VIROLOGY 252, 39–45 (1998)
ARTICLE NO. VY989442

Mucosal Immunisation with Papillomavirus Virus-like Particles Elicits Systemic and Mucosal Immunity in Mice

Xiao Song Liu, Ibtissam Abdul-Jabbar, Ying Mei Qi,[1] Ian H. Frazer, and Jian Zhou[2]

Centre for Immunology and Cancer Research, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4012, Australia

Received May 7, 1998; returned to author for revision June 1, 1998; accepted September 22, 1998

It has been shown previously that recombinant virus-like particles (VLPs) of papillomavirus can induce VLP-specific humoral and cellular immune responses following parenteral administration. To test whether mucosal administration of bovine papillomavirus type 1 (BPV1) VLPs could produce mucosal as well as systemic immune responses to VLPs, 50 μg chimeric BPV1 VLPs containing an HPV16 E7 CTL epitope (BPVL1/E7 VLP) was administered intranasally to mice. After two immunisations, L1-specific serum IgG and IgA were observed. L1-specific IgG and IgA were also found in respiratory and vaginal secretions. Both serum and mucosal antibody inhibited papillomavirus VLP-induced agglutination of RBC, indicating that the antibody induced by mucosal immunisation may recognize conformational determinants associated with virus neutralisation. For comparison, VLPs were given intramuscularly, and systemic and mucosal immune responses were generally comparable following systemic or mucosal delivery. However, intranasal administration of VLP induced significantly higher local IgA response in lung, suggesting that mucosally delivered HPV VLP may be more effective for mediating local mucosal immune responses. Intranasal immunisation with HPV6b L1 VLP produced VLP-specific T proliferative responses in splenocytes, and immunisation with BPVL1 VLP containing an HPV16 E7 CTL epitope induced E7-specific CTL responses. We conclude that immunisation with papillomavirus VLPs via mucosal and intramuscular routes, without adjuvant, can elicit specific antibody at mucosal surfaces and also systemic VLP epitope specific T cell responses. These findings suggest that mucosally delivered VLPs may offer an alternative HPV VLP vaccine strategy for inducing protective humoral immunity to anogenital HPV infection, together with cell-mediated immune responses to eliminate any cells which become infected.

INTRODUCTION

Viral and bacterial pathogens, including human immunodeficiency virus (HIV), human papillomavirus (HPV), herpes simplex virus (HSV), *Chlamydia trachomatis,* and *Neisseria gonorrhoeae,* are sexually transmitted diseases that enter the body through the genital mucosa. Therefore, a major goal of vaccines against these diseases should be to elicit an immune response at the level of the genital mucosa, which may block, or at least limit, entry of the pathogen. Ideally, a vaccine should also stimulate a cytotoxic T cell (CTL) response, which would offer improved protection by clearing infected cells when infectious virus penetrates the first line of defense, the neutralising antibodies.

Papillomavirus (PV) major capsid protein can self-assemble into virus-like particles (VLPs) when expressed in eukaryotic and prokaryotic systems (Zhou *et al.,* 1991; Kirnbauer *et al.,* 1992; Hagensee *et al.,* 1993; Schiller and Lowy, 1996; Nardelli-Haefliger *et al.,* 1997). PV VLPs have been used to elicit high titres of systemic neutralizing antibodies which provide protection from experimental challenge with infectious virus in animal papillomavirus models. As papillomavirus infection is via the mucosal route, it is of interest to examine if papillomavirus VLPs can be used as a vaccine to elicit mucosal and systemic immune responses.

A C-terminal truncation mutant of L1 protein has been observed to form VLPs (Paintsil *et al.,* 1996), and up to 60 amino acids can be fused into this region without disrupting its ability to form VLPs (Müller *et al.,* 1997). Thus it is possible to fuse different defined CTL epitopes, or other immunogenic epitopes, to the C terminus of PV L1 sequence to construct vaccines against different diseases. The defined H-2D^b-restricted HPV16 E7 CTL epitope (aa 318–327) (Feltkamp *et al.,* 1993) has been fused to the C terminus of BPV1 L1 sequence and expressed in insect cells by recombinant baculovirus. It was demonstrated that this recombinant BPV1 L1 protein could spontaneously self-assemble into VLPs with a morphology very similar to that of wild-type L1 VLPs and that these VLPs elicit immunity to PV and to the incorporated CTL epitope when administered systemically to mice (Greenstone *et al.,* 1998; Peng *et al.,* 1998). In this paper, we show

[1] Present address: Center for Molecular and Cellular Biology, University of Queensland, St. Lucia 4072, Brisbane, Australia.

[2] To whom reprint requests should be addressed at Centre for Immunology and Cancer Research, University of Queensland, Department of Immunology, Princess Alexandra Hospital, Brisbane, QLD 4012, Australia. (Fax) +61-7-3240 2048. E-mail: jzhou@medicine.pa.uq.edu.au.

