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Papillomavirus virus-like particles as vehicles for the delivery of epitopes or genes

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Summary. Papillomaviruses (PVs) are simple double-strand DNA viruses whose virion shells are T = 7 icosahedrons and composed of major capsid protein L1 and minor capsid protein L2. L1 alone or together with L2 can self-assemble into virus-like particles (VLPs) when expressed in eukaryotic or prokaryotic expression systems. Although the VLPs lack the virus genome DNA, their morphological and immunological characteristics are very similar to those of nature papillomaviruses. PV VLP vaccination can induce high titers of neutralizing antibodies and can effectively protect animals or humans from PV infection. Moreover, PV VLPs have been good candidates for vehicles to deliver epitopes or genes to target cells. They are widely used in the fields of vaccine development, neutralizing antibody detection, basic virologic research on papillomaviruses, and human papillomavirus (HPV) screening. Besides the structural biology and immunological basis for PV VLPs used as vehicles to deliver epitopes or genes, this review details the latest findings on chimeric papillomavirus VLPs and papillomavirus pseudoviruses, which are two important forms of PV VLPs used to transfer epitopes or genes.

Abbreviations

PV Papillomavirus; *VLPs* virus-like particles; *cVLPs* chimeric virus-like particles; *HPV* human papillomavirus; *DCs* dendritic cells; *LCs* Langerhans cells; *IL* interleukin; *TNF* tumor necrosis factor; *INF* interferon; *CTLs* cytotoxic T lymphocytes

Introduction

Papillomaviruses (PVs), members of the family *Papillomaviridae*, are simple double-strand DNA viruses that infect squamous or mucosal epithelia and produce

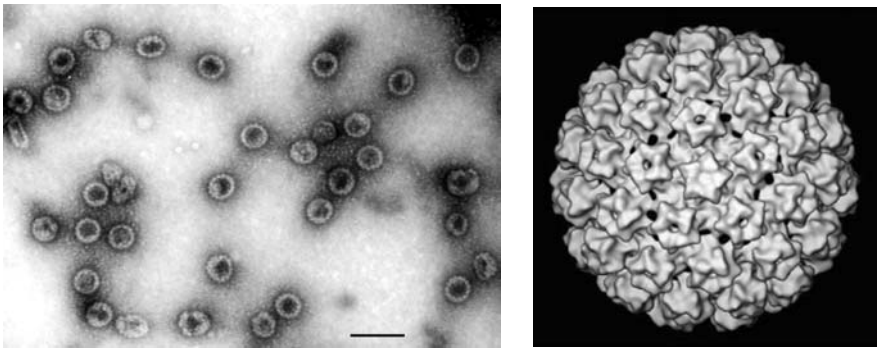
a range of epithelial neoplasms, both benign and malignant, in most animals and humans. Until now, 118 PV types have been completely described, and many new types have been detected by preliminary data such as subgenomic amplicons. More than 80 human papillomavirus (HPV) types have been defined, and many of these HPV types are ubiquitous and globally distributed (http://hpv-web.lanl.gov/stdgen/virus/cgi-bin/hpv_organisms.cgi?dbname=hpv). The detailed information on PV classification has been reviewed by de Villers EM et al. [15]. The papillomavirus virion shells consist of L1 protein (the major capsid protein) and L2 protein (the minor capsid protein). L1 protein alone or together with L2 protein can self-assemble into virus-like particles (VLPs) when expressed in eukaryotic or prokaryotic expression systems. zur Hausen et al. [95] observed that infection with HPVs might be responsible for human cervical cancer, which was confirmed by numerous subsequent studies. Because cervical cancer is the second-most common cancer in women, more attention has been paid to HPV research, especially on PV VLP-based epitope or gene delivery [73, 82], effective prophylactic and therapeutic vaccine development [28, 58], the life cycle of PVs [17], the mechanism of virus evasion from host-cell control [96], and the epidemiology of HPV infections [2, 53]. We will focus on the structural biology and immunological characteristics of PV VLPs and the latest studies on chimeric papillomavirus VLPs and papillomavirus pseudoviruses, which are two important forms of PV VLPs used as vehicles to deliver epitopes or genes.

Structural biology and immunological characteristics of papillomavirus VLPs

Structural biology characteristics of papillomavirus VLPs

The papillomavirus virion contains 72 pentamers of L1 protein, centered on the vertices of a $T = 7$ icosahedral lattice [83], while L2 protein is present at about 1/30 of the abundance of L1 [38]. The diameter of the PV particles is about 50–60 nm. Although the PV VLPs lack the virus genome DNA, they are also composed of 72 pentamers of L1 protein, and their morphological characterstics are very similar to those of the PV virions as well (Fig. 1).

Because it is very difficult to obtain large amounts of PV viruses by *in vitro* culture and to obtain suitable crystals of PV virons or VLPs, the crystallographic study of PV particles has been limited. Chen et al. [13] truncated the 10 N-terminal residues of HPV16 L1 to overcome the irregularity of the shells formed by the intact L1 for successful crystallization and expressed the truncated HPV16 L1 in *E. coli*. The truncated L1 could assemble into a 12-pentamer, $T = 1$ icosahedron, named "small VLP", whose outer diameter was 318 Å , and the X-ray crystallographic analysis was performed at 3.5 Å resolution. The L1 monomer contains 12 β-strands, 6 loops, and 5 helices (Fig. 2). These loops extend towards the outer surface of the capsid, and the loop sequences are quite variable among different papillomavirus types [13]. Mutagenesis of the hypervariable loop domains has revealed the binding sites of several neutralizing monoclonal antibodies against



A B

Fig. 1. Structure of papillomaviruses (PVs) and PV virus-like particles (VLPs). **A**. Electron micrograph of negative-stained human papillomavirus (HPV)16 L1 VLPs with 1% uranyl acetate solution. Bar represents 100 nm. **B**. The structure model of bovine papillomavirus(BPV) [83]

