Nasal Immunization of Mice with Human Papillomavirus Type 16 (HPV-16) Virus-Like Particles or with the HPV-16 L1 Gene Elicits Specific Cytotoxic T Lymphocytes in Vaginal Draining Lymph Nodes

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Human papillomavirus type 16 (HPV-16) infects the genital tract and is closely associated with the development of cervical cancer. HPV-16 initiates infection at the genital mucosal surface; thus, mucosal immune responses are likely to contribute to defense against HPV-16 infection. However, little information is available regarding the induction of immune responses in the genital tract mucosa. In this study, we evaluated the potential of intranasally administered papillomavirus vaccines to elicit both systemic and vaginal immune responses. HPV-16 virus-like particles (VLPs) produced by self-assembly of L1 protein and the HPV-16 L1 gene cloned into a mammalian expression vector were used as vaccines. Intranasally administered VLPs induced serum immunoglobulin G (IgG) and vaginal IgA secretory antibodies. Very weak serum IgG and vaginal IgA responses were found after DNA immunization. Both splenic and vaginal lymphocytes could be activated by intranasal immunization with VLPs and the HPV-16 L1 gene. Activated CD4+ Th1-like T cells were shown to synthesize gamma interferon, and activated CD8+ T cells were demonstrated to be cytotoxic.

The principal aim of prophylactic vaccines is to prevent genital HPV infections and HPV-associated genital tumors; thus, an effective vaccine ideally should stimulate immune responses in mucosal tissues and associated lymph nodes (LN). Such responses include the secretion of immunoglobulin A (IgA), which mediates virus neutralization to induce complete inactivation of the virus before any cells become infected. The induction of mucosal IgA responses strongly depends on help provided by CD4+ T cells, and the costimulatory molecules and cytokines that they express may play a major role in mucosal immune responses (20, 49). These T cells residing in mucosal response-inducing sites may provide a mechanism to eliminate cells undergoing productive viral infection and to prevent virus dissemination if the mucosal barrier is breached. Recent studies have provided evidence that the female reproductive tract has the characteristics of mucosal effector tissues, with IgA-producing cells, T-lymphocyte subpopulations, and secretory components (15, 27, 38, 44), indicating that a mucosal immunization strategy should elicit the secretion of both antibodies and cytotoxic T lymphocytes (CTL) in the genital tract.

Based on the concept of a common mucosal immune system (37), intranasal immunization with particular antigens has already been proven to generate specific cellular and humoral responses to numerous immunogens and to confer protection (10, 18, 55, 62). The mucosal response was shown to be enhanced by cholera toxin (CT), which has great potential as an adjuvant in intranasal vaccinations (5, 10, 55). Another candidate approach to induce mucosal immunity consists of the administration of plasmid DNA containing a viral gene (43). DNA administered intranasally is effective at inducing mucosal antibody responses (25, 61) and at conferring partial protection against genital tract pathogens (61).

Only limited information is available concerning HPV-specific mucosal immunity in the female reproductive tract. Mu-
cosal immunity is considered essential for protection against invading pathogens, and it is therefore important to find optimal administration routes that elicit protection in the genital mucosa. Experiments with mice have shown that systemic immunization with HPV type 1 (HPV-1) VLPs does not induce cervical IgA (17), whereas i.n. administration of HPV-6b and bovine papillomavirus type 1 VLPs induces both IgG and IgA in vaginal secretions (30). In addition, anti-HPV-11 VLP cervicovaginal IgG elicited after intramuscular immunization of monkeys is sufficient to neutralize HPV-11 in the athymic nude mouse xenograft model (41, 53). These results illustrate that immunization with papillomavirus VLPs via mucosal and systemic routes triggers both a systemic T-cell immune response and neutralizing antibodies at mucosal surfaces. However, none of these models is adequate to address the question of whether vaginal immunoglobulins are sufficient to protect the genital mucosa and whether VLPs elicit T-cell responses at the mucosal level.

The abilities of HPV-16 VLPs to activate systemic humoral and cellular immunity in mice when injected subcutaneously and to induce a vaginal IgA response when administered intranasally have already been described (1, 14, 41). Neutralizing antibodies have also been obtained after intramuscular immunization of rabbits with DNA coding for the L1 protein of cottontail rabbit papillomavirus (13).