0042-6822/98 $25.00

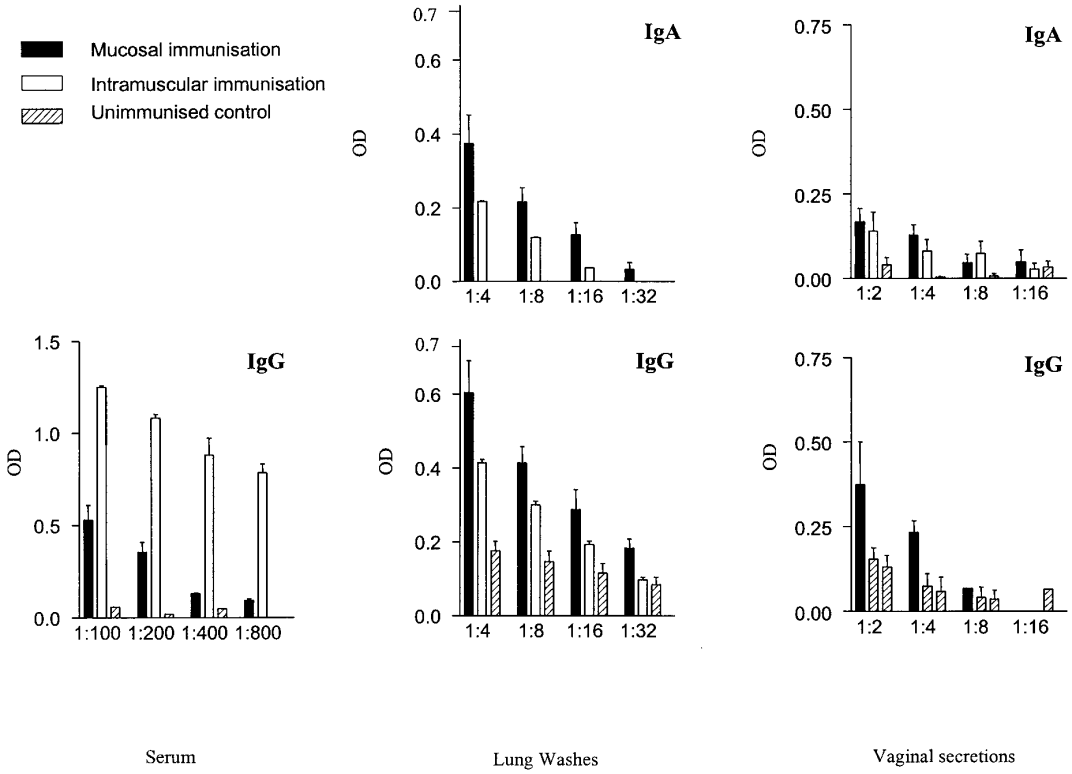

FIG. 1 Capture ELISA assays for detection of BPV-specific antibodies in sera and mucosal secretions from mice received 50 μg BPVL1/E7 VLP. Diluted (*x* axis) sera and mucosal secretions are incubated with BPV1 VLPs coated on 96-well plates, and the VLP-specific IgA (top) or IgG (panel) antibodies were detected with HRP-linked goat anti-mouse antibodies. The OD values are shown on the *y* axis.

that mice immunised with BPVL1/E7 VLP intranasally can also elicit cellular and humoral immunity and that the humoral immunity extends to production of VLP-specific antibodies at mucosal surface.

RESULTS

Humoral and secretory antibody responses in mice following nasal immunisation with BPVL1/E7 and BPVL1/HIVP18 VLPs

Purified BPVL1/E7 (BPV1 VLPs incorporating CTL epitopes from HPV16E7) and BPVL1/HIVP18 VLPs (BPVL1 VLP containing a HIV gp120 CTL epitope) have been examined by immunoblotting and electromicroscopy. The morphology of those VLPs is similar to that of BPVL1 VLP (data not shown), indicating that the insertion of HPV16 E7 or HIV gp120 epitope did not interfere with the VLP formation. Mice immunised twice intranasally or intramuscularly with the hybrid VLP developed significant anti-BPVL1 antibody titres in serum by 7 days after the second immunisation. VLP (50 μg) produced serum IgG, and the titre of serum IgG antibody to BPVL1 VLPs in the intramuscularly immunized group was higher than that of sera from mice that received VLPs intranasally (Fig. 1), indicating that both muscular and mucosal administration of papillomavirus VLPs can stimulate a systemic HPV-specific antibody response and that incorporation of HPV16 E7 and HIV CTL epitopes did not abolish the immunogenicity of the BPV1 L1 protein conformational epitopes. As secretion of IgA at mucosal surface may play a role in protecting the host from pathogens that infect mucosal membranes, we examined whether immunisation with VLPs intranasally or intramuscularly, without adjuvant, could elicit mucosal IgA antibody production. Our data showed that mice immunised by alternative route in both groups developed mucosal IgA antibody at local (lung) and remote (vaginal tract) mucosal surfaces (Fig. 1). The IgA titre in lung washes from mice immunised intranasally was higher than that from the intramuscular immunised group. The IgA titres in vaginal washes from mice immunised by either route both was relatively low. IgG titres in lung and vaginal secretions were also detected following immunisation by either route, with the IgG titre in lung being higher than that of vaginal washes. However, the IgG and IgA titres were higher in the sera from mice immunized with VLP intramuscularly. The absolute IgA reactivity with VLPs in vaginal mucosal washings was relatively low, as also indicated by HA inhibition assays (see below), suggesting that mucosal delivery of VLPs can induce protective local and remote mucosal as well as systemic immunity to HPV.

