Papillomavirus-Like Particle Vaccines

John T. Schiller, Douglas R. Lowy

Papillomavirus-like particle (VLP)-based subunit vaccines have undergone rapid development over the past 8 years. Three types are being investigated. The most basic type is composed of only the L1 major capsid protein and is designed to prevent genital human papillomavirus (HPV) infection by inducing virus-neutralizing antibodies. On the basis of positive results in animal models, clinical trials of this type of vaccine for HPV16, and other types, are currently under way. Preliminary results have been encouraging in that systemic immunization with the L1 VLPs induced high serum titers of neutralizing antibodies without substantial adverse effects. The second type of vaccine incorporates other papillomavirus polypeptides into the VLPs as L1 or L2 fusion proteins. These chimeric VLPs are designed to increase the therapeutic potential of an HPV vaccine by inducing cell-mediated responses to nonstructural viral proteins, such as E7. Studies in mice indicate that these vaccines generate potent antitumor cytotoxic lymphocyte (CTL) responses while retaining the ability to induce high-titer neutralizing antibodies. It is likely that prophylactic and therapeutic clinical trials of chimeric VLPs will be initiated in the near future. The third type of VLP-based vaccine is designed to induce autoantibodies against central self-antigens by incorporating self-peptides into the outer surface of VLPs, a process that could have therapeutic potential in various disease settings unrelated to HPV infection. In a recent proof of concept study, a peptide from an external loop of mouse CCR5 protein was inserted into a neutralizing epitope of L1. In mice, the particles generated by this chimeric L1 were able to induce high titers of CCR5 antibodies that specifically recognized the surface of CCR5-transfected cells and blocked *in vitro* infection of an M-tropic human immunodeficiency virus strain. [J Natl Cancer Inst Monogr 2000;28: 50–4]

Very strong biologic, clinical, and epidemiologic evidence exists that sexually transmitted human papillomavirus (HPV) infections cause most cervical cancers *(1).* This infectious etiology provides an opportunity to prevent a major cause of cancer deaths in women through vaccination. The desire to prevent or treat genital HPV infection through immunization has led investigators to employ a number of strategies to develop candidate HPV vaccines *(2).* This report will focus on the development of one of these strategies, papillomavirus-like particle (VLP)-based subunit vaccines. In general, VLP-based vaccines are attractive for combating viral infections because they retain the highly immunogenic array of repetitive epitopes found on the surface of authentic virions, yet VLPs are devoid of the potentially harmful viral genomes. Preclinical *in vitro* and animal studies of papillomavirus VLPs, composed of only the L1 major virion protein, have moved this candidate to the forefront of vaccines to prevent HPV infection [reviewed in *(3)*]. They have also prompted attempts to develop second-generation VLP-based vaccines that incorporate polypeptides of other viral and cellular proteins into the VLPs. In these cases, the VLPs are used as vehicles to facilitate immune presentation of additional antigens to both the cellular and humoral arms of the immune system (Fig. 1). Some of these second-generation vaccines are being developed with the goal of improving the effectiveness against HPV infection, whereas others have the goal of combating other diseases.

Prophylactic Vaccines

Prophylactic vaccines against viruses are thought to function primarily through the induction of virion-neutralizing antibodies that prevent infection *(4).* It has been difficult to employ this strategy to develop an HPV vaccine. HPV virions cannot be propagated efficiently enough in cultured cells to serve as a source of antigen for a vaccine *(5).* Even if they could be easily propagated, they would be unattractive as a prophylactic vaccine, because their genomes contain oncogenes. Subunit vaccines that lack the viral genome are, therefore, much more attractive candidates. However, early attempts to develop virion protein-based subunit vaccines in animal papillomavirus models were only minimally successful. This minimal success is because neutralizing antibodies predominantly recognize conformational epitopes of the L1 major capsid protein, and the early vaccines used denatured virion proteins or peptides [*(6)* and references therein]. The methodologic breakthrough in prophylactic vaccine development was the finding that L1 alone could self-assemble into VLPs that are structurally and antigenically very similar to authentic virions. This finding was first shown in a bovine papillomavirus type 1 (BPV1) model *(7)* and later confirmed for HPV VLPs as suitable serologic assays became available. VLPs have been generated in a variety of cultured cells, including those from mammals, insects, yeast, and even bacteria *(3).*