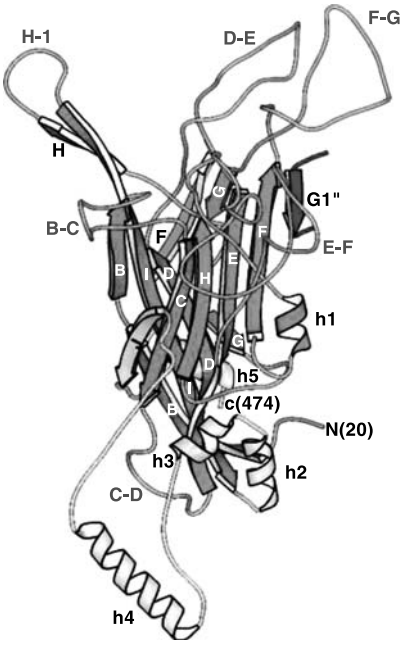


Fig. 2. The human papillomavirus (HPV) 16 L1 monomer structure [13]. Secondary structural elements are labeled, with letters *B–J* for β-strands and h1–h5 for the 5 α-helices. Loops between strands are labeled *B–C*, *C–D*, etc. The first and the last residues are marked N (20) and C (474), respectively. A primed label (*F′*) denotes a segment of polypeptide chain from the clockwise neighbor within the pentamer; a double-primed label (*G″*), one from the anticlockwise neighbor

HPV11 [47–49] or HPV6 [50], showing that many neutralizing epitopes lie in the loop domains.

Immunological characteristics of papillomavirus VLPs

Though papillomavirus VLPs lack the virus genome DNA, they are structurally and immunologically similar to the infectious viruses as judged by electron microscopic imaging studies and their ability to bind type-specific and conformation-dependent monoclonal antibodies. Many studies have demonstrated that

vaccination with PV VLPs by different methods can induce high titers of type-specific neutralizing antibodies (IgG and IgA) and effectively protect animals [5, 37, 38, 41, 44, 49, 57, 60, 79] and humans [1, 6, 7, 19, 21, 27, 32, 40, 54, 64, 65, 71, 87, 99] from papillomaviruses infection. The phase II randomized, multicentre, double-blind placebo-controlled clinical trials of bivalent (HPV16 and HPV18) VLPs vaccine and quadrivalent (HPV6, 11, 16 and 18) VLPs vaccine, which were conducted by GlaxoSmithKline and Merck, respectively, have been finished. Both vaccines were very effective in protecting women against incident and persistent HPV infections [32, 87]. What's more, since the VLPs do not contain the viral genome, they are not harmful. So PV VLPs are considered good candidates for both prophylactic and therapeutic vaccines against PV infection. Besides their safety and high immunogenicity, the following characteristics make PV VLPs good candidates for vehicles to deliver epitopes or genes.

Papillomavirus VLPs themselves are good "adjuvants" and can induce strong T-helper cell responses. A lot of studies have demonstrated that PV VLPs can activate dendritic cells (DCs) [3, 42, 70, 90–93]. DCs are a family of professional antigen-presenting cells (APCs) with a unique capacity to initiate and modulate cell-mediated immune responses. A wide range of stimuli, such as infectious virus and inflammatory cytokines, can induce DC maturation, which is associated with the up-regulation of co-stimulatory molecules (reviewed in [39, 66]). The activated DCs then migrate to regional lymph nodes and induce T cell activation. Immature DCs can efficiently bind and rapidly internalize PV VLPs, which is followed by DC maturation. DCs activated by PV VLPs can induce the secretion of the proinflammatory cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α, IL-8, and interferon(INF)-α [42, 43]. Although Langerhans cells (LCs) can effectively bind and take up HPV VLPs, they can not be activated by HPV VLPs [22–24]. But a recent study showed that heterologous PV VLPs immune complexes could activate human Langerhans cells [25]. Both DCs and LCs incubated with the VLPs immune complexes could up-regulate surface activation markers and increase secretion of IL-12 p70 and IL-15. So both DCs and LCs can be activated by PV VLPs using certain immunization strategies. In addition, papillomavirus VLPs could bind to a wide variety of cell types [51, 88] through certain molecules, such as CD16 [90], heparan sulfate [14, 18, 30, 34, 68], and α_6 integrin [20, 94]. Therefore, the epitopes or genes encapsidated in the VLPs will be well delivered to certain immunocytes.

Chimeric papillomavirus VLPs

Chimeric papillomavirus VLPs, which means PV VLPs composed of capsid proteins (L1 or L1/L2) fused with foreign epitopes or polypeptides in certain areas, are one of the important forms of PV VLPs used to deliver epitopes or polypeptides. Some of the different strategies for construction of chimeric PV VLPs are summarized in Table 1.