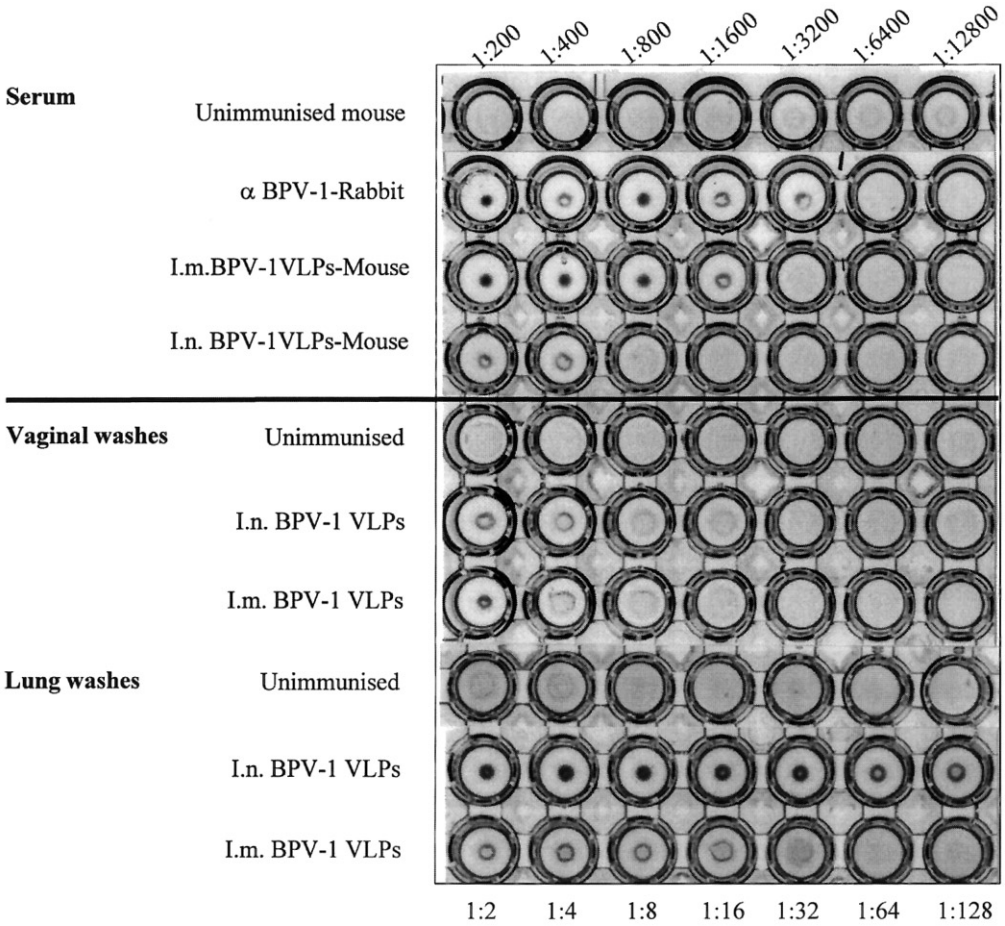

FIG. 2. Inhibition of BPV-1-VLP-induced agglutination of mouse erythrocytes by serum, vaginal, and lung washings from mice immunised intranasally or intramuscularly with 50 μg BPVL1/E7 hybrid VLPs and the tested dilutions of sera (top) or mucosal washes (bottom) are indicated. The assay was performed as described under Materials and Methods. All tested sera from VLP-immunised mice inhibited agglutination. Rabbit anti-BPV1 virus antibody was used as a positive control and preimmune mouse serum, vaginal, and lung washing samples were used as negative controls.

VLPs induce neutralising antibodies

To assess whether the serum and mucosal antibodies of animals intranasally or intramuscularly immunised with 50 μg BPVL1/E7 VLP had neutralizing activity, serum and mucosal secretions were tested in a hemagglutination inhibition assay which has been held to correlate with virus neutralisation (Roden *et al.,* 1995). BPVL1/E7 VLP were mixed with serial dilutions of serum or of vaginal/lung washes of immunised mice. A rabbit anti-BPV1 antiserum was used as a positive control. The serum of immunised mice showed hemagglutination inhibition (HAI) activity. The diluted vaginal secretion washes of the both intramuscularly and intranasally immunized mice showed HAI activity at a dilution of 1:4. However, the diluted lung secretion washes of the intranasally immunized group showed HAI activity at a dilution of 1:128 and the HAI activity of intramuscularly immunized group was 1:16. No HAI activity was found in the sera or secretions from nonimmunised mice (Fig. 2).