Because HPVs do not infect animals, studies of protection from virus challenge after VLP vaccination were conducted with the use of animal-type viruses and VLPs in their animal host species. Three animal models have been used: cutaneous challenge of domestic rabbits with cottontail rabbit papillomavirus (CRPV) *(8–10),* oral mucosal challenge of dogs with canine oral papillomavirus *(11),* and oral mucosal challenge of cattle with bovine papillomavirus type 4 (BPV4) *(12).* In these studies, purified VLPs were administered parenterally, and challenge virus was applied to an abraded epithelium to expose the proliferating basal keratinocytes to infection. In each model, vaccination with high nanogram to low microgram doses of L1 VLPs induced high titers of virion antibodies and protection from experimental challenge with high-dose virus. In most experiments, approximately 90% of the control subjects developed papillomas

Affiliation of authors: Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD

Correspondence to: John T. Schiller, Ph.D., National Institutes of Health, Bldg. 36, Rm. 1D-32, Besthesda, MD 20892 (e-mail: schillej@dc37a.nci.nih.gov).

See "Notes" following "References."

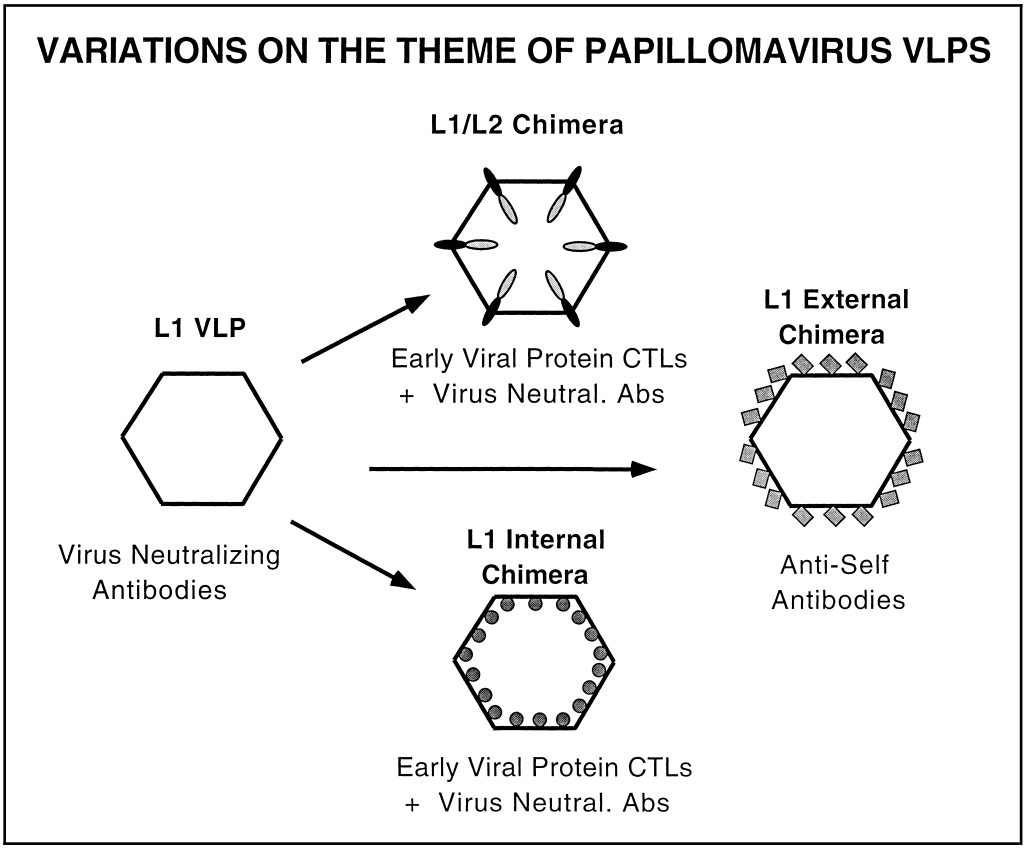

Fig. 1. The type of papillomavirus-like particle is indicated above each particle. The applicable immune effector functions generated by each type of particle are indicated below. The non-virion polypeptides are depicted in gray tone.