It is clear that mutants of PV L1 with some residues in the C-terminus truncated can also self-assemble into VLPs [59]. As a result, epitopes or short polypeptides,

Table 1. Different construction strategies of chimeric papillomavirus VLPs

Construction strategy	Epitopes or polypeptides	References
Fusing epitopes or polypeptides to the C terminus of the C-terminal truncated PVs L1	HPV16 E7 (partial and full length gene)*	[29, 52, 74]
	HPV16 E7 CTL epitope (RAHYNIVTF)	[62]
	HIV-IIIB gp160 CTL epitope (RGPGRAFVTI)	[62]
	A fusion polypeptide containing HPV16 E7 B cell epitope (QAEPD), HPV16 E7 CTL epitope (RAHYNIVTF), HIV-IIIB gp120 CTL epitope (RGPGRAFVTI), HIV-IIIB Nef CTL epitope (PLTFGWCFKL), and HIV-IIIB RT CTL epitope (VIYQYMDDL)	[45]
	P1A epitope (LPYLGWLVF)	[55]
Replacing certain sequences of PVs L1 by epitopes or polypeptides	HPV16 E7 (partial and full length gene)	[52]
	SV40 tag	[52]
	HPV16 L2 (LVEETSFIDAGAP)	[86]
	HIV-1 gp41 B cell epitope (LELDKWAS)	[76]
	HIV-1 gp41 epitope (ELDKWA)	[98]
	Mouse CCR5 epitope (HYAANEWVFGNIMCKV)	[10]
Inserting epitopes or polypeptides into some areas of the PVs L1	HPV16 $E7_{1-50}$	[52]
	HBc epitope (DPASRE)	[72]
	HIV-1 gp41 B cell epitope (LELDKWAS)	[76]
Fusing epitopes or polypeptides to the C terminus of PV L2	HPV16 E7 (partial length gene)	[16, 25, 31, 70, 89]
Replacing certain sequences of PVs L2 by epitopes or polypeptides	HPV16 E1E2E7 fusion protein	[81]

PVs papillomaviruses, *HPV* human papillomavirus, *CTLs* cytotoxic T lymphocytes, *HIV* human immunodeficiency virus, *HBc* hepatitis B core antigen

*The description or amino acid sequence of the polypeptide is parenthesized

up to 60 amino acids, can be fused to the C-terminus of the truncated PV L1 without disrupting the assembly of VLPs [52]. Until now, many epitopes or short polypeptides have been added to the C-terminus of the truncated PV L1 (see Table 1), and these chimeric VLPs (cVLPs) can induce good immune responses against not only the epitopes or polypeptides but also the VLP shells. Because the size of epitopes or polypeptides fused to the C-terminus of the truncated PV L1 is limited, large proteins cannot be delivered using this strategy. However, these large proteins can be delivered well by replacing certain areas of L1 or L2 that are not important for VLPs assembly, or by fusing them to the C-terminus of L2 (see Table 1). L1 is the major capsid protein, and it has been shown that some residues in the L1 protein, such as Asp_{202}, Cys_{175}, and Cys_{428} of HPV16 L1, are very important for VLP formation. Therefore, those important residues should not be included in the target replacing area. However, there may be a few potent important residues in L1. When some areas of L1 were replaced by large proteins, the efficiency of VLP assembly has been reduced to some extent [76]. While L2

is the minor capsid protein, it is not as important as L1 for VLPs assembly. So replacing some sequences of L2 by large genes or fusing large proteins to the C-terminus of L2 may have less influence on VLP formation than that of L1. Tobery et al. [81] showed that replacing the area of HPV16 $L2_{70-390}$ by HPV16E1E2E7, approximately 130 kDa, did not disrupt VLP assembly and that the chimeric VLPs had good immunogenicity. In addition, co-expression of L1 and L2 can enhance the efficiency of VLP formation compared with L1 alone [38]. However, in HPV16 VLPs the ratio of L1 to L2 molecules is about 30:1 [38], that is to say an L1-epitope fusion would theoretically result in 360 copies of the epitopes being delivered to the cytosol of an APC, compared to the delivery of 12 copies of the epitopes by an L2-epitope fusion.

Besides the construction strategies described above, there is another L1-loop-based construction strategy. Structural biology research on PV VLPs has shown that the loop regions of L1 are exposed on the outer surface of VLPs. Some epitopes fused into these loop regions by insertion or replacement might be displayed on the outer surface of cVLPs, so these epitopes could be efficiently delivered and well recognized by the immunocytes, and could induce epitope-specific immune responses [72] (Table 1).

Given that cervical cancer is one of the leading causes of female cancer mortality worldwide, especially in developing countries, many studies are focused on the development of effective prophylactic and therapeutic vaccines. It has been well demonstrated that expression of early protein E7 is required for the maintenance of the proliferative state of HPV-infected cells [80, 85]. E7 is considered a tumor antigen and a potential target for activated T cells in the development of strategies for immune therapy, and many forms of E7 vaccines, such as purified protein; DNA vaccine; recombinant vaccinia virus and chimeric VLPs, have been developed as well. PV L1E7 or L1/L2E7 cVLPs are considered candidates for "dual-purpose" vaccines inducing neutralizing antibodies, which prevent infection, and cytotoxic T lymphocytes (CTLs) that can eradicate established infections. Many studies showed that immunization of a few different forms of L1E7 or L1/L2E7 cVLPs could induce both strong CTL responses and high-titer, type-specific neutralizing antibodies against the VLP shells (see the related references listed in Table 1).

Papillomavirus pseudoviruses

Besides the chimeric papillomavirus VLPs, papillomavirus pseudoviruses are another important form to deliver genes using PV VLPs shells. Although the DNA molecules encapsidated in PV pseudoviruses are not PV genome DNA, PV pseudoviruses have very similar structural and immunological characteristics to native PVs. PV pseudoviruses could be produced by the following methods illustrated in Fig. 3. Firstly, PV pseudoviruses could be generated by a cell-free system *in vitro* [36, 56, 75, 82, 97] (Fig. 3A). PV VLPs were able to package unrelated plasmid containing target genes by the following process: The purified PV VLPs could be disassembled by different methods, such as incubation of VLPs with 5% 2-mercaptoethanol at 4 °C for 16 h or with 50 mM Tris–HCl buffer

