPV infection it is clear that a systemic antibody response can protect against genital infection [2], it is likely that a more comprehensive understanding of the workings of the innate and adaptive immune system at the genital mucosa, and their mechanisms of protection, is necessary for the rational design of vaccines against these and other sexually transmitted infections (STIs).

Virus-like particles (VLPs) are a promising technology for the development of vaccines. VLPs are comprised of viral structural proteins, which, when overexpressed, can spontaneously self-assemble into non-infectious particles that are structurally indistinguishable from the virus from which they were derived, but lack a viral genome. VLPs make excellent vaccines because they induce strong and long-lasting immune responses. They can be used as stand-alone vaccines that target the viruses from which they are derived and as scaffolds for increasing the immunogenicity of heterologous antigens. Two VLP-based vaccines that target Hepatitis B virus (HBV) and Human Papillomavirus (HPV) are clinically approved, and other VLP vaccines targeting Norwalk virus and Influenza virus are currently in clinical trials. VLPs have also been used as platforms for the development of vaccines for infectious and chronic diseases, as well as diseases of addiction [3].

VLPs evoke strong antibody responses because of their highly dense, multivalent structures. This arrangement of proteins provides a dense, repetitive display of epitopes that is highly stimulatory to B cells [4]. Unlike non-particulate subunit vaccines, VLPs can induce strong antibody responses at low doses and without the use of exogenous adjuvants. However, VLPs can also be loaded with adjuvants to increase their potency [5].

We have been interested in assessing the ability of VLP-based vaccines to induce mucosal immune responses. Previously, we developed a bacteriophage VLP-based vaccine targeting the HIV coreceptor CCR5 and showed in a rat model that administration of a nebulized form of this vaccine to the respiratory tract induced both local (in the lung) and systemic IgG and IgA antibody responses [6]. This vaccination protocol also induced a weak but measurable IgA antibody response in the genital tract of vaccinated rats. Here, we asked whether direct vaccination of the genital tract could lead to enhanced antibody responses at this local mucosal site, as well as induce antibody responses in serum. Moreover, we asked if genital vaccination could provide protection in mouse model from infection with pseudovirions derived from a prototypical STI, HPV. We assessed the ability of two HPV vaccines to provide protection from HPV pseudovirus infection upon immunization of the genital tract. These include HPV16 VLPs, which induce type-specific neutralizing antibodies against the viral major capsid protein L1, and bacteriophage VLPs displaying a peptide from the HPV minor capsid protein, L2, which induce neutralizing antibodies that are capable of blocking infection by a broad spectrum of HPV types [7].

2. Materials and Methods

2.1 VLP and Phage preparations

Q β bacteriophage were prepared by infecting a culture of *E. coli* strain A/ λ . Phage were precipitated from clarified supernatant at 4°C overnight after addition of NaCl to 0.5M and PEG 8000 to 10%. Precipitate was collected by centrifugation and then dissolved in TNME (0.1M NaCl, 10 mM Tris-HCl, 0.1 mM MgCl₂, 0.01 mM EDTA). Phage were then purified by isopycnic centrifugation in CsCl as previously described [8]. Production and purification of human papillomavirus type 16 (HPV16) L1/L2 VLPs using 293TT cells were accomplished using previously reported methods [9, 10] and were generously supplied by Michelle Ozbun (University of New Mexico). Recombinant bacteriophage PP7 VLPs

displaying a peptide representing amino acids 17–31 from the minor capsid protein (L2) of HPV16 were constructed and purified as described [11].

2.2 Mice Immunizations

Groups of three to five Balb/c mice were vaccinated with Q β bacteriophage, recombinant 16L2-PP7 VLPs, or HPV16 L1/L2-VLPs. Mice given vaccine intramuscularly were immunized with 10 μ g of VLPs formulated 1:1 (volume:volume) in incomplete Freund's adjuvant, with the exception of mice receiving the 16L2-PP7 vaccine, which did not contain any adjuvant.

Prior to genital immunization the vaginal epithelia was disrupted either by mechanical or chemical abrasion. Both methods of disruption were performed as previously described [12, 13]. Briefly, mechanical disruption was conducted immediately prior to immunization and performed using a cytobrush (Cooper Surgical). Chemical disruption was conducted 6 hours prior to immunization and performed by introducing 30 μ l of a 4% nonoxynol-9 in 3% carboxymethylcellulose (CMC) in distilled water solution into the genital tract.