In vitro T cell proliferation in response to nasal immunisation with HPV6b L1 VLPs

T cell proliferation to viral capsid antigens has been observed in animals experimentally infected with papillomavirus. We examined whether mice immunised with VLP via the mucosal route could produce VLP-specific systemic T cell responses. Mice were immunised with 30 μg HPV6b L1 VLPs via the nostrils and were boosted once after 14 days. In two independent experiments, splenocytes were cultured in the presence of denatured HPV6b L1 protein for 4 days, and T cell proliferation assessed as [^{3}H]thymidine incorporation. The stimulation index (SI) for splenocytes exposed to 20 μg/ml VLPs was 5.6 $\pm$ 1.0 (3610 $\pm$ 621/643 $\pm$ 64) or 7.2 $\pm$ 2.1 (4667 $\pm$ 1372) to 40 μg/ml VLPs, which was significantly greater than that for splenocytes exposed to ovalbumin [1.6 $\pm$ 0.1 (1064 $\pm$ 72/643 $\pm$ 64)], demonstrating that intranasal immunisation with HPV6b L1 VLPs induced a specific T helper response to the HPV6b L1 protein.

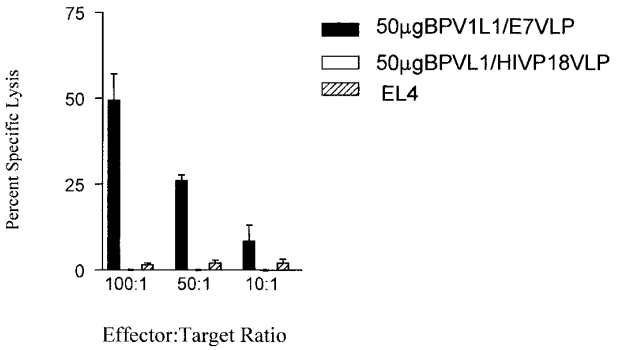

FIG. 3. Specific killing of HPV16-E7-transformed EL-4 cell line (C-2) by CTL bulk cultures generated from mice immunised twice intranasally with hybrid BPVL1/E7 VLP. Splenocytes were cultured with IL-2 for 3 days and used as effector cells in a standard ^{51}Cr release assay. Specific ^{51}Cr release is shown for effectors from mice intranasally immunised with 50 μg BPV1-HPV16 E7 VLPs or with 50 μg BPV1/HIVP18 VLPs. Lysis of untransfected EL-4 cells was, in each case, $<$5%.

Hybrid BPVL1/HPV16 E7 VLPs can induce a CTL response

VLP delivered systematically induces CTL response to defined CTL epitopes incorporated into the VLP L1 protein (Greenstone *et al.,* 1998; Peng *et al.,* 1998). To test if VLP delivered by the mucosal route could similarly induce specific T-cell-mediated cytotoxicity activity, the CTL response to a defined H-2D^b-restricted CTL epitope (HPV16 E7) incorporated within the hybrid BPV-1 VLPs was observed. C57B1/6J mice were immunised intranasally with 50 μg of BPVL1/E7 or BPVL1/HIVP18 VLPs on Days 0 and 14. On Day 28, splenocytes were harvested and expanded *in vitro* for 3 days in the presence of rIL-2. CTL activity of the effector T cells was tested on the E7-transformed EL-4 cells (C2 cell line) or parent EL-4 cells, a negative control to confirm that the cell lysis is not caused by LAK activity after IL-2 stimulation, by a standard ^{51}Cr release assay. A CTL response was detected in the BPVL1/E7 groups immunised with 50 μg BPVL1/E7 VLPs (Fig. 3). Mice immunised with BPVL1/HIVP18 VLPs did not produce a C2-specific CTL response, as expected. These results demonstrate that immunisation with papillomavirus VLPs through the intranasal route can elicit systemic antigen-specific CTL responses.

DISCUSSION

In this study, we have shown that mucosal delivery of VLPs in mice induces both mucosal and systemic humoral immunity and additionally induces cellular immunity to incorporated T epitopes. The low titre of the vaginal mucosal antibody responses observed reflects in part the significant dilution factor ($>$50-fold) inherent in the technique of vaginal washing. Responses at remote mucosal surfaces are also held to be of lower titre than those at the immunisation site, as our data and other studies have suggested (Hopkins *et al.,* 1995). Measurement of vaginal responses following immunisation at remote mucosal sites was held desirable for practical reasons. Although vaginal immunisation is feasible (Uehling *et al.,* 1997), a local inflammatory response following intravaginal immunisation might result in local serum exudation, and therefore assay results obtained following immunisation by this route might not reflect local secretory antibody.

Several vaccine candidates induce humoral mucosal immunity against papillomavirus. HPV16 E7 recombinant *Streptococcus gordonii* (Di Fabio *et al.,* 1998) or *Salmonella typhimurium* (Londono *et al.,* 1996) and HPV L1 recombinant vaccinia (Hagensee *et al.,* 1995) and *S. typhimurium* (Nardelli-Haefliger *et al.,* 1997) each elicit mucosal antibody in mice. A double nasal immunisation with L1 recombinant *Salmonella* was effective in inducing L1-specific antibodies that recognized native but not disassembled VLPs and neutralized HPV16 pseudotyped virions in an *in vitro* infectivity assay (Nardelli-Haefliger *et al.,* 1997). Conformation-dependent anti-VLP IgA and IgG were also detected in oral and vaginal secretions. African green monkeys immunised with HPV11 virus-like particles in aluminum adjuvant by intramuscular injection mount a high-titre HPV11 VLP-specific antibody response, and HPV11-neutralizing IgG antibodies were also observed in cervical vaginal secretions, but IgA antibody was not detected (Lowe *et al.,* 1997). Thus the mode and route of delivery are likely to influence the nature and magnitude of the induced immune response following immunisation with HPV VLPs.