We report a study to establish whether the intranasal administration of either HPV-16 L1 protein VLPs or the HPV-16 L1 gene in combination with CT elicits both systemic and mucosal humoral and cellular immune responses.

MATERIALS AND METHODS

HPV VLP vaccine preparation. Recombinant HPV-16 L1 VLPs were prepared as previously described (28, 56). Briefly, the HPV-16 L1 coding sequence from HPV strain Sen32 (57) was cloned into the pBluescript vector (Invitrogen, San Diego, Calif.). The expression vector obtained was cotransfected into SF-21 cells together with Autographa californica multiple nuclear polyhedrosis virus genomic DNA. SF-21 cells were infected with a recombinant baculovirus selected by 2.5 mg of CT at 15-day intervals.

Immunization. Mice received three doses of 10 μg of HPV-16 L1 VLPs combined with 2.5 μg of CT (Sigma, Saint-Quentin Fallavier, France) or 100 μg of HPV-16 L1 DNA alone or combined with 2.5 μg of CT at 15-day intervals. Each vaccine dose was diluted to 20 μl in 0.15 M NaCl and was instilled into the nostrils with a micropipette. Mice were either anesthetized with diethyl ether (Prolabo, Fontenay sous Bois, France) or conscious. Control mice received 10 μg of the last five VLPs or HBE buffer combined with 2.5 μg of CT. Splenocytes and vaginal lymphocytes were harvested 15 days after the last vaccine dose. Each experiment was repeated twice.

Biological fluids. Serum blood samples were obtained by retroorbital puncture just before vaccination and 1 week after each injection. Vaginal secretions were obtained by washing the vaginal lumen with 50 μl of PBS containing 0.1% phenylmercuric alcohol (Sigma).

Purification of splenocytes and iliac LN cells. Mice were sacrificed 8 days after the last immunization. Splenocytes and vaginal lymphocytes were purified 1 day after RT-qPCR assay, 2 × 10⁵ cells were seeded in triplicate in wells of flat-bottom microplates (Nunc, Cergy Pontoise, France), 5% fetal calf serum (FCS) (Life Technologies), 2 mM l-glutamine ( Gibco), 100 IU of penicillin per ml, and 100 μg of streptomycin (Gibco). For the lymphoproliferation assay, 2 × 10⁵ g of HPV-16 VLPs per ml. After 4 days, cells were resuspended in HBSS-10% FCS and washed twice before separation. Thirty million cells were incubated with a rat anti-mouse Thy-1.2 monoclonal antibody conjugated with fluorescein isothiocyanate (Pharmingen). Lymphocytes were incubated 30 min at room temperature with sheep sera to eliminate nonspecific reactions. Cells were stained with 0.15 M NaCl and was instilled into the nostrils with a micropipette. Mice were either anesthetized with diethyl ether (Prolabo, Fontenay sous Bois, France) or conscious.

Mice. Seven groups of 6- to 10-week-old female BALB/c mice (HFA Credo, St. Germain l’Arbresle, France) were used in the immunization studies. Each experimental group was composed of five to seven mice. Experimental groups for HPV DNA vaccine preparation.

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Cytotoxicity assay. Thiglycolate-elicted macrophages were used as target cells. Peritoneal macrophages from three mice were collected 4 to 7 days after thiglycolate peritoneal endotoxemia. Macrophages were washed three times in HBSS and dispensed in culture medium at a concentration of 3 × 10⁵ cells/ml well into round-bottom tissue culture plates (Falcon, Lincoln Park, N.J.). They were incubated for at least 4 h at 37°C in 5% CO₂ and then radiolabeled with ³¹Cr (1 μCi/well; specific activity, 469 μCi/mg; NECN) for 3 h. After washing, radiolabeled target cells were sensitized with 15 μg of HPV-16 VLPs per ml and incubated overnight. Macrophages were washed just before contact with effector cells, which were purified iliac LN or vaginal cells. After purification, the effector cells (10⁵/ml) were activated over 4 days with 15 μg of HPV-16 VLPs per ml in plates containing nonradiolabeled macrophages (10⁷/ml). The activated cells were then removed by repeating just before the cytotoxicity assay, and Thy-1.2- or CD8⁻ cells were eluted by washing with PBS-FCS. Both positive and negative cells were analyzed by flow cytometry with a FACS sorter (Becton Dickinson, Le Pont de Claix, France). The mini-MACS separation yielded a pure population of Thy-1.2⁺ and CD8⁻ cells.