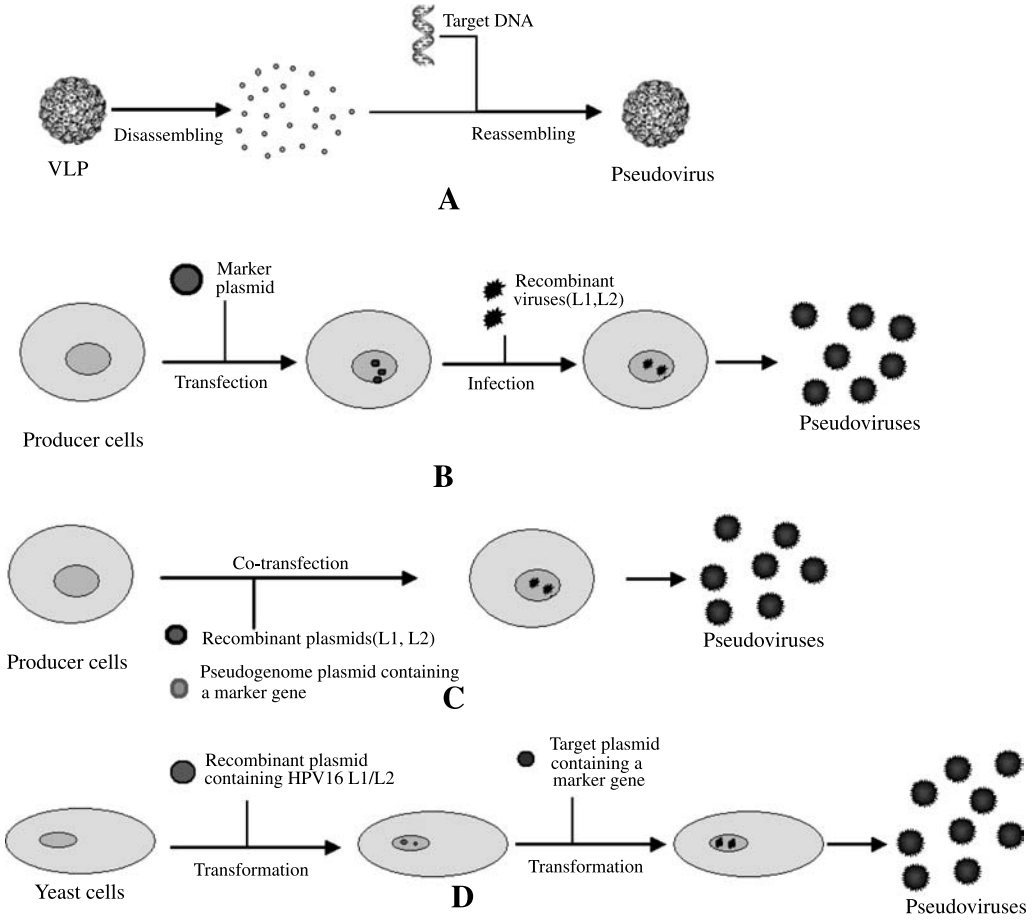


Fig. 3. Different strategies of producing papillomavirus (PV) pseudoviruses. **A**. Using a cell-free system to produce pseudoviruses *in vitro*. Purified PV virus-like particles (VLPs) were disrupted under certain conditions. The disassembled VLPs were co-cultured with the plasmids containing target genes and could reassemble into pseudoviruses. **B**. Recombinant-virus-based production of pseudoviruses. The producer cells were transfected by the plasmid containing one marker gene. Then the transfected cells were infected by recombinant viruses containing capsid proteins L1 or L1/L2, which was followed by the formation of pseudoviruses. **C**. Producer cells were co-transfected by two recombinant plasmids containing capsid proteins L1 and L2, respectively, and the pseudogenome plasmid containing a marker gene, then the pseudoviruses were produced by the transfected producer cells. **D**. Producing pseudoviruses in *Saccharomyces cerevisiae*. Yeast cells were transformed with a recombinant plasmid containing HPV16 L1/L2 and subsequently with the target plasmid containing a marker gene. Pseudoviruses then could be generated by yeast cells transformed with both plasmids

(pH 7.5) containing 150 mM NaCl, 1 mM EGTA and 20 mM dithiothreitol at room temperature for 30 min. Then the plasmid containing the target gene was co-cultured with disassembled VLPs. The preparation was then incubated with $CaCl_2$ (25 mM) and 20% dimethyl sulfoxide at room temperature for 1 h to form

the pseudoviruses. The second method to produce pseudoviruses was based on certain recombinant viruses, and is illustrated in Fig. 3B. HPV16 pseudoviruses were generated in hamster BPHE-1 cells by using a recombinant Semliki Forest virus [67], and those of HPV 33 and BPV 1 were produced in COS-7 or COS-1 cells by using a recombinant vaccinia virus [73, 84, 46, 100, 101]. The third method has been reported very recently and it seems to be a better method with higher production efficiency [8, 9, 61] (Fig. 3C). Briefly, producer cells, including C127, HaCaT, and 293TT, were co-transfected by two recombinant plasmids containing L1 and L2, respectively, and the pseudogenome plasmid containing a marker gene, then the pseudoviruses were produced by the transfected producer cells. Buck et al. [8] showed that the titers of pseudoviruses produced by this method could reach the level of several billion transducing units per milliliter, which was at least 10-million-fold greater than that of the previous method [67]. Finally, infectious HPV16 peseudoviruses could also be generated in yeast using the two-step-transformation method [69] (Fig. 3D). *Saccharomyces cerevisiae* strain 1699 was transformed with an yeast expression plasmid containing HPV16 L1/L2 so that it could produce HPV16 VLPs, which was followed by being transformed with a target plasmid containing the green fluorescent protein (GFP) gene as a marker gene. Subsequently, the yeast cells transformed with both plasmids could generate HPV16 pseudoviruses.