We chose to deliver VLP via the nasal route as, following four mucosal routes of immunization—oral, nasal, rectal, and vaginal—the highest titres of mucosal antibody were obtained with intranasal immunisation (Hopkins *et al.,* 1995), whereas vaginal immunisation gave a high or low response depending on the estrous status of the mice at the time of immunisation. The mechanism by which nasal immunisation induces mucosal and systemic immunity is not clear. Antigen at mucosal surfaces is taken up by mucosal dendritic cells and carried to the local mucosal lymphoid tissues, where specific T and B cells are induced (Husband, 1993). These can move into cervical and uterine tissues (Roche and Crum, 1991; Mestecky *et al.,* 1994; Crowley-Nowick *et al.,* 1997), where polymeric IgA antibodies are produced and transported across the epithelium. Intraepithelial dendritic cells in the bronchial epithelium may play a major role in antigen presentation by taking up antigens in the respiratory epithelium and carrying them to distant draining lymph nodes, where priming for CTL response occurs (Hamilton-Easton and Eichelberger, 1995). Papillomaviruses bind to epithelial cells through α6 integrins (Evander *et al.,* 1997), and activated dendritic cells also express α6 integrin (Price *et al.,* 1997). This latter finding

may explain why nasal immunisation can trigger both local and systemic antibody and systemic T cell responses. Currently, mucosal T cell responses to papillomavirus VLPs are under investigation in our laboratory.

Papillomavirus VLPs have been shown to have strong immunogenicity in the absence of adjuvants (Roden *et al.,* 1994; Christensen *et al.,* 1996). We and others have previously shown that hybrid papillomavirus VLPs containing HPV16 E7 and HIV CTL epitopes can be used as carriers to present antigens without adjuvant to both MHC class I and class II pathways, providing a promising strategy for the design of vaccines to prevent virus infection (Greenstone *et al.,* 1998; Peng *et al.,* 1998). In this study, we have demonstrated that papillomavirus VLPs can also prime antibody and cellular immune responses via the mucosal route, without adjuvant, suggesting that preservation of the VLP structure to allow induction of conformation-dependent neutralizing antibody responses and to facilitate VLP uptake by antigen-presenting cells may assist effective design of PV vaccines designed to induce local mucosal immunity.

CTL play a role in controlling the initial stage of viral infection by eliminating virus-infected cells. CTL are effective in the genital tract for prevention of vaginal infection, as demonstrated for herpes simplex virus (HSV) by adoptive transfer of $CD8^+$ genital LN cells from donors immunised with live attenuated HSV (McDermott *et al.,* 1989). Mucosal immunisation of macaques with VLPs together with adjuvant elicits virus-specific T cells in the genital or rectal mucosa as well as draining lymph nodes (Di Fabio *et al.,* 1998). We immunised mice intranasally with 50 μg different VLPs and observed a specific CTL response as well as both systemic and mucosal antibody responses in those animals.

Vaccines based on injection of purified papillomavirus VLPs have been shown to induce type-specific protection from experimental infection by cottontail rabbit papillomavirus in domestic rabbits and by canine oral papillomavirus in dogs (Breitburd *et al.,* 1995; Suzich *et al.,* 1995). Passive transfer experiments indicated that protection is due to the production of virion-neutralizing antibodies. Thus HPV16 VLP-specific antibodies in cervical secretions could be expected to similarly prevent sexually transmitted HPV16 infection in humans. However, it is difficult to predict whether secretory IgA produced by mucosal lymphoid tissues or serum IgG or a combination of the two will be most effective in preventing genital HPV infection. Therefore induction of both local and systemic antibody responses may be desirable in a prophylactic genital HPV vaccine, while specific T cell responses may help to eliminate any cells which are infected with virus that is not neutralised. Mucosal delivery of PV VLP provides an alternative administration route for a vaccine to induce both mucosal and systematic immunity to HPV infection. It would therefore be worthwhile to consider this route in evaluating potential vaccines against HPV infection in man.

MATERIALS AND METHODS

Mice and cell lines

Adult female C57BL/6J mice were purchased from Animal Resource Centre (ARC, Australia). The HPV16 E7 transfected EL4 cell line designated C2 (Tindle *et al.,* 1995) and the parent EL4 line were maintained in complete RPMI 1640 medium plus 10% fetal bovine serum (FBS, CSL, Australia).

Construction of recombinant baculovirus and purification of VLPs

Production and purification of 6b L1 VLPs (Park *et al.,* 1993) and of BPVL1/E7 and BPV1/HIV1 gp160P18 hybrid recombinant baculoviruses (Peng *et al.,* 1998) from SF-9 cells infected with the appropriate recombinant baculoviruses have been described. In brief, VLPs were purified from insect cell nuclei by CsCl gradient centrifugation, and a band with a density of 1.28 g/ml was collected and dialysed extensively against PBS. The samples were then used for analysis by transmission electron microscopy, and for Western immunoblotting to confirm the identity and integrity of the VLPs.