Immunofluorescence labeling and flow cytometry. Splenic and vaginal lymphocytes were phenotyped with anti-CD22 (B lymphocytes), anti-CD4⁺, anti-CD8⁻, and anti-Thy-1.2 (predominantly T lymphocytes) antibodies conjugated to fluorescein (Pharmingen). Lymphocytes were incubated 30 min at room temperature with sheep sera to eliminate nonspecific reactions. Cells were stained with the appropriate antibodies (diluted 1:1,000) for 1 h at 4°C. After washing, phenotype analysis was performed by flow cytometry.
**RESULTS**

Intranasal instillation of HPV-16 L1 VLPs without anesthesia induced serological antibody responses when associated with CT. A dose-effect experiment was carried out with HPV-16 L1 VLPs and CT (Fig. 1). HPV-16 L1 VLPs alone failed to induce detectable antibodies against VLPs, but a serum IgG response to HPV-16 L1 VLPs was found in all groups of mice intranasally instilled with HPV-16 L1 VLPs combined with CT. Antibody titers increased with VLP doses and CT concentrations. The highest titer (1,980) was observed when mice received 10 μg of VLPs combined with 2.5 μg of CT. Antibody responses after intranasal instillation remained significantly lower than those obtained after subcutaneous immunization, whatever the CT or HPV-16 L1 VLP concentration (data not shown). The dose chosen for all subsequent intranasal immunizations was 10 μg of HPV-16 L1 VLPs combined with 2.5 μg of CT. As anesthetization was shown (1, 41) to increase the immune response after intranasal immunization, both anesthetized and conscious groups were included in the experimental schedule.

VLPs administered intranasally with CT triggered serum IgG and vaginal IgA antibody responses. Serum anti-HPV-16 L1 VLP IgG antibodies were detected in anesthetized mice 2 weeks after the first immunization. No anti-HPV-16 L1 VLP antibodies were detected in the sera of conscious mice at that time. Serum IgG antibodies were first detected in conscious mice 15 days after the second immunization at a titer of 500; the value in anesthetized mice was 5,000 (data not shown). After the third immunization, the titer of anti-VLP antibodies remained lower in the conscious group (4,000) than in the anesthetized group (16,000) (Fig. 2A). Anti-HPV antibodies were not detected in the sera of mice immunized with HBC VLPs (control group).

Among mice receiving HPV-16 L1 DNA, low levels of anti-HPV-16 IgG antibodies (titer, 116) were detected in the sera of anesthetized mice immunized with HPV-16 L1 DNA together with CT. Anti-HPV antibodies were not detected in the sera of mice receiving HBC DNA (control group).

IgA antibody responses were induced in the vaginal secretions of mice immunized with VLPs under anesthesia soon after the second immunization (data not shown). No anti-HPV-16 antibodies were detected in conscious mice at that time. The highest titer of IgA antibodies (200) was observed after the third immunization in the group of mice receiving HPV-16 L1 VLPs under anesthesia (Fig. 2B). Three immunizations were necessary to induce vaginal IgA antibody responses in the conscious group; nevertheless, titers remained lower than those in the anesthetized group. Anti-HPV-16 IgA antibodies were not detected in the HBC VLP control group. A very low IgA antibody response (titer, 16) was observed in vaginal secretions from the anesthetized mice immunized with HPV-16 L1 DNA combined with CT, but the titer obtained was higher than those obtained in the conscious mice and the mice receiving HBC DNA. Vaginal anti-HPV-16 IgA antibodies could not be detected in any of the immunization groups.

L1 VLPs and L1 DNA induced systemic cellular immune responses. The development of cellular immune responses after immunization was monitored by enumeration of splenocytes and by phenotype characterization (Table 1). The number of splenocytes in immunized mice was higher than the number in nonimmunized mice (7 × 10^9 versus 1 × 10^8 cells/ml). The variations in splenocyte phenotypes were similar in the anesthetized and conscious HPV-16 L1 VLP-treated groups. Compared to that in nonimmunized mice, the number of B cells in mice immunized with VLPs was greatly increased (6.7 × 10^7 versus 28 × 10^6). The number of T cells was higher in mice immunized with VLPs, but to a lesser extent (7 × 10^7 in the nonimmunized group versus 22 × 10^6 in the immunized group). The increase in the number of T cells was mainly due to an increase in the number of CD4^+ T cells.