For intravaginal immunizations 25 μ g of VLPs were applied without exogenous adjuvant. For each animal receiving the vaccine in gel form, 25 μ g of VLPs were added to 3% CMC in a total volume 30 μ l. VLPs were highly concentrated to ensure that the optimal viscosity was maintained. Using a positive displacement pipette (Gilson, Middleton, WI), the VLP-in-CMC gel was delivered to the vaginal tract. For each animal receiving the aerosolized vaccine, 25 μ g of VLPs in PBS (in a volume not to exceed 50 μ l) were loaded into a microsprayer high-pressure syringe (Penn-Century) and delivered to the vaginal tract.

Mice were vaccinated according to the following schedule. In general, most groups of mice were immunized on days 0 and 14. The group immunized with 16L2-PP7 VLPs received boosts on days 42 and 200. One of the groups immunized with HPV VLPs received a single immunization at day 0. In all studies, serum samples and vaginal lavages (approximately 0.2 mL in PBS) were collected prior to immunization and weekly thereafter. To minimize fluctuations in whole IgA levels [14], all mice received subcutaneous injections of Depo-Provera (3.0 mg in 0.1 mL) 4 days prior to immunization, and then every ten days until sacrifice, to synchronize the estrous cycle. Synchronization was verified by microscopic examination of cells obtained by vaginal lavage.

2.3 Quantifying antibody responses

Sera and vaginal lavages were collected at the time points indicated, and all samples analyzed by ELISA for antibodies specific to the appropriate VLP. ELISA experiments were performed as previously described [6]. Specifically, Immulon II ELISA plates (Thermo Biosystems) were coated overnight at 4°C with 500 ng of HPV16 VLPs, Q β bacteriophage, or PP7 VLPs per well. For analysis of L2-specific antibody in sera from animals immunized with 16L2-PP7 VLPs, wells were coated in three steps (using volumes of 50 μ l for each step). First, 0.5 μ g of streptavidin (Sigma Aldrich) was applied to each well for 2 h at room temperature. After washing with PBS, 0.5 μ g of the crosslinker SMPH (Pierce Endogen) was added to each well for 2 h at room temperature. Following washing, 0.5 μ g of an synthetic L2 peptide representing HPV16 L2 amino acids 14–40 and containing a C-terminal cysteine residue for conjugation to streptavidin (SATQLYKTCKQAGTCPPDIIPKVEGKTGGC, with a disulfide bridge between Cys9 and Cys15; Designer BioScience) was added to each well, and plates were left overnight at 4°C. The next day, after blocking wells with PBS-0.5% milk (blocking buffer) for 2 h at room temperature, mouse serum was serially diluted in blocking buffer and applied to wells for 2.5h at room temperature. Reactivity to the target antigen was determined by using

horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (Jackson ImmunoResearch) or HRP-labeled goat anti-mouse IgA (Open Biosystems) at a dilution of 1:4000 in blocking buffer and incubated for 1 h at room temperature. Upon development using ABTS, the optical density at 405nm (OD₄₀₅) was determined using a Thermo Max microplate reader (ThermoLab Systems). Values greater than twice the background were considered positive. ELISAs for antibody in vaginal lavage samples were conducted as above, incorporating the following changes: the blocking was performed with 0.5% BSA in PBS and the lavage samples were applied directly to the wells at a single, 1:1 dilution in PBS.

2.4 HPV pseudovirus challenge

HPV16 pseudovirions (PsV) encapsidating a luciferase reporter plasmid were generated as previously described [12, 13, 15]. Prior to inoculation, the vaginal epithelium was chemically disrupted as described above. Six hours later, 3×10^6 IU HPV16 PsV formulated in 3% CMC was applied to the genital tract of each animal using a positive displacement pipette.

Infection was detected as a bioluminescent signal using a live animal imager as follows. Forty-eight hours after pseudoviral challenge, anesthetized mice were intravaginally instilled with 20 μ L of the luciferase reporter substrate, XenoLight D-Luciferin Potassium Salt (Caliper Life Sciences) and then imaged using a IVIS Lumina II instrument (Caliper Life Sciences). Images were taken three minutes after installation of luciferin at medium binning with a 5-minute exposure. The average radiance per mouse was measured in photons/s/cm²/sr for a set region (of equal dimensions) between mice. Statistical significance was determined by comparing log-transformed radiance values by one-tailed T-test.