at the site of inoculation, whereas at least 90% of the vaccinated subjects showed no evidence of infection. High-titer antibodies and protection were seen even after vaccination in the absence of adjuvant *(8,9,11).* However, protection was obtained only after vaccination with the homologous VLP type *(8,9,11).* For instance, rabbits vaccinated with BPV1 VLPs were not protected from CRPV challenge. Although the L2 minor capsid protein is incorporated into VLPs when co-expressed with L1, there were no detectable differences in the titers of virion antibodies or in the degree of protection generated after vaccination with L1 or with L1 and L2 VLPs *(8,12).* Protection could be passively transferred to naive animals via immune sera or purified immunoglobulin G, indicating that neutralizing antibodies were sufficient to confer protection from experimental challenge *(8,11)* (Table 1).

Clinical Trials

The positive results of the animal vaccine studies have prompted the National Institutes of Health (NIH), and at least two pharmaceutical companies, to begin clinical trials of HPV VLP vaccines. The early-phase NIH trials are a collaboration of the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and The Johns Hopkins Center for Immunization Research. They use HPV16 L1 VLPs generated in recombinant baculovirus-infected insect cells. A placebo-controlled, dose-escalation phase I trial compared intramuscular injection of the VLPs either alone, in alum, or in MF59 adjuvant *(13).* The vaccine was administered in three 10-μg or 50-μg doses at 0, 1, and 4 months. Preliminary analyses of the results (unpublished) are encouraging in that the vaccine was consistently immunogenic and well tolerated. All of the 60 subjects who received the VLPs seroconverted by 1 month after the second dose, as measured in an HPV16 VLP-based enzyme-linked immunosorbent assay (ELISA), whereas none of the 12 control subjects seroconverted during the course of the study. Preliminary analysis suggests that both adjuvants increased the titers of VLP antibodies after low-dose (10 μg) VLP vaccination. However, at the higher dose (50 μg), the highest geometric mean titer (GMT) was seen for the group injected with VLPs without adjuvant. The relative neutralizing titers obtained in an HPV16 pseudovirion neutralization assay appear to parallel ELISA titers for both group GMTs and individuals within groups *(14).* In the individuals receiving VLPs alone or VLPs plus alum, reactogenicity to the vaccine was minimal, with transient mild pain at the site of injection being the most frequent side effect. Reactogenicity did not increase with vaccine dose or boosting. The side effects in the individuals receiving VLPs plus MF59 were somewhat greater, with more frequent reports of mild or moderate transient pain at the site of injection. A phase II trial of the 50 μg VLPs without adjuvant formulation is currently in progress.

The appropriate valency of a prophylactic HPV vaccine is

Table 1. Summary of papillomavirus-like particle (VLP) vaccine trials in animals*

Vaccine	Protection
L1 VLPs	Yes
L1/L2 VLPs	Yes
VLPs without adjuvant	Yes
Denatured VLPs	No
Heterologous VLPs	No
Immune serum	Yes

*Cottontail rabbit papillomavirus VLPs in rabbits, canine oral papillomavirus VLPs in dogs, and bovine papillomavirus type 4 VLPs in cattle.

currently under debate. On the basis of *in vitro* neutralization and hemagglutination assays of HPV VLP sera raised in animals, it is assumed that protection in people will be predominantly genotype specific *(14–18).* Because its goal is proof of concept, the NIH prophylactic vaccine program involves only VLPs of HPV16, the type found in approximately 50% of cervical cancers. However, many other types are also detected in cervical cancer *(19),* and an HPV vaccine for general distribution will likely contain multiple VLP types. Types 18, 31, and 45, along with 16, account for approximately 80% of cancers worldwide *(19),* so most or all of these types will likely be included in a commercial vaccine. The question of cross-interference in the elicitation of antibodies to specific VLP types in polyvalent formulations will need to be addressed during development of this type of vaccine.

Good reasons exist to consider including VLPs of nononcogenic genital HPVs in a polyvalent prophylactic HPV vaccine as well. HPV6, and HPV11 to a lesser extent, induce most genital warts *(20).* Although genital warts very rarely undergo malignant progression, they cause substantial morbidity. A vaccine that targets genital warts would make the vaccine more attractive to men, because men, as well as women, suffer from these lesions. In contrast, the overall incidence of HPV-induced cancers is much lower in men than in women, although a substantial proportion of penile and anal cancers in men are attributed to HPV infection *(1).* Vaccination of both men and women is likely to increase the effectiveness of a prophylactic vaccination program by increasing herd immunity and breaking the cycle of venereal transmission.