Western immunoblot analysis

Protein samples were diluted in SDS–PAGE sample buffer, boiled at 100°C for 10 min, and electrophoresed through a 10% SDS–PAGE gel, and then transferred to nitrocellulose membrane. The membrane was blocked with 5% skim milk in PBS and probed with the anti-L1 monoclonal antibody MC15 (Kulski *et al.,* 1997) at a dilution of 1:2000. Bound antibody was detected by incubation of the membrane with horseradish peroxidase-conjugated, sheep anti-mouse antibody (Silenus, Australia) at a dilution of 1:1000 and visualised using enhanced chemiluminescence (ECL; Amersham).

Transmission electron microscopy

CsCl gradient-purified and dialysed samples were mounted onto carbon-coated grids, stained with 2% ammonium molybdate, pH 6.2, and examined with an Hitachi H-800 electron microscope.

Immunisation of mice

Four- to 8-week-old female mice were immunised on Days 0 and 21 with 50 μg of VLPs intranasally. Alternatively, mice were immunised intramuscularly and boosted at Day 21. Sera and mucosal secretions were collected after a further 7 days and stored at −70°C until use. Vaginal secretions were collected by washing the vaginal tracts with 100 μl of sterile PBS, and respiratory

secretions were collected by repeatedly flushing the lungs with 300 μl sterile PBS 7 days after a booster immunisation as previously described (VanCott *et al.,* 1998). The vaginal and lung washes were cleared by centrifugation at 13,000 rpm to remove any tissue and cellular debris and stored at −70°C until analyzed. For the hemagglutination inhibition assay, the washes were incubated in a 56°C water bath for 30 min before testing.

Cytotoxic T lymphocyte assays

Mice immunised as described above were sacrificed on Day 35, spleens were removed, and a single cell suspension of spleen cells was cultured in complete RMPI 1640 medium supplemented with 10 μ/ml rIL-2 (Sigma) for 3 days. For HPV-E7-specific CTL assays, EL4 or EL4.E7 target cells were washed once with serum-free complete RPMI and labeled with 100 μCi of $^{51}Cr/10^7$ cells. Excess ^{51}Cr was removed using the FBS underlay technique. Effector cells and labeled target cells were plated into 96-well round-bottom plates at various effector/target ratios and incubated at 37°C, 5% CO_2 for 5 h. Supernatant (100 μl) was collected from each well for counting with a gamma counter. The percentage of specific lysis was calculated as specific lysis = (sample release − spontaneous release)/(maximum release − spontaneous release) × 100. Maximum release was generated by adding 100 μl of 10% SDS to 100 μl of target cells, and spontaneous release was assayed from 100 μl of target cells incubated with 100 μl of medium. Assays were performed in triplicate, and spontaneous ^{51}Cr release from the various targets did not exceed 15%. Each set of results represents the means from two independent experiments.

T proliferation response assay

T proliferation assays were carried out as described (Tindle *et al.,* 1991). In brief, splenocyte suspensions were treated with an NH_4Cl solution to lyse the red blood cells. White blood cells were harvested by centrifugation at 800 rpm for 5 min and were washed three times with DMEM without FCS. The washed cells were resuspended in 20 ml of complete DMEM with 10% FCS and diluted to a concentration of 10^6 cells/ml. Cell suspension (200 μl) was added to each well of 96-well tissue-culture plates. Cells in test wells were stimulated by addition of antigen in a total volume of 10–20 μl/well, using four replicates, for 4 days in a humidified tissue-culture incubator. Cellular proliferation was assessed by the incorporation of [^{3}H]thymidine at 0.4 mCi/well over a period of 18 h. The stimulation index (SI) was calculated as the ratio of the mean counts per minute of [^{3}H]thymidine incorporated in the presence of stimulating antigens or peptides to the mean counts per minute of incorporated [^{3}H]thymidine obtained in the absence of stimulating antigens.

ELISA assays for anti-VLP IgA and serum IgG

Measurements of total and VLP-specific IgA in serum, lung and vaginal secretions were performed in flat-bottom microtitre plates, as previously described (Peng *et al.,* 1998). Briefly, a rabbit anti-BPV1 antiserum (Peng *et al.,* 1998) was used to coat the plates. After blocking with 5% milk/PBS, 50 μl BPV1L1/E7 VLPs at a concentration of 10 μg/ml was added to each well and incubated at 37°C for 90 min. Sera and mucosal washes were tested by twofold serial dilution in 5% milk/PBS. Bound antibody was detected with horseradish peroxidase-conjugated sheep anti-mouse IgG and goat anti-mouse IgA α chain, at a dilution of 1:1000, followed by incubation with OPD in the presence of hydrogen peroxide. The reaction was stopped with 3 N HCl and absorbances were measured at 450 nm.