3. Results

3.1 Immunization of the genital tract with Q β induces specific IgG in the serum

Initially we asked whether direct immunization of the genital tract could elicit systemic and/or local antibody responses. To this end, we assessed the responses to genital vaccination with naked virus particles or VLPs using several different strategies. It had been shown previously that the ability of HPV pseudovirus (PsV) to infect the genital tract and HPV VLP vaccination of the genital tract to induce antibody responses were greatly enhanced by abrasion of the genital tract prior to infection [12, 16]. It is hypothesized that disruption of the vaginal epithelia prior to vaccination or infection allows for maximum uptake of the particles and exposure to resident antigen presenting cells and lymphocytes in the genital mucosa. Therefore, we investigated the efficacy of two separate disruption methods, chemical and mechanical disruption. Mechanical disruption was accomplished by scraping the genital tract with a cytobrush immediately preceding immunization. Chemical disruption was accomplished by applying a solution of 4% nonoxynol-9 in 3% CMC to the genital tract six hours prior to immunization. Treatment with nonoxynol-9 has been shown to cause abrasions in the genital tract; these abrasions ultimately create a microenvironment that is more permissive to infection than when left untreated [12, 16, 17]. In addition, we investigated two vaccine application techniques. In the first, the vaccine was delivered to mice in a gel format, with VLPs suspended in 3% CMC. In the second, VLPs were delivered directly to the genital tract as an aerosol using a high-pressure microsyringe. In each case, mice were immunized twice at a two-week interval with a dose of 25 μ g of Q β bacteriophage without adjuvant. For comparison, one group of mice received intramuscular immunizations, using 10 μ g of Q β plus incomplete Freund's adjuvant. Because we were somewhat concerned that CMC could have an inhibitory effect on the induction of antibody responses, we also intramuscularly immunized a group of mice with Q β suspended in 3% CMC.

As shown in Fig. 1, the intramuscular route induced the highest titer anti-Q β IgG antibodies in the sera (Fig. 1a). Mice intramuscularly immunized with Q β solubilized in 3% CMC elicited similar antibody titers as when we immunized with Q β without CMC, indicating that CMC has no inhibitory effect (data not shown). Vaginal immunization also resulted in systemic anti-Q β antibody responses (Fig. 1b–d). Based on a comparison of mice given vaccine after mechanical disruption of the genital tract, aerosol administration proved more effective than gel administration at inducing antibodies in the sera, although these differences were not statistically significant (by two-tailed T-test). The mechanical abrasion group immunized with a VLP-containing gel (Fig. 1b) had the lowest titers; one mouse in this group failed to induce an antibody response. Mechanical disruption followed by aerosol immunization induced antibody responses in all four mice (Fig. 1c), but only one mouse had especially high antibody titers. Chemical disruption of the vaginal epithelia followed by aerosol application produced the highest antibody responses, approximately 10- to 100-fold higher than the mice in the mechanical abrasion groups. Pairwise comparison at individual time points showed significant differences between the chemical/aerosol group and the mechanical/gel group ($p < 0.05$ at days 7 and 14, and $p < 0.01$ at days 21, 28, and 48, by two-tailed T-test of log-transformed titers). In the chemical/aerosol group (Fig. 1d), the peak geometric mean antibody titers were approximately 10-fold lower than in the animals given vaccine via the intramuscular route, although this difference was less at the final timepoint. Anti-Q β antibodies levels were maintained through the end of this study, five weeks following the second immunization.