Therapeutic VLP Vaccines

Studies in mice indicate that papillomavirus VLPs can induce L1-specific cell-mediated immune (CMI) responses *(21),* in addition to inducing high titers of virion antibodies. However, the virion proteins are not expressed at a detectable level in the proliferating basal keratinocytes of virus producing lesions or in the dedifferentiated cells of HPV-induced dysplasias and cancers *(22).* Therefore, it is unlikely that CMI responses to the virion proteins will induce regression of established lesions. In an attempt to generate effective CMI against papillomavirus-infected cells, papillomavirus VLPs have been generated in which polypeptides of nonstructure viral proteins are incorporated into the VLPs as fusion proteins of L1 or L2 [reviewed in *(23)*].

Chimeric VLPs that contain the entire HPV16 E7 oncoprotein fused to L2, or the N-terminus of E7 fused to L1, have been generated and shown to induce antigen-specific protection of mice from lethal challenge with E7-expressing tumor cells *(24–26).* Protection was obtained after a single injection of 10 μg of VLPs in the absence of adjuvant. The chimeric VLPs could also act therapeutically to induce regression of established tumors *(26).* The antitumor immune response to the chimeric VLPs appears to be primarily mediated by $CD8^-$ cytotoxic lymphocytes. *In vitro* E7-specific cytotoxic lymphocyte (CTL) activity was detected in lymphocytes from chimeric VLP-vaccinated mice *(25,26).* Also, good protection was observed in major histocompatibility complex class II knockout or natural killer cell-depleted mice, but no protection was seen in β_2 microglobulin or perforin knockout mice *(24).* It is unclear how the VLPs are routed for class I presentation. It might involve an endocytic pathway that the virus normally uses to enter the cell during the infectious process.

L1 and L2 chimeras for E7 produced similar results in mice, so it is unclear whether L1 or L2 chimeric VLPs would be preferable for testing in humans. L1 chimeras have the theoretical advantage in delivering more copies of the target antigen per VLP than L2 chimeras (360 for L1 versus 12 for L2). L2 chimeras have the theoretical advantage of being able to incorporate larger polypeptides and thereby increasing the number of epitopes for immune recognition. It would seem reasonable to continue testing both types of chimeras.

Several alternative strategies for generating CMI responses to E7 have been developed *(2).* From a safety standpoint, protein-based strategies for generating CTLs to oncoproteins, such as E7, are preferable to gene transfer-based strategies, because transfer of oncogenes might theoretically be tumorigenic. An attractive feature of VLPs is their ability to induce CTL responses without the addition of strong nonspecific immune stimulators. It is likely that early-phase trials of chimeric VLPs that contain nonstructural papillomavirus polypeptides will begin shortly. Future efficacy trials could be done in several settings. The chimeric VLPs might be effective in treating clinically apparent HPV-induced neoplastic lesions. Although the initial safety studies may be done in cancer patients, there is also considerable interest in attempting to induce regression of HPV-induced premalignant cervical dysplasias by chimeric VLP vaccination. Chimeric VLPs also have the potential to function as a combined prophylactic-therapeutic vaccine, because the insertion of the additional polypeptide did not appear to diminish the ability of the chimeric VLPs to induce high titers of virion-neutralizing antibodies *(24).* It is possible that chimeric VLPs could increase the effectiveness of a prophylactic vaccine by eliminating early subclinical infections that break through, despite the presence of neutralizing antibodies.

The National Cancer Institute is contemplating a prophylactic vaccine trial of an HPV16 chimera in which the entire E7 and E2 is fused to the C-terminus of L2. E2 was included because basal cells in benign lesions may express more E2 than E7, and because it simply increases the number of viral epitopes for generated CMI responses. To address concerns that a fusion protein that contains the two nonstructural viral proteins might have adverse effects on cells, mutations were introduced to inactivate the Rb binding activity of E7 and the sequence-specific transcription activating activity of E2. Fusion of E2 to E7 did not inhibit the ability of the chimeric VLPs to generate potent antitumor responses against E7 in a standard mouse tumor model (our unpublished results). A similar HPV6 chimera is being generated for eventual use in genital wart therapy trials.