Although PV VLPs composed of L1 alone can package unrelated plasmid DNA *in vitro* [4], the efficiency is enhanced to a great extent by co-expressing L1 and L2 in the procedure of pseudoviruses production [36, 67, 84, 100]. In addition, Zhao et al. showed [101] that BPV1 E2 protein could enhance the packaging of plasmid DNA into BPV1 pseudoviruses. Psuedogenome encapsidation within L1/L2 capsids was largely sequence-independent, and plasmids completely lacking PV sequences could be packaged efficiently, providing they were less than 8 kb in size. PV pseudoviruses were structurally indistinguishable from native viruses and could efficiently infect many cell types [82]. Moreover, these infections of PV pseudoviruses could be inhibited by type-specific antiserum against the same type of PV VLPs [36, 67, 82, 84]. So, if the plasmids encapsidated by the PV pseudoviruses contained a certain marker gene, such as GFP [73, 82, 69], β-galactosidase [82, 36, 84, 77], or secreted alkaline phosphatase (SEAP) [8, 61, 84], these pseudoviruses could be used to analyze the papillomavirus internalization pathways, to detect the level of neutralizing antibodies, and to test the reactivity of human sera in population-based HPV screening. What's more, Pastrana et al. [61] showed that the pseudovirus-based papillomavirus neutralization assay was high-throughput and appeared to be as sensitive as, but more specific than, a standard VLP-based enzyme-linked immunosorbent assay (ELISA).

It is clear that using appropriate adjuvants can enhance vaccine-induced specific immune responses. Oh et al. [56] showed that the mucosal and systemic immunogenicity of HPV16 VLPs could be enhanced by encapsidating IL-2 gene adjuvant. Vaccination of the pseudoviruses encapsidaed with IL-2 gene could elicit much higher titers of vaginal and salivary HPV16 L1-specific IgA and serum IgG,

IgG_1, and IgG_{2a} antibodies than those of HPV16 VLPs alone. In addition, PV pseudoviruses encoding IL-2 could fully restore mucosal and systemic immune responses to vaccinations in aged mice [26]. In aged mice, immunization with PV pseudoviruses encapsidating the IL-2 gene could activate specific Th cells, induce specific antibodies and CTL responses, and protect against mucosal viral challenge.

PV VLPs were shown to induce strong T-helper responses and the concurrent T-helper responses to the VLPs might enhance the CTL responses against the antigen encoded by the plasmid in the pseudoviruses. Shi et al. [75] showed that PV pseudoviruses could efficiently pseudoinfect mucosal and system lymphoid tissues and reach Peyer's patches, lamina propria, and the spleen when administrated orally. By systemic immunization, PV pseudoviruses induced stronger CTL responses than plasmid DNA vaccines alone, and by oral immunization, pseudoviruses could generate specific mucosal and systemic CTL responses and protect mice against mucosal challenge. Women infected with human immunodeficiency virus (HIV) are at a greater risk of being co-infected with HPV. HIV-positive women have a 3–5 times greater risk of developing intraepithelial lesions and a 3–4.5 times greater risk of developing invasive neoplasia [63]. Zhang et al. [97] showed that oral immunization with PV pseudoviruses encoding HIV-1 Gag could induce mucosal and systemic Gag-specific CTL responses which could effectively lyse Gag-expressing target cells. Furthermore, pseudovirus vaccination could generate Gag-specific IFN-γ-producing T cells, serum IgG, and mucosal IgA. It would seem that PV pseudoviruses may be better candidates for prophylactic and therapeutic vaccines to some infectious diseases.

In summary, PV pseudoviruses are valuable tools for determination of neutralizing antibody and analysis of papillomavirus internalization pathways and are good candidates for vaccine development.

Conclusions

Papillomavirus VLPs are good vehicles for the efficient delivery of epitopes or genes to target cells. Now, chimeric papillomavirus VLPs and papillomavirus pseudoviruses have been widely used in the fields of basic virological studies of papillomavirus, development of various vaccines, neutralizing antibody detection, and population-based HPV screening. In order to enhance vaccine immunogenicity, there are still other strategies based on PV VLPs, such as fusing adjuvant molecules to the capsid proteins to form chimeric VLPs (our unpublished data), encapsidating nonmethylated CpG motifs into VLPs derived from the hepatitis B core antigen or the bacteriophage Qβ [78], chemically binding adjuvant molecules (subunit B of cholera toxin [35]) or antigenic peptides (TNF-α [11, 12], epitope of influenza type A M2 protein [33]) to PV VLPs. What's more, DNA molecules can directly bind to PV VLPs to form a VLP/DNA complex, which is another way to transfer genes using PV VLPs [4]. It is expected that more types of PV VLP-based vaccines with higher immunogenicity and better epitopes or gene delivery systems will be developed in the future.