Hemagglutination inhibition assay

The method used was as previously described (Roden *et al.,* 1995). Fresh mouse blood (1 ml) was collected in a tube containing 1000 units of heparin. The blood sample was suspended in 9 ml of PBS and 1 mg BSA and centrifuged at 1500 rpm for 5 min. The supernatant was aspirated, and the red blood cells (RBC) were resuspended in 10 ml PBS and 1 mg BSA and centrifuged at 1500 rpm for 5 min. After being washed three times, the final RBC pellet was diluted to 1% (v/v) in PBS + 1 mg/ml BSA. One hundred microliters of this RBC suspension was mixed with the same volume of BPV1L1/E7 VLPs and added to a 96-well round-bottom plate, incubated at 4°C for 3 h, and photographed. For the antibody inhibition assay, the sera were incubated with 4 vol of 25% v/v mouse RBC overnight at 4°C, and cleared by centrifugation at 1000 rpm at 4°C. The sera and mucosal washes were heat-inactivated at 56°C for 30 min and incubated with BPVL1/E7 VLPs at room temperature for 1 h with gentle rocking, before mixing with mouse RBCs.

ACKNOWLEDGMENTS

This work was funded in part by grants from the Queensland Cancer Fund, Maye Bequest, and Princess Alexandra Hospital Research and Development Foundation.

REFERENCES

Breitburd, F., Kirnbauer, R., Hubbert, N. L., Nonnenmacher, B., Trin-Dinh-Desmarquet, C., Orth, G., Schiller, J. T., and Lowy, D. R. (1995). Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J. Virol.* 69, 3959–3963.

Christensen, N. D., Reed, C. A., Cladel, N. M., Han, R., and Kreider, J. W. (1996). Immunization with viruslike particles induces long-term protection of rabbits against challenge with cottontail rabbit papillomavirus. *J. Virol.* 70, 960–965.

Crowley-Nowick, P. A., Bell, M. C., Brockwell, R., Edwards, R. P., Chen, S., Partridge, E. E., and Mestecky, J. (1997). Rectal immunization for

induction of specific antibody in the genital tract of women. *J. Clin. Immunol.* 17, 370–379.

Di Fabio, S., Medaglini, D., Rush, C. M., Corrias, F., Panzini, G. L., Pace, M., Verani, P., Pozzi, G., and Titti, F. (1998). Vaginal immunization of Cynomolgus monkeys with *Streptococcus gordonii* expressing HIV-1 and HPV 16 antigens. *Vaccine* 16, 485–492.

Evander, M., Frazer, I. H., Payne, E., Qi, Y. M., Hengst, K., and McMillan, N. A. (1997). Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. *J. Virol.* 71, 2449–2456.

Feltkamp, M. C. W., Smits, H. L., Vierboom, M. P. M., Minnaar, R. P., De Jongh, B. M., Wouter Drijfhout, J., Ter Schegget, J., Melief, C. J. M., and Kast, W. M. (1993). Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur. J. Immunol.* 23, 2242–2249.

Greenstone, H. L., Nieland, J. D., De Bruijn, M. L., Kirnbauer, R., Roden, R. B., Lowy, D. R., Kast, W. M., and Schiller, J. T. (1998). Chemeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. *Proc. Natl. Acad. Sci. USA* 95, 1800–1805.

Hagensee, M. E., Carter, J. J., Wipf, G. C., and Galloway, D. A. (1995). Immunization of mice with HPV vaccinia virus recombinants generates serum IgG, IgM, and mucosal IgA antibodies. *Virology* 206, 174–182.

Hagensee, M. E., Yaegashi, N., and Galloway, D. A. (1993). Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins. *J. Virol.* 67, 315–322.

Hamilton-Easton, A., and Eichelberger, M. (1995). Virus-specific antigen presentation by different subsets of cells from lung and mediastinal lymph node tissues of influenza virus-infected mice. *J. Virol.* 69, 6359–6366.

Hopkins, S., Kraehenbuhl, J. P., Schödel, F., Potts, A., Peterson, D., De Grandi, P., and Nardelli-Haefliger, D. (1995). A recombinant *Salmonella typhimurium* vaccine induces local immunity by four different routes of immunization. *Infect. Immun.* 63, 3279–3286.

Husband, A. J. (1993). Novel vaccination strategies for the control of mucosal infection. *Vaccine* 11, 107–112.

Kirnbauer, R., Booy, F., Cheng, N., Lowy, D. R., and Schiller, J. T. (1992). Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc. Natl. Acad. Sci. USA* 89, 12180–12184.

Kulski, J. K., Sadleir, J. W., Kelsall, S. R., Cicchini, M. S., Shellam, G. R., Peng, S. W., Qi, Y. M., Galloway, D. A., Zhou, J., and Frazer, I. H. Type specific and genotype cross reactive B epitopes of the L1 protein of HPV16 defined by a panel of monoclonal antibodies. *Virology* (in press).

Londono, L. P., Chatfield, S., Tindle, R. W., Herd, K., Gao, X. M., Frazer, I., and Dougan, G. (1996). Immunisation of mice using Salmonella typhimurium expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. *Vaccine* 14, 545–552.

Lowe, R. S., Brown, D. R., Bryan, J. T., Cook, J. C., George, H. A., Hofmann, K. J., Hurni, W. M., Joyce, J. G., Lehman, E. D., Markus, H. Z., Neeper, M. P., Schultz, L. D., Shaw, A. R., and Jansen, K. U. (1997). Human papillomavirus type II (HPV-11) neutralizing antibodies in the serum and genital mucosal secretions of African green monkeys immunized with HPV-11 virus-like particles expressed in yeast. *J. Infect. Dis.* 176, 1141–1145.