Anesthesia and CT were essential for lymphocyte stimulation in the group receiving HPV-16 L1 DNA. The number of lymphocytes was higher in mice receiving L1 DNA combined with CT under anesthesia, whatever the lymphocyte population considered. The greatest increase was in T cells (7 × 10^7 in the nonimmunized group versus 2 × 10^8 in the anesthetized immunized group) due to increases in CD4^+ and CD8^+ T cells.

Splenocytes from mice immunized with VLPs under anesthesia displayed a specific proliferative response (65,000 cpm) following in vitro incubation with 25 μg of HPV-16 L1 VLPs per ml (Fig. 3). Proliferative responses occurred in both groups of mice immunized with HPV-16 L1 VLPs and were higher than those in the HBC VLP control group (17,000 cpm).
Splenocytes from mice immunized with HPV-16 L1 DNA displayed a specific proliferative response. The proliferative response was enhanced by anesthesia, with 46,000 cpm observed in the anesthetized group and 28,000 cpm observed in the conscious group. Splenocytes from HBc DNA-immunized mice did not proliferate.

IFN-γ production by activated splenocytes was monitored after 72 h of in vitro stimulation of 2 × 10⁶ splenocytes with 15 µg of HPV-16 L1 VLPs per ml. IFN-γ was released into culture supernatants by splenocytes purified from mice immunized with HPV-16 L1 VLPs under anesthesia (418 pg/ml) (Table 2) but was not detected in the HBc VLP control group. Splenic lymphocytes from mice immunized intranasally with HPV-16 L1 DNA released IFN-γ whether the DNA was given with anesthesia (655 pg/ml) or without anesthesia (506 pg/ml).

L1 VLPs and L1 DNA induced a vaginal cellular immune response. The number of vaginal lymphocytes was increased (7 × 10⁵ cells/ml) in all of the immunized groups compared to the nonimmunized group (2 × 10⁵ to 7 × 10⁵ cells/ml), with an increase in the numbers of B and T cells in the group receiving HPV-16 L1 VLPs under anesthesia and in the group immunized with HPV-16 L1 DNA, whatever the immunization protocol. The difference between the L1 VLP-immunized group and the L1 DNA-immunized group depended on the cell subset stimulated. After L1 VLP immunization, the increase in the number of CD4⁺ T cells might have explained the change in the number of T cells, whereas after L1 DNA immunization, the change in the number of T cells was explained by the increase in the number of CD8⁺ T cells. Vaginal lymphocytes stimulated in vivo in the VLP-immunized group displayed a

FIG. 2. IgG and IgA antibody responses in BALB/c mice immunized intranasally with HPV-16 L1 VLPs, HBc VLPs, HPV-16 L1 DNA, or HBc DNA. (A) The IgG antibody response in serum samples was assayed 7 days after the third immunization by an ELISA with HPV-16 L1 VLP-coated plates. (B) The IgA antibody response in vaginal secretions of mice was tested 7 days after the third immunization.
specific proliferative response (12,000 cpm) about two times higher than that of vaginal lymphocytes from the nonimmunized and HBc VLP control groups (7,000 cpm) (Fig. 4). Anesthesia enhanced vaginal lymphocyte stimulation, since a two-fold increase in proliferation was obtained in anesthetized mice (6,000 and 12,000 cpm, respectively). The proliferative response was greatly increased in vaginal lymphocytes from mice immunized with HPV-16 L1 DNA and CT under anesthesia (33,000 cpm). This response was greater than that obtained in conscious mice (18,000 cpm). Proliferation was antigen specific, since lymphocytes from mice immunized with HBc DNA did not proliferate.

IFN-γ production (Table 2) was monitored with $2 \times 10^4$ vaginal cells. IFN-γ production was detected in mice immunized with HPV-16 L1 VLPs combined with CT under anesthesia (60 pg/ml). No IFN-γ was detected in stimulated vaginal lymphocytes from the other mice.