Chimeric papillomavirus VLPs containing polypeptides of nonpapillomavirus targets are also being investigated in preclinical studies. One approach is to incorporate polypeptides of other sexually transmitted diseases (STDs). With the provision that induction of neutralizing antibodies is sufficient for protection against genital HPV infection, this strategy could produce a vaccine that provides protection against both HPV and another STD at little or no increase in the cost of production or administration. A second approach involves incorporating cellular tumor antigens into the VLPs. This strategy was recently shown to induce therapeutic antitumor immune responses in a mouse model *(27).* Immunization of mice with an immunodominant peptide derived from the P815 tumor-associated antigen P1A

induces specific T-cell tolerance, resulting in progressive outgrowth of a normally regressing P815 tumor line. In contrast, immunization with an L1 chimera that contains this same P1A peptide did not induce tolerance. Rather, it protected mice from lethal challenge with a progressor P815 line. Vaccination with this chimeric VLP also functioned therapeutically to suppress the growth of established tumors and to increase survival of the tumor-bearing mice.

Autoantibody-Inducing Vaccines

As exemplified above, the mammalian immune system has clearly evolved to produce a strong antibody response to viruses and VLPs that mimic them. In contrast, it has evolved to normally be tolerant to self-antigens exposed to the circulating immune system. In part, the humoral immune system may distinguish between self (safe) and nonself (dangerous) on the basis of epitope arrangement, with the highly ordered repetitive arrangement of virion surface determinants being especially immunogenic *(28).* It was, therefore, of interest to determine whether a central self-antigen, to which the immune system was normally tolerant, could induce an antibody response if it was presented in the ordered context of a papillomavirus VLP. To test this possibility, the first external loop of the mouse CCR5 chemokine receptor (which is primarily expressed on macrophages and memory T cells) was cloned into an immunodominant-neutralizing epitope of BPV1 L1 *(29).* The chimeric VLPs assembled into particles, but they were smaller than those of wild-type L1 VLPs, containing an estimated 12 capsomeres rather than 72, and they did not induce BPV-neutralizing antibodies. Nevertheless, vaccination of mice expressing an identical CCR5 sequence resulted in high-titer antibodies that recognized the CCR5 peptide in ELISA. The ability of the chimeric L1 to generate CCR5 autoantibodies depended on the arrangement of the antigen, because no CCR5 antibodies were generated if the chimeric particles were denatured prior to vaccination (Table 2). The antibodies generated against the chimeric particles recognized the native CCR5, because the sera specifically bound cells transfected with mouse CCR5 and inhibited binding of RANTES, a CCR5 ligand. In contrast, antibodies generated against the same CCR5 peptide coupled to keyhole-limpet hemocyanin as a carrier bound the peptide in an ELISA but did not recognize cell surface CCR5 and did not block ligand binding, indicating that autoantibodies to the native structure were not generated by the latter immunogen.

Because human CCR5 is the co-receptor for macrophage-tropic HIV strains, it was possible to determine if the antibodies generated to CCR5 by the chimeric particles could inhibit HIV infection. Although mouse CCR5 cannot function as an HIV co-receptor, a hybrid CCR5 in which the first external loop of the mouse protein replaces the corresponding loop in the human protein can function as a co-receptor. M-tropic HIV (BaL strain) infection of cells carrying this recombinant CCR5 was effectively neutralized by sera from the chimeric VLP-vaccinated mice but not by sera from wild-type VLP-vaccinated mice (Table 2). These results establish that, in principle, mammals can be induced to synthesize neutralizing autoantibodies to virus cell–surface receptors. Whether this strategy can be effective at preventing or controlling viral infection *in vivo* remains to be determined.