Acknowledgments

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References

1. Ault KA, Giuliano AR, Edwards RP, Tamms G, Kim LL, Smith JF, Jansen KU, Allende M, Taddeo FJ, Skulsky D, Barr E (2004) A phase I study to evaluate a human papillomavirus (HPV) type 18 L1 VLP vaccine. Vaccine 22: 3004–3007
2. Baseman JG, Koutsky LA (2005) The epidemiology of human papillomavirus infections. J Clin Virol 32 [Suppl 1]: S16–S24
3. Bontkes HJ, Ruizendaal JJ, Kramer D, Meijer CJ, Hooijberg E (2005) Plasmacytoid dendritic cells are present in cervical carcinoma and become activated by human papillomavirus type 16 virus-like particles. Gynecol Oncol 96: 897–901
4. Bousarghin L, Combita-Rojas AL, Touze A, El Mehdaoui S, Sizaret PY, Bravo MM, Coursaget P (2002) Detection of neutralizing antibodies against human papillomaviruses (HPV) by inhibition of gene transfer mediated by HPV pseudovirions. J Clin Microbiol 40: 926–932
5. Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G, Schiller JT, Lowy DR (1995) Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. J Virol 69: 3959–3963
6. Brown DR, Bryan JT, Schroeder JM, Robinson TS, Fife KH, Wheeler CM, Barr E, Smith PR, Chiacchierini L, DiCello A, Jansen KU (2001) Neutralization of human papillomavirus type 11 (HPV-11) by serum from women vaccinated with yeast-derived HPV-11 L1 virus-like particles: correlation with competitive radioimmunoassay titer. J Infect Dis 184: 1183–1186
7. Brown DR, Fife KH, Wheeler CM, Koutsky LA, Lupinacci LM, Railkar R, Suhr G, Barr E, Dicello A, Li W, Smith JF, Tadesse A, Jansen KU (2004) Early assessment of the efficacy of a human papillomavirus type 16 L1 virus-like particle vaccine. Vaccine 22: 2936–2942
8. Buck CB, Pastrana DV, Lowy DR, Schiller JT (2004) Efficient intracellular assembly of papillomaviral vectors. J Virol 78: 751–757
9. Buck CB, Thompson CD, Pang YY, Lowy DR, Schiller JT (2005) Maturation of papillomavirus capsids. J Virol 79: 2839–2846
10. Chackerian B, Lowy DR, Schiller JT (1999) Induction of autoantibodies to mouse CCR5 with recombinant papillomavirus particles. Proc Natl Acad Sci USA 96: 2373–2378
11. Chackerian B, Lowy DR, Schiller JT (2001) Conjugation of a self-antigen to papillomavirus-like particles allows for efficient induction of protective autoantibodies. J Clin Invest 108: 415–423
12. Chackerian B, Lenz P, Lowy DR, Schiller JT (2002) Determinants of autoantibody induction by conjugated papillomavirus virus-like particles. J Immunol 169: 6120–6126
13. Chen XS, Garcea RL, Goldberg I, Casini G, Harrison SC (2000) Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. Mol Cell 5: 557–567
14. Combita AL, Touze A, Bousarghin L, Sizaret PY, Munoz N, Coursaget P (2001) Gene transfer using human papillomavirus pseudovirions varies according to virus genotype and requires cell surface heparan sulfate. FEMS Microbiol Lett 204: 183–188

15. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004) Classification of papillomaviruses. Virology 324: 17–27
16. Da Silva DM, Schiller JT, Kast WM (2003) Heterologous boosting increases immunogenicity of chimeric papillomavirus virus-like particle vaccines. Vaccine 21: 3219–3227
17. Doorbar J (2005) The papillomavirus life cycle. J Clin Virol 32 [Suppl 1]: S7–S15
18. Drobni P, Mistry N, McMillan N, Evander M (2003) Carboxy-fluorescein diacetate, succinimidyl ester labeled papillomavirus virus-like particles fluoresce after internalization and interact with heparan sulfate for binding and entry. Virology 310: 163–172
19. Emeny RT, Wheeler CM, Jansen KU, Hunt WC, Fu TM, Smith JF, MacMullen S, Esser MT, Paliard X (2002) Priming of human papillomavirus type 11-specific humoral and cellular immune responses in college-aged women with a virus-like particle vaccine. J Virol 76: 7832–7842
20. Evander M, Frazer IH, Payne E, Qi YM, Hengst K, McMillan NA (1997) Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. J Virol 71: 2449–2456
21. Evans TG, Bonnez W, Rose RC, Koenig S, Demeter L, Suzich JA, O'Brien D, Campbell M, White WI, Balsley J, Reichman RC (2001) A Phase 1 study of a recombinant viruslike particle vaccine against human papillomavirus type 11 in healthy adult volunteers. J Infect Dis 183: 1485–1493
22. Fausch SC, Da Silva DM, Rudolf MP, Kast WM (2002) Human papillomavirus virus-like particles do not activate Langerhans cells: a possible immune escape mechanism used by human papillomaviruses. J Immunol 169: 3242–3249
23. Fausch SC, Da Silva DM, Kast WM (2003) Differential uptake and cross-presentation of human papillomavirus virus-like particles by dendritic cells and Langerhans cells. Cancer Res 63: 3478–3482
24. Fausch SC, Fahey LM, Da Silva DM, Kast WM (2005) Human papillomavirus can escape immune recognition through Langerhans cell phosphoinositide 3-kinase activation. J Immunol 174: 7172–7178
25. Fausch SC, Da Silva DM, Kast WM (2005) Heterologous papillomavirus virus-like particles and human papillomavirus virus-like particle immune complexes activate human Langerhans cells. Vaccine 23: 1720–1729
26. Fayad R, Zhang H, Quinn D, Huang Y, Qiao L (2004) Oral administration with papillomavirus pseudovirus encoding IL-2 fully restores mucosal and systemic immune responses to vaccinations in aged mice. J Immunol 173: 2692–2698
27. Fife KH, Wheeler CM, Koutsky LA, Barr E, Brown DR, Schiff MA, Kiviat NB, Jansen KU, Barber H, Smith JF, Tadesse A, Giacoletti K, Smith PR, Suhr G, Johnson DA (2004) Dose-ranging studies of the safety and immunogenicity of human papillomavirus Type 11 and Type 16 virus-like particle candidate vaccines in young healthy women. Vaccine 22: 2943–2952
28. Frazer IH (2004) Prevention of cervical cancer through papillomavirus vaccination. Nat Rev Immunol 4: 46–54
29. Freyschmidt EJ, Alonso A, Hartmann G, Gissmann L (2004) Activation of dendritic cells and induction of T cell responses by HPV 16 L1/E7 chimeric virus-like particles are enhanced by CpG ODN or sorbitol. Antivir Ther 9: 479–489
30. Giroglou T, Florin L, Schafer F, Streeck RE, Sapp M (2001) Human papillomavirus infection requires cell surface heparan sulfate. J Virol 75: 1565–1570
31. Greenstone HL, Nieland JD, de Visser KE, De Bruijn ML, Kirnbauer R, Roden RB, Lowy DR, Kast WM, Schiller JT (1998) Chimeric papillomavirus virus-like particles

elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. Proc Natl Acad Sci USA 95: 1800–1805
32. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A, Zahaf T, Innis B, Naud P, De Carvalho NS, Roteli-Martins CM, Teixeira J, Blatter MM, Korn AP, Quint W, Dubin G (2004) Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet 364: 1757–1765
33. Ionescu RM, Przysiecki CT, Liang X, Garsky VM, Fan J, Wang B, Troutman R, Rippeon Y, Flanagan E, Shiver J, Shi L (2006) Pharmaceutical and immunological evaluation of human papillomavirus virus like particle as an antigen carrier. J Pharm Sci 95: 70–79
34. Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU, Keller PM (1999) The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J Biol Chem 274: 5810–5822
35. Kang SM, Yao Q, Guo L, Compans RW (2003) Mucosal immunization with virus-like particles of simian immunodeficiency virus conjugated with cholera toxin subunit B. J Virol 77: 9823–9830
36. Kawana K, Yoshikawa H, Taketani Y, Yoshiike K, Kanda T (1998) In vitro construction of pseudovirions of human papillomavirus type 16: incorporation of plasmid DNA into reassembled L1/L2 capsids. J Virol 72: 10298–10300
37. Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT (1992) Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proc Natl Acad Sci USA 89: 12180–12184
38. Kirnbauer R, Taub J, Greenstone H, Roden R, Durst M, Gissmann L, Lowy DR, Schiller JT (1993) Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. J Virol 67: 6929–6936
39. Klagge IM, Schneider-Schaulies S (1999) Virus interactions with dendritic cells. J Gen Virol 80 (Pt 4): 823–833
40. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, Chiacchierini LM, Jansen KU (2002) A controlled trial of a human papillomavirus type 16 vaccine. N Engl J Med 347: 1645–1651
41. Kowalczyk DW, Wlazlo AP, Shane S, Ertl HC (2001) Vaccine regimen for prevention of sexually transmitted infections with human papillomavirus type 16. Vaccine 19: 3583–3590
42. Lenz P, Day PM, Pang YY, Frye SA, Jensen PN, Lowy DR, Schiller JT (2001) Papillomavirus-like particles induce acute activation of dendritic cells. J Immunol 166: 5346–5355
43. Lenz P, Lowy DR, Schiller JT (2005) Papillomavirus virus-like particles induce cytokines characteristic of innate immune responses in plasmacytoid dendritic cells. Eur J Immunol 35: 1548–1556
44. Liu WJ, Gissmann L, Sun XY, Kanjanahaluethai A, Muller M, Doorbar J, Zhou J (1997) Sequence close to the N-terminus of L2 protein is displayed on the surface of bovine papillomavirus type 1 virions. Virology 227: 474–483
45. Liu WJ, Liu XS, Zhao KN, Leggatt GR, Frazer IH (2000) Papillomavirus virus-like particles for the delivery of multiple cytotoxic T cell epitopes. Virology 273: 374–382
46. Liu Y, Frazer IH, Liu WJ, Liu XS, McMillan N, Zhao KN (2001) Efficiency of delivery of DNA to cells by bovine papillomavirus type-1 L1/L2 pseudovirions. Appl Microbiol Biotechnol 56: 150–156

47. Ludmerer SW, Benincasa D, Mark GE III (1996) Two amino acid residues confer type specificity to a neutralizing, conformationally dependent epitope on human papillomavirus type 11. J Virol 70: 4791–4794
48. Ludmerer SW, Benincasa D, Mark GE III, Christensen ND (1997) A neutralizing epitope of human papillomavirus type 11 is principally described by a continuous set of residues which overlap a distinct linear, surface-exposed epitope. J Virol 71: 3834–3839
49. Ludmerer SW, McClements WL, Wang XM, Ling JC, Jansen KU, Christensen ND (2000) HPV11 mutant virus-like particles elicit immune responses that neutralize virus and delineate a novel neutralizing domain. Virology 266: 237–245
50. McClements WL, Wang XM, Ling JC, Skulsky DM, Christensen ND, Jansen KU, Ludmerer SW (2001) A novel human papillomavirus type 6 neutralizing domain comprising two discrete regions of the major capsid protein L1. Virology 289: 262–268
51. Muller M, Gissmann L, Cristiano RJ, Sun XY, Frazer IH, Jenson AB, Alonso A, Zentgraf H, Zhou J (1995) Papillomavirus capsid binding and uptake by cells from different tissues and species. J Virol 69: 948–954
52. Muller M, Zhou J, Reed TD, Rittmuller C, Burger A, Gabelsberger J, Braspenning J, Gissmann L (1997) Chimeric papillomavirus-like particles. Virology 234: 93–111
53. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348: 518–527
54. Nardelli-Haefliger D, Lurati F, Wirthner D, Spertini F, Schiller JT, Lowy DR, Ponci F, De Grandi P (2005) Immune responses induced by lower airway mucosal immunisation with a human papillomavirus type 16 virus-like particle vaccine. Vaccine 23: 3634–3641
55. Nieland JD, Da Silva DM, Velders MP, de Visser KE, Schiller JT, Muller M, Kast WM (1999) Chimeric papillomavirus virus-like particles induce a murine self-antigen-specific protective and therapeutic antitumor immune response. J Cell Biochem 73: 145–152
56. Oh YK, Sohn T, Park JS, Kang MJ, Choi HG, Kim JA, Kim WK, Ko JJ, Kim CK (2004) Enhanced mucosal and systemic immunogenicity of human papillomavirus-like particles encapsidating interleukin-2 gene adjuvant. Virology 328: 266–273
57. Orozco JJ, Carter JJ, Koutsky LA, Galloway DA (2005) Humoral immune response recognizes a complex set of epitopes on human papillomavirus type 6 l1 capsomers. J Virol 79: 9503–9514
58. Pagliusi SR, Teresa AM (2004) Efficacy and other milestones for human papillomavirus vaccine introduction. Vaccine 23: 569–578
59. Paintsil J, Muller M, Picken M, Gissmann L, Zhou J (1996) Carboxyl terminus of bovine papillomavirus type-1 L1 protein is not required for capsid formation. Virology 223: 238–244
60. Pastrana DV, Vass WC, Lowy DR, Schiller JT (2001) NHPV16 VLP vaccine induces human antibodies that neutralize divergent variants of HPV16. Virology 279: 361–369
61. Pastrana DV, Buck CB, Pang YY, Thompson CD, Castle PE, FitzGerald PC, Kruger KS, Lowy DR, Schiller JT (2004) Reactivity of human sera in a sensitive, high-throughput pseudovirus-based papillomavirus neutralization assay for HPV16 and HPV18. Virology 321: 205–216
62. Peng S, Frazer IH, Fernando GJ, Zhou J (1998) Papillomavirus virus-like particles can deliver defined CTL epitopes to the MHC class I pathway. Virology 240: 147–157
63. Pinto AP, Baggio HC, Guedes GB (2005) Sexually-transmitted viral diseases in women: clinical and epidemiological aspects and advances in laboratory diagnosis. Braz J Infect Dis 9: 241–250