McDermott, M. R., Graham, F. L., Hanke, T., and Johnson, D. C. (1989). Protection of mice against lethal challenge with herpes simplex virus by vaccination with an adenovirus vector expressing HSV glycoprotein B. *Virology* 169, 244–247.

Mestecky, J., Kutteh, W. H., and Jackson, S. (1994). Mucosal immunity in the female genital tract: Relevance to vaccination efforts against the human immunodeficiency virus. *AIDS Res. Hum. Retroviruses* 10 (suppl. 2), S11–S20.

Müller, M., Zhou, J. A., Reed, T. D., Rittmüller, C., Burger, A., Gabelsberger, J., Braspenning, J., and Gissmann, L. (1997). Chimeric papillomavirus-like particles. *Virology* 234, 93–111.

Nardelli-Haefliger, D., Roden, R. B., Benyacoub, J., Sahli, R., Kraehenbuhl, J. P., Schiller, J. T., Lachat, P., Potts, A., and De Grandi, P. (1997). Human papillomavirus type 16 virus-like particles expressed in attenuated *Salmonella typhimurium* elicit mucosal and systemic neutralizing antibodies in mice. *Infect. Immun.* 65, 3328–3336.

Paintsil, J., Müller, M., Picken, M., Gissmann, L., and Zhou, J. (1996). Carboxyl terminus of bovine papillomavirus type-1 L1 protein is not required for capsid formation. *Virology* 223, 238–244.

Park, D. S., Selvey, L. A., Kelsall, S. R., and Frazer, I. H. (1993). Human papillomavirus type 16 E6, E7 and L1 and type 18 E7 proteins produced by recombinant baculoviruses. *J. Virol. Methods* 45, 303–318.

Peng, S. W., Frazer, I. H., Fernando, G. J., and Zhou, J. (1998). Papillomavirus virus-like particles can deliver defined CTL epitopes to the MHC class I pathway. *Virology* 240, 147–157.

Price, A. A., Cumberbatch, M., Kimber, I., and Ager, A. (1997). $\square_6$ integrins are required for Langerhans cell migration from the epidermis. *J. Exp. Med.* 186, 1725–1735.

Roche, J. K., and Crum, C. P. (1991). Local immunity and the uterine cervix: Implications for cancer-associated viruses. *Cancer Immunol. Immunother.* 33, 203–209.

Roden, R. B. S., Hubbert, N. L., Kirnbauer, R., Breitburd, F., Lowy, D. R., and Schiller, J. T. (1995). Papillomavirus L1 capsids agglutinate mouse erythrocytes through a proteinaceous receptor. *J. Virol.* 69, 5147–5151.

Roden, R. B. S., Weissinger, E. M., Henderson, D. W., Booy, F., Kirnbauer, R., Mushinski, J. F., Lowy, D. R., and Schiller, J. T. (1994). Neutralization of bovine papillomavirus by antibodies to L1 and L2 capsid proteins. *J. Virol.* 68, 7570–7574.

Schiller, J., and Lowy, D. R. (1996). Papillomavirus-like particles and HPV vaccine development. *Semin. Cancer Biol.* 7, 373–382.

Suzich, J. A., Ghim, S. J., Palmer-Hill, F. J., White, W. I., Tamura, J. K., Bell, J. A., Newsome, J. A., Jenson, A. B., and Schlegel, R. (1995). Systemic immunzation with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc. Natl. Acad. Sci. USA* 92, 11553–11557.

Tindle, R. W., Croft, S., Herd, K., Malcolm, K., Geczy, A. F., Stewart, T., and Fernando, G. J. P. (1995). A vaccine conjugate of "ISCAR" immunocarrier and peptide epitopes of the E7 cervical cancer-associated protein of human papillomavirus type 16 elicits specific Th1-and Th2-type responses in immunized mice in the absence of oil-based adjuvants. *Clin. Exp. Immunol.* 101, 265–271.

Tindle, R. W., Fernando, G. J., Sterling, J. C., and Frazer, I. H. (1991). A "public" T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes. *Proc. Natl. Acad. Sci. USA* 88, 5887–5891.

Uehling, D. T., Hopkins, W. J., Balish, E., Xing, Y. N., and Heisey, D. M. (1997). Vaginal mucosal immunization for recurrent urinary tract infection: Phase II clinical trial. *J. Urol.* 157, 2049–2052.

VanCott, T. C., Kaminski, R. W., Mascola, J. R., Kalyanraman, V. S., Wassef, N. M., Alving, C. R., Ulrich, J. T., Lowell, G. H., and Birx, D. L. (1998). HIV-1 neutralizing antibodies in the genital and respiratory tracts of mice intranasally immunized with oligomeric gp160. *J. Immunol.* 160, 2000–2012.

Zhou, J., Sun, X. Y., Stenzel, D. J., and Frazer, I. H. (1991). Expression of vaccinia recombinant HPV 16 L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology* 185, 251–257.