**HPV-16 L1 VLPs and HPV-16 L1 DNA induced local cytotoxicity.** Iliac LN, vaginal, and splenic lymphocytes obtained from mice immunized intranasally under anesthesia with HPV-16 L1 VLPs or with HPV-16 L1 DNA were restimulated for 4 days in vitro with HPV-16 L1 VLPs and then assayed for cytotoxicity. The effector/target ratio was 10:1 due to the low number of cells obtained from mouse vaginas. Lymphocytes purified from iliac LNs exhibited specific cytotoxicity when mice were immunized with HPV-16 L1 VLPs (14%) or with HPV-16 L1 DNA (17%) (Table 3). No cytotoxic activity was detected with vaginal and splenic lymphocytes. CD8$^+$ T cells from iliac LNs were cytotoxic (Table 4) in mice immunized with L1 VLPs (9%) or with L1 DNA (19%). CD4$^+$ T cells did not show cytotoxic activity.

**DISCUSSION**

Genital papillomavirus infections occur when basal cells of the genital squamous epithelium are exposed to viruses. A prophylactic vaccine against HPV infections and associated diseases should therefore provide protective immunity at the site of pathogen entry. One important component in the efforts to develop a vaccine against genital papillomaviruses is the

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**TABLE 1.** Splenic and vaginal lymphocyte subsets in mice immunized intranasally with HPV-16 L1 VLPs or HPV-16 L1 DNA

<table>
<thead>
<tr>
<th>Immunization group</th>
<th>Anesthesia</th>
<th>Splenic</th>
<th>Vaginal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B (10^5)</td>
<td>T (10^5)</td>
</tr>
<tr>
<td>Nonimmunized</td>
<td>No</td>
<td>700 ± 141</td>
<td>700 ± 99</td>
</tr>
<tr>
<td>VLPs</td>
<td>Yes</td>
<td>3,000 ± 75</td>
<td>2,000 ± 128</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>4,000 ± 158</td>
<td>2,000 ± 112</td>
</tr>
<tr>
<td>DNA</td>
<td>Yes</td>
<td>900 ± 51</td>
<td>1,500 ± 42</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>900 ± 78</td>
<td>200 ± 22</td>
</tr>
</tbody>
</table>

CT was included in all immunized groups.
definition of routes and conditions of immunization that stimulate mucosal immune responses as well as systemic immune responses. Detection of cell-mediated immunity and antibody-secreting cells in genital tissues following virus infections in humans and monkeys (32) has provided evidence that an appropriate vaccine strategy might be able to elicit both antibodies and CTL in the genital tract (38) and could provide protection. Such lymphocytes have already been detected in the vaginal mucosa and draining LNs after mucosal immunization (16, 23, 35).

The concept of the common mucosal immune system (37) implies that an immune response may occur in mucosal sites other than the site of vaccine administration. Indeed, some intranasally administered antigens have already been shown to induce a specific IgA antibody response in the genital tract (12, 16, 31, 36, 60). Some reports have also demonstrated that intranasal administration of a DNA vaccine leads to vaginal secretion of IgA antibodies and to major histocompatibility complex (MHC)-restricted CTL in genital LNs (6, 24, 25, 31, 50). Virus-neutralizing IgA antibodies are important in preventing local infection and disease, since binding of IgA to the virus in the mucosa can prevent attachment to epithelial cells. Systemic immunization of African green monkeys with HPV-11 VLPs resulted in transudated cervicovaginal IgG antibodies that only partially neutralized HPV-11 infection in vitro (33), suggesting that IgG alone may be insufficient for long-lasting protection.

In the present study, we observed that intranasally administered HPV-16 L1 VLPs combined with CT induced anti-VLP IgG antibodies, in agreement with the results obtained for other viral antigens (19, 29, 34, 40, 42, 52). The IgG response observed was CT dose dependent, with a 10-fold increase in IgG immune response corresponding to a 5-fold increase in the CT concentration in the vaccine. In contrast, an increase in the dose of HPV-16 L1 VLPs did not enhance the immune response proportionally. In contrast to the results of Liu et al. (30), we failed to detect any intestinal or vaginal IgA after parenteral vaccination (data not shown) as well as after intranasal immunization, regardless of the antigen and CT doses used. In our study, the highest serum reactivity was obtained

### Table 2. Quantification of IFN-γ in culture supernatants of splenic and vaginal lymphocytes from mice immunized with HPV-16 L1 VLPs, HBc VLPs, HPV-16 L1 DNA, or HBc DNAa

<table>
<thead>
<tr>
<th>Immunization group</th>
<th>Anesthesia</th>
<th>IFN-γ release (pg/ml) from the following lymphocytes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Splenic (2 × 10⁶ cells)</td>
</tr>
<tr>
<td>Nonimmunized</td>
<td>No</td>
<td>&lt;20</td>
</tr>
<tr>
<td>VLPs</td>
<td>Yes</td>
<td>418</td>
</tr>
<tr>
<td>Control (HBc)</td>
<td>Yes</td>
<td>&lt;20</td>
</tr>
<tr>
<td>DNA</td>
<td>Yes</td>
<td>655</td>
</tr>
<tr>
<td>Control (HBc)</td>
<td>Yes</td>
<td>506</td>
</tr>
</tbody>
</table>

a The limit of detection of the assay was 20 pg/ml.

b CT was included in all immunized groups.