3.2 Vaginal immunization induces local mucosal IgG and IgA responses

The ability of the genital vaccines to elicit a systemic response prompted us to determine whether they could also elicit a robust mucosal immune response, and ask how that response compared to the one elicited by the intramuscular vaccine. To assess the ability of intramuscular and vaginal (chemical/aerosol) immunization with Q β to elicit a genital mucosal antibody responses, we performed vaginal lavages at the time points indicated and measured Q β -specific IgG or IgA in these secretions. As shown in Fig. 2, intramuscular administration was able to elicit secreted IgG in the genital tract (panel A) but only low levels of IgA (panel B). Vaginal immunization (chemical disruption followed by aerosol administration) of Q β elicited similar IgG and higher IgA levels compared to intramuscular immunization. Secreted IgA antibody was detected at the final timepoint, five weeks after the booster vaccination. The other vaginal immunization protocols failed to consistently induce detectable IgG or IgA in vaginal lavage samples in the mice tested (data not shown), reflecting the overall weaker response observed using these protocols. Based on these results, we concluded that the optimal protocol for vaccine delivery to the genital tract was to chemically disrupt the vaginal epithelia prior to immunization via an aerosol spray.

3.3 Vaginal immunization with HPV16 VLPs

The next question we addressed was whether the type of VLP used in a genital vaccine influenced the magnitude of the immune response. We hypothesized that a VLP derived from a virus that ordinarily infects at the genital mucosa may be able to preferentially cross this epithelial barrier and be recognized by resident immune cells. Alternatively, it is possible that an STI pathogen may have evolved mechanisms for evading immune recognition. As our previous results showed that immunization with the Q β bacteriophage VLP, a bacteriophage that does not infect the mammalian genital tract, was successful at inducing both mucosal and systemic antibodies, we repeated these experiments (using only the aerosolized vaccine) with HPV16 VLPs. HPV16 infects at the genital mucosa, is carcinogenic, and HPV16 L1-VLPs are one of the components of the current HPV vaccines. At days 0 and 14 following chemical disruption of the vaginal epithelia, mice were immunized intravaginally via aerosol with 25 μ g of HPV VLPs without exogenous adjuvant.

Sera were collected at the time points indicated and analyzed by end-point dilution ELISA for specific IgG. As shown in Fig. 3a, HPV16 VLPs also elicited high-titer antibodies in the serum, with geometric mean end-point dilution titers peaking around 10^5 . We followed animals immunized with HPV16 VLPs for one month following the second vaccination. During this period geometric mean antibody titers dropped approximately 10-fold. These antibody titers were similar to IgG levels seen in mice immunized in a similar manner with Q β bacteriophage (Fig. 1d).

Vaginal lavages were collected and analyzed by ELISA for IgG and IgA specific antibodies against HPV16 VLPs. As shown in Fig. 3b & 3c we detected both isotypes in lavage samples from all five of the immunized mice. Similar to what we observed in the sera, mice continued to secrete IgG and IgA five weeks after the second immunization. In general, the levels of vaccine-specific secreted IgG and IgA were higher in animals immunized with HPV16 VLPs as compared to Q β bacteriophage (Fig. 2).

3.4 A single intravaginal dose of HPV16 VLPs confers protection from HPV pseudovirus infection

Next, we asked whether intravaginal immunization with HPV16 VLPs could protect against HPV16 pseudovirus infection of the genital tract. Because we detected both IgA and IgG responses after a single intravaginal vaccination, we asked whether a single dose was sufficient to provide protection from challenge. A group of five mice was given a single vaginal dose of HPV16 VLPs via the chemical abrasion/aerosol immunization protocol. Vaginal lavages and sera were collected prior to application and 20 days following immunization. As shown in Fig. 4, a single immunization was sufficient for the induction of low-titer serum IgG in all 5 mice, and mucosal IgG and IgA in 4 out of 5 animals. Serum titers were several logs lower than the peak antibody level observed in the group that received two immunizations (Fig. 3).

Vaccinated mice (and a group of unimmunized mice) were genitally challenged with HPV16 pseudovirus on day 24. Consistent with the immune responses observed prior to challenge, 4 out of 5 animals were completely protected against infection (Fig. 5). The mouse that was partially susceptible to infection was the same animal that failed to elicit mucosal HPV-specific IgA and IgG antibodies in the genital tract. While this mouse also had low antibody titers in the sera (titer = 40), a second animal had equally low titers in the sera, yet displayed moderate levels of specific antibody in its vaginal washes (IgG, OD₄₀₅=1.131; IgA, OD₄₀₅=0.701), and was protected against infection, suggesting that in this animal mucosal antibody responses against HPV mediated protection. Thus, these data suggest that specific antibodies at the site of infection could alone be sufficient for protection from HPV pseudovirus challenge. In addition, these data indicate that a single immunization with HPV VLPs can induce antibody levels in the sera and mucosa that are sufficient to confer protection against challenge.