The general safety of autoantibody induction as an approach to immunotherapy must obviously be considered and could vary considerably, depending on the cellular target. A potential advantage of targeting CCR5 is that it appears to be a nonessential protein. Individuals who are homozygous for a defective CCR5 gene are phenotypically normal, except that they have a substantially decreased risk of HIV infection *(30,31).* It is noteworthy that the mice producing CCR5 autoantibodies were outwardly healthy at 6 months after vaccination and did not exhibit signs of immunopathology at autopsy *(29).* There was also no decline in the numbers of macrophages or T-cell subsets that express CCR5 in comparison to control animals. Although we did not test for autoreactive T cells, we would not expect to break T-cell tolerance to CCR5. T cells that recognize central autoantigens are strongly selected against during development of the immune system. Of interest, the levels of CCR5 antibodies had begun to slowly decline by 6 months postvaccination, and the relative decline paralleled the decline in L1 antibodies for individual animals. This result suggests that exposure of the vaccinated animals to self-CCR5 does not result in continuous stimulation of the CCR5-specific B cells, presumably because the cellular protein remains in a context that continues to be ignored. The parallel decline in antibodies to the viral antigen also suggests that the presence of the cellular CCR5 does not specifically attenuate the CCR5-specific response generated against the chimeric VLPs. If autoantibody induction proves safe, this approach to immunotherapy could have diverse applications. For instance, it could potentially be an effective alternative to monoclonal antibody therapy in instances in which cell surface or soluble molecules, such as HER2/neu or tumor necrosis factor α, are known to be important mediators of disease.

Table 2. Induction of CCR5 autoantibodies

Sera to	Antibody assay				
	L1 VLP ELISA	CCR5 ELISA	CCR5 FACS	HIV neutralization	BPV neutralization
L1 VLP	+	−	−	−	+
CCR5-L1 VLP	+	+	+	+	−
CCR5-L1 denatured	+	−	NT	NT	−
KLH-CCR5	−	+	−	−	NT

VLP = papillomavirus-like particle; ELISA = enzyme-linked immunosorbent assay; FACS = fluorescence-activated cell sorter; HIV = human immunodeficiency virus; BPV = bovine papillomavirus; NT = not tested; KLH = keyhole-limpet hemocyanin.

In summary, papillomavirus VLP-based vaccines are being developed according to the theory that the mammalian immune system has evolved to efficiently recognize the ordered surface of nonenveloped icosahedral virions as foreign or dangerous and to generate a variety of potent immune responses to them. Therefore, vaccines that mimic the outer structural features of virions should be highly antigenic. This concept is strongly supported by the studies described in this report. Low-dose vaccination with VLPs induced both high-titer antibodies and CTLs to viral antigens without the addition of an adjuvant (Fig. 1). Even high-titer antibodies to a central self-antigen could be generated when it was presented in the context of a VLP. Given their potential for safety, it is likely that a number of clinical trials for this type of subunit vaccine will be conducted in the future.

References

(1) International Agency for Research on Cancer. Monograph on Cancer, Human Papillomavirus. Vol. 64. Geneva (Switzerland): World Health Organization; 1995.