### Table 3. Cytotoxic activities of splenic, iliac LN, and vaginal lymphocytes purified from 20 mice immunized intranasally with HPV-16 L1 VLPs or HPV-16 L1 DNA

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>% of the following lymphocytes with cytotoxic activity:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Splenic</td>
</tr>
<tr>
<td>Nonimmunized</td>
<td>5</td>
</tr>
<tr>
<td>HPV-16 L1 VLPs</td>
<td>4</td>
</tr>
<tr>
<td>HPV-16 L1 DNA</td>
<td>3</td>
</tr>
</tbody>
</table>

![Diagram](http://jvi.asm.org/)

**FIG. 4.** In vitro proliferation of vaginal lymphocytes from mice immunized intranasally with HPV-16 L1 VLPs, HBc VLPs, HPV-16 L1 DNA, or HBc DNA and restimulated with 15 μg of HPV-16 L1 VLPs per ml. Error bars show standard deviations.
TABLE 4. Cytotoxic activities of iliac LN CD8⁺ and CD8⁻ cells from mice immunized with HPV-16 L1 VLPs or HPV-16 L1 DNA

<table>
<thead>
<tr>
<th>Iliac LN lymphocytes</th>
<th>% of cytotoxic iliac LN cells from mice immunized with HPV-16 L1:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VLPs</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>9</td>
</tr>
<tr>
<td>CD8⁻</td>
<td>0</td>
</tr>
</tbody>
</table>

In accordance with these findings, we showed that intranasally administered HPV-16 L1 VLPs enhanced specific CD4⁺ T-cell populations in both the spleen and the vagina. These CD4⁺ T cells appeared to produce IFN-γ, known to inhibit viral infection. The CD8⁺ T-cell population remained stable. In vitro proliferation and IFN-γ synthesis by mucosal cells in response to antigenic stimulation provide strong evidence for putative local immune reactivity. In the HSV type 2 model, MHC class II expression was up-regulated more rapidly when antigenic stimulation, thus explaining why virus-specific CTL and localized in genital LNs are effective in the clearance of virus from the vagina (21, 35, 46).

In conclusion, the HPV-16 L1 DNA vaccine strongly promotes the stimulation of vaginal CD8⁺ T cells, which are essential for the elimination of virus-infected cells. However, the HPV-16 L1 DNA vaccine does not seem to be a good candidate for a prophylactic vaccine when given intranasally, since it induces weak humoral immunity. However, such a vaccine could be given in addition to an HPV-16 L1 VLP vaccine to increase its ability to eliminate infected cells. On the other hand, an intranasally administered E6 or E7 DNA vaccine should be considered for therapeutic use, since the L1 DNA vaccine induces a very strong T-cell immune response in the vagina. Our results also provide evidence that an intranasal HPV-16 L1 VLP vaccine induces a vaginal IgA response and activates vaginal Th1-like CD8⁺ T-cell-mediated cytotoxicity. Thus, intranasally administered HPV VLPs probably constitute the vaccine formulation and route of administration of choice to obtain maximum protection at the site of virus entry.

Anesthesia and CT are, of course, not suitable for human vaccination against HPV. However, an aerosol with VLPs could be administered by inhalation to target the BALT, and CT could be replaced by its nontoxic B subunit, which elicits both IgG and IgA production in the genital tract when given intranasally to humans (3). On the other hand, protective efficacy against vaginal infection of immunized mice with Chlamydia trachomatis is correlated with the production of specific vaginal IgG and IgA antibodies and a T-cell immune response (47). Thus, if VLP vaccines currently being investigated for intramuscular immunization of humans are not completely efficient against natural HPV infections, an HPV vaccine containing nontoxic CT as an adjuvant and administered via an aerosol might be an alternative.
REFERENCES