3.5 Vaginal immunization with a recombinant VLP elicits immune responses against both the platform and a displayed target peptide

As described in the introduction, VLPs can be used as stand-alone vaccines, but they can also be used as platforms for displaying heterologous peptides in a highly immunogenic multivalent format. To this end, we asked whether a recombinant VLP displaying HPV-derived neutralizing epitope could also elicit antibody responses upon intravaginal immunization. We used a recombinant VLP in which a peptide (amino acids 17–31) representing a broadly cross-neutralizing epitope derived from the HPV16 minor capsid protein, L2, was displayed on the surface of VLPs from the RNA bacteriophage, PP7 [11].

We characterized the magnitude of the mucosal and systemic immune response following aerosol immunization of the genital tract with 16L2-PP7 VLPs.

At days 0, 14, 56, and 195 mice were either immunized intramuscularly (10 µg) or intravaginally via aerosol (25 µg without adjuvant) with 16L2-PP7 VLPs. Unlike the other intramuscular immunizations described in this manuscript, in this experiment intramuscular immunizations were also administered without using adjuvant, in order to better compare the two vaccination strategies (as no adjuvant was present in the vaginal aerosol preparation). Sera were collected prior to immunization and at the timepoints indicated, and analyzed for IgG antibodies against both VLP platform (PP7) and a synthetic peptide representing the HPV16 L2 sequence. Both the intramuscular and vaginal vaccination approaches elicited high-titer IgG antibodies against both the PP7 platform and the L2 peptide in the sera (Fig. 6), though titers were approximately 10-fold higher in the IM immunized group. In the intramuscular immunization group, high-titer IgG against the displayed L2 peptide was achieved after a single immunization, whereas animals immunized via the vaginal route did not have significant anti-L2 IgG until at least 2 weeks after their second immunization (Fig. 6b & d). As we showed previously [11], control mice immunized with non-recombinant PP7 VLPs did not induce antibodies that cross-reacted with the L2 peptide (data not shown). We also measured IgG and IgA against both the VLP platform and the L2 peptide in vaginal lavage samples. Anti-VLP antibody levels were similar to the data reported elsewhere in this manuscript (anti-PP7 antibody titers were similar to the anti-Qβ antibody levels shown in Fig. 1; data not shown). However, we were unable to detect anti-L2 IgG or IgA above background levels (data not shown), perhaps reflecting the weaker responses to this single epitope in the context of a polyclonal antibody response.

3.6 Vaginal immunization of the mouse genital tract with recombinant L2-VLPs confers protection from pseudovirus infection

Eight days after the final boost, vaccinated mice and three unimmunized controls were challenged with HPV16 pseudovirus. As we previously reported [11], intramuscular immunization with 16L2-VLPs protected mice from pseudovirus infection (Fig. 7). Aerosol immunization of the genital tract conferred only partial, but statistically significant, protection to the group, with two of the mice showing complete protection (no signal), two showing no protection, and one mouse showing an intermediate degree of protection. This was not surprising based on the pre-challenge antibody titers that we measured; the two mice in the vaginal immunization group with the highest luciferase signal had the lowest anti-L2 antibody titers. Nevertheless, these data indicate that vaginal immunization can also be effective at inducing antibody responses against heterologous antigens displayed on the surface of VLPs.

4. Discussion

Many microbial pathogens use mucosal surfaces as sites of entry into the host. Despite this fact, most current vaccines are administered parenterally and do not elicit mucosal immune responses. In part, this reflects the difficulties in inducing consistent immune responses by vaccination of mucosal surfaces using vaccines other than live attenuated strains. Moreover, the mucosal immune system is largely compartmentalized, making it difficult to develop mucosal vaccination strategies that elicit immune responses at locations other than the site of immunization.