(2) Frazer I. Strategies for immuoprophylaxis and immunotherapy of papillomaviruses. Clin Dermatol 1997;15:285–97.
(3) Schiller J, Lowy D. Papillomavirus-like particles and HPV vaccine development. Semin Cancer Biol 1996;7:373–82.
(4) Robbins JB, Schneerson R, Szu SC. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J Infect Dis 1995;171:1387–98.
(5) Hagensee M, Galloway D. Growing human papillomaviruses and virus-like particles in the laboratory. Papillomavirus Report 1993;4:121–4.
(6) Lin YL, Borenstein LA, Selvakumar R, Ahmed R, Wettstein FO. Effective vaccination against papilloma development by immunization with L1 or L2 structural protein of cottontail rabbit papillomavirus. Virology 1992;187: 612–9.
(7) Kirnbauer R, Booy F, Cheng N, Lowry DR, Schiller JT. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci U S A 1992;89:12180–4.
(8) Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, et al. Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 1995;69:3959–63.
(9) Christensen ND, Reed CA, Cladel NM, Han R, Kreider JW. Immunization with viruslike particles induces long-term protection of rabbits against challenge with cottontail rabbit papillomaviruses. J Virol 1996;70:960–5.
(10) Jansen KU, Rosolowsky M, Schultz LD, Markus HZ, Cook JC, Donnelly JJ, et al. Vaccination with yeast-expressed cottontail rabbit papillomavirus (CRPV) virus-like particles protects rabbits from CRPV-induced papilloma formation. Vaccine 1995;13:1509–14.
(11) Suzich JA, Ghim S, Palmer-Hill FJ, White WI, Tomura JK, Bell JA, et al. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc Natl Acad Sci U S A 1995;92:11553–7.
(12) Kirnbauer R, Chandrachud L, O'Neil B, Wagner ER, Grindlay GJ, Armstrong A, et al. Virus-like particles of bovine papillomavirus type 4 in prophylactic and therapeutic immunization. Virology 1996;219:37–44.
(13) Ott G, Barchfeld GL, Van Nest G. Enhancement of humoral response against human influenza vaccine with the simple submicron oil/water emulsion adjuvant MF59. Vaccine 1995;13:1557–62.
(14) Roden RB, Greenstone HL, Kirnbauer R, Booy FP, Jessie J, Lowy DR, et al. *In vitro* generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. J Virol 1996;70:5875–83.
(15) Christensen ND, Kirnbauer R, Schiller JT, Ghim SJ, Schlegal R, Jenson AB, et al. Human papillomavirus types 6 and 11 have antigenically distinct strongly immunogenic conformationally dependent neutralizing epitopes. Virology 1994;205:329–35.
(16) Roden RB, Hubbert NL, Kirnbauer R, Christensen ND, Lowy DR, Schiller JT. Assessment of the serological relatedness of genital human papillomaviruses by hemagglutination inhibition. J Virol 1996;70:3298–301.
(17) Unckell F, Streeck RE, Sapp M. Generation and neutralization of pseudovirions of human papillomavirus type 33. J Virol 1997;71:2934–9.
(18) White WI, Wilson SD, Bonnez W, Rose RC, Koenig S, Suzich JA. *In vitro* infection and type-restricted antibody-mediated neutralization of authentic human papillomavirus type 16. J Virol 1998;72:959–64.
(19) Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide prospective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796–802.
(20) Greer CE, Wheeeler CM, Lander MB, Beutner K, Coyne MY, Liang H, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particle in patients with genital warts. J Clin Microbiol 1995;33:2058–63.
(21) De Bruijn ML, Greenstone HL, Vermeulen H, Melief CJ, Lowy DR, Schiller JT, et al. L1-specific protection from tumor challenge elicited by HPV16 virus-like particles. Virology 1998;250:371–6.
(22) Laimins LA. The biology of human papillomaviruses: from warts to cancer. Infect Agents Dis 1993;2:74–86.
(23) Da Silva D, Velders MP, Rudolf MP, Schiller JT, Kast WM. Papillomavirus virus-like particles as anticancer vaccines. Curr Opin Mol Ther 1999; 1:82–8.
(24) Greenstone HL, Nieland JD, de Visser KE, De Bruijn ML, Kirnbauer R, Roden RB, et al. Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. Proc Natl Acad Sci U S A 1998;95:1800–5.
(25) Peng S, Frazer IH, Fernando GJ, Zhou J. Papillomavirus-like particles can deliver defined CTL epitopes to the MHC class I pathway. Virology 1998; 240:147–57.
(26) Schafer K, Muller M, Faath S, Henn A, Osen W, Zentgraf H, et al. Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumor protection. Int J Cancer 1999;81:881–8.
(27) Nieland JD, Da Silva DM, Velders MP, de Visser KE, Schiller JT, Muller M, et al. Chimeric papillomavirus virus-like particles induce a murine self-antigen-specific protective and therapeutic antitumor immune response. J Cell Biochem 1999;73:145–52.
(28) Bachmann MF, Rohrer UH, Kundig TM, Burki K, Hengartner H, Zinkernagel RM. The influence of antigen organization on B cell responsiveness. Science 1993;262:1448–51.
(29) Chackerian B, Lowy DR, Schiller JT. Induction of autoantibodies to mouse CCR5 with recombinant papillomavirus particles. Proc Natl Acad Sci U S A 1999;96:2373–8.
(30) Liu R, Paxton WA, Choe S, Ceradini D, Martin SR, Horuk R, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86:367–77.
(31) Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722–5.

NOTES

Presented at the Third National AIDS Malignancy Conference.

The NIH clinical trials discussed here are supported by the NIH Office of Research on Women's Health and the NIH Office of Research on Minority Health.