It has been particularly difficult to induce immune responses in the genital tract. A number of studies, including our own, have reported that immunization of the respiratory tract can also induce a weaker secondary response in the genital mucosa [6, 18–22]. Sublingual immunization can also induce immune responses in the female genital tract [15, 23]. There

have only been a few other recent studies that have shown that direct immunization of the genital tract can induce local and systemic immune responses. Other studies have used live, attenuated recombinant *Salmonella* strain expressing HPV16 L1 [24], the anionic polymer PRO 2000 to potentiate genital responses to an HIV envelope-based antigen [25], and HPV pseudovirions for gene delivery of expression plasmids encoding respiratory syncytial virus M-M2 genes [26]. In the latter study it was found that intravaginal immunization also induced IgA in nasal secretions, suggesting that lymphocytes or APCs are able to migrate between these two compartments. Interestingly, both nasal and vaginal vaccination of human volunteers with a cholera vaccine were successful at inducing anti-cholera IgG and IgA responses in the genital tract, but nasal administration required substantially higher doses [27].

In what is probably the most complete study of genital vaccination, Fraillery and colleagues showed that three weekly intravaginal instillations of the HPV16 VLPs plus nonoxydyl-9 can induce high titer genital and systemic neutralizing antibody responses against HPV [16]. Vaginal application of VLPs in the absence of nonoxydyl-9, but with other adjuvants (such as imiquimod, Resiquimod, and MPL), failed to induce antibody responses. Here, we show that this strategy can be generalized and be used with other VLPs and VLP-platform-based immunogens. Q β bacteriophage and VLPs derived from HPV and PP7 bacteriophage are suitable for vaccination of the genital tract and the induction of local and systemic antibody responses. Unlike HPV, Q β and PP7 are not human pathogens and have not evolved to infect the mammalian genital tract. Nevertheless, intravaginal immunization with these VLPs elicited similar serum antibody responses as HPV VLPs. HPV VLPs did induce higher local IgA responses in the genital tract than the phage platforms. Notably, both HPV and Q β VLPs have been shown to induce activation of dendritic cells (DCs) [28–30], suggesting that differential DC activation is unlikely to account for our observations.

As others have shown using HPV pseudovirus vectors [26] and HPV VLPs [16], the ability to induce antibody responses was dependent on physical disruption of the vaginal epithelium. In our study, we found that chemical disruption using nonoxynol-9 was more effective than mechanical abrasion. In addition, we found that aerosol application of our vaccine was more effective than gel-based application. In particular, aerosolization of particles resulted in higher titer antibody responses in the serum and in vaginal secretions. A possible explanation for this observation is that the gel application was unable to consistently access the upper genital tract (the endocervix and uterus). This region is characterized by a simple columnar epithelial layer that is distinguished by infiltrating immune cells and the presence of IgA transport mechanisms [31]. In contrast, the vagina and ectocervix are covered by a thicker stratified epithelial layer, are sparsely populated with APCs and lymphocytes, and lack the ability to secrete IgA [32]. Presumably, the smaller droplet size and distributive force posited by the aerosolized vaccine would encourage distribution into the upper genital tract.

HPVs are the most prevalent STIs world-wide, and cause significant morbidity and mortality. The existing parenterally administered HPV vaccines are effective at preventing genital HPV infection. This protection is likely mediated by serum IgG that can transudate or exudate into the genital tract. In our studies, intramuscular immunization with HPV VLPs or VLPs displaying an epitope from the minor capsid protein L2 induced IgG in the serum and in vaginal secretions that were protective upon HPV pseudovirus challenge. We also showed that intravaginal vaccination with VLPs displaying L1 and, to a lesser extent, L2, also protected mice from pseudovirus challenge. Notably, a single mucosal administration of HPV16 VLPs was sufficient to confer protection from genital challenge with HPV16 pseudovirions. The success of the single immunization may in part be due to the VLP platform used, as our studies suggest that HPV VLPs may be somewhat more potent at

generating high titer antibody in the genital mucosa than the bacteriophage platforms that we used.

Clearly, the vaginal immunization methods described in the paper would face significant clinical hurdles. These include the use of Depo-Provera and nonoxynol-9 to thin and abrade the vaginal epithelia, and the invasive use of a microspray device. Thus, alternative approaches for delivery will need to be developed. Notably, we showed that VLPs could induce antibody responses at relatively low doses and without using exogenous adjuvant. Taken together, these studies provide proof-of-principle data supporting the use of VLPs or VLP-based immunogens as genital vaccines against STIs.

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Fig. 1.

Antibody responses in the serum of mice immunized with Q β bacteriophage via the intramuscular and intravaginal routes. Mice were immunized on days 0 and 14 (shown by the arrows) with Q β bacteriophage. Sera were collected at the time points indicated and IgG antibody titers determined by end-point dilution ELISA. Each data point indicates the end-point dilution IgG titer in an individual mouse. Bars reflect the geometric mean titer of the group at each timepoint. Mice were immunized by (A) intramuscular injection of 10 μ g of Qs plus incomplete Freund's adjuvant, or (B–D) intravaginal administration of 25 μ g of Q β without adjuvant using three different protocols. These include: (B) mechanical disruption of the vaginal epithelium followed by gel administration, (C) mechanical disruption followed by aerosol administration, and (D) chemical disruption followed by aerosol administration.

Fig. 2.

Anti-Q β IgG and IgA levels in vaginal washes of immunized mice. Mice were vaccinated via the intramuscular (open circles) or vaginal (chemical disruption/aerosol administration; closed circles) route as described in Fig. 1. Arrows denote the dates that the mice were immunized. Vaginal lavages were obtained, diluted with an equal volume of PBS, and then IgG (panel A) and IgA (panel B) antibody levels determined by ELISA. The data shows the mean ELISA values for each group of mice; error bars represent SEM.

Fig. 3.

Antibody responses in mice intravaginally immunized with HPV16 VLPs. Mice were immunized on days 0 and 14 (shown with arrows) with 25 μ g VLPs. Shown are: (A) End-point dilution titers of IgG in the serum, (B) IgG levels in vaginal lavage, and (C) IgA levels in vaginal lavage.

Fig. 4.

Antibody responses in mice given a single intravaginal aerosol immunization of 25 μ g of HPV16 VLPs via the genital tract is sufficient for inducing systemic and mucosal antibodies. Mice were immunized at day 0, sera and vaginal lavages were collected on days 0 and 20, and samples were analyzed by ELISA for HPV16 specific antibodies. Shown are: (A) End-point dilution titers of IgG in the serum, (B) IgG levels in vaginal lavage, and (C) IgA levels in vaginal lavage. The data point denoted with a grey circle indicates the single mouse in the vaccinated group that was poorly protected from HPV16 pseudovirus challenge (see Fig. 5).

Fig. 5.

Mice immunized with a single intravaginal dose of HPV16 VLPs are protected from vaginal challenge with HPV16 pseudovirions. Three and a half weeks after being vaccinated, five mice were intravaginally challenged with 3×10^6 IU of HPV16 pseudovirus containing a luciferase reporter. As a control, a group of four unimmunized mice were also infected. Luciferase activity was quantitated 48 h after infection by taking images 3 minutes after post-installation of luciferin. Images were then analyzed by drawing an equally sized region of interest for each mouse (shown in a single mouse in panel B) and measuring average radiance (photons/s/cm²/sr) within this region. Shown are (A) mice intravaginally immunized with HPV16 VLPs and (B) control mice. Note that the luminescence scales are shown to the right of each set of images, and these scales vary. (C) A graph showing the radiance for each individual mouse in each group. Bars reflect the geometric mean radiance for each group. A comparison of the log-transformed radiance values was statistically significant (*, $p < 0.05$) by one-tailed T-test.

Fig. 6. Serum IgG antibody responses in mice immunized intramuscularly (A, B) or intravaginally (C, D) with PP7 VLPs displaying a peptide derived from HPV16 L2. Mice were immunized on days 0, 14, 56, and 195 (shown with arrows) with 10 μ g (intramuscular) or 25 μ g (intravaginal) of VLPs without adjuvant. Sera were collected and analyzed via end-point dilution ELISA for (A, C) PP7- and (B, D) L2 peptide specific IgG.

Fig. 7.

Aerosol immunization of the genital tract with 16L2-PP7 VLPs vaccine confers only partial protection from infection. The mice described in Fig. 6 and five controls were infected with HPV16 pseudovirions encapsidating a luciferase reporter plasmid. Average radiance values were measured 48 h after infection. A comparison of the log-transformed radiance values was statistically significant (*, $p < 0.05$; **, $p < 0.005$) by one-tailed T-test.