64. Pinto LA, Edwards J, Castle PE, Harro CD, Lowy DR, Schiller JT, Wallace D, Kopp W, Adelsberger JW, Baseler MW, Berzofsky JA, Hildesheim A (2003) Cellular immune responses to human papillomavirus (HPV)-16 L1 in healthy volunteers immunized with recombinant HPV-16 L1 virus-like particles. J Infect Dis 188: 327–338
65. Pinto LA, Castle PE, Roden RB, Harro CD, Lowy DR, Schiller JT, Wallace D, Williams M, Kopp W, Frazer IH, Berzofsky JA, Hildesheim A (2005) HPV-16 L1 VLP vaccine elicits a broad-spectrum of cytokine responses in whole blood. Vaccine 23: 3555–3564
66. Reis e Sousa, Sher A, Kaye P (1999) The role of dendritic cells in the induction and regulation of immunity to microbial infection. Curr Opin Immunol 11: 392–399
67. Roden RB, Greenstone HL, Kirnbauer R, Booy FP, Jessie J, Lowy DR, Schiller JT (1996) In vitro generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. J Virol 70: 5875–5883
68. Rommel O, Dillner J, Fligge C, Bergsdorf C, Wang X, Selinka HC, Sapp M (2005) Heparan sulfate proteoglycans interact exclusively with conformationally intact HPV L1 assemblies: basis for a virus-like particle ELISA. J Med Virol 75: 114–121
69. Rossi JL, Gissmann L, Jansen K, Muller M (2000) Assembly of human papillomavirus type 16 pseudovirions in Saccharomyces cerevisiae. Hum Gene Ther 11: 1165–1176
70. Rudolf MP, Fausch SC, Da Silva DM, Kast WM (2001) Human dendritic cells are activated by chimeric human papillomavirus type-16 virus-like particles and induce epitope-specific human T cell responses in vitro. J Immunol 166: 5917–5924
71. Ruiz W, McClements WL, Jansen KU, Esser MT (2005) Kinetics and isotype profile of antibody responses in rhesus macaques induced following vaccination with HPV 6, 11, 16 and 18 L1-virus-like particles formulated with or without Merck aluminum adjuvant. J Immune Based Ther Vaccines 3: 2
72. Sadeyen JR, Tourne S, Shkreli M, Sizaret PY, Coursaget P (2003) Insertion of a foreign sequence on capsid surface loops of human papillomavirus type 16 virus-like particles reduces their capacity to induce neutralizing antibodies and delineates a conformational neutralizing epitope. Virology 309: 32–40
73. Sapp M, Selinka HC (2005) Pseudovirions as specific tools for investigation of virus interactions with cells. Methods Mol Biol 292: 197–212
74. Schafer K, Muller M, Faath S, Henn A, Osen W, Zentgraf H, Benner A, Gissmann L, Jochmus I (1999) Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles: induction of cytotoxic T cells and specific tumor protection. Int J Cancer 81: 881–888
75. Shi W, Liu J, Huang Y, Qiao L (2001) Papillomavirus pseudovirus: a novel vaccine to induce mucosal and systemic cytotoxic T-lymphocyte responses. J Virol 75: 10139–10148
76. Slupetzky K, Shafti-Keramat S, Lenz P, Brandt S, Grassauer A, Sara M, Kirnbauer R (2001) Chimeric papillomavirus-like particles expressing a foreign epitope on capsid surface loops. J Gen Virol 82: 2799–2804
77. Stauffer Y, Raj K, Masternak K, Beard P (1998) Infectious human papillomavirus type 18 pseudovirions. J Mol Biol 283: 529–536
78. Storni T, Ruedl C, Schwarz K, Schwendener RA, Renner WA, Bachmann MF (2004) Nonmethylated CG motifs packaged into virus-like particles induce protective cytotoxic T cell responses in the absence of systemic side effects. J Immunol 172: 1777–1785
79. Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA, Newsome JA, Jenson AB, Schlegel R (1995) Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. Proc Natl Acad Sci USA 92: 11553–11557

80. Tan TM, Ting RC (1995) In vitro and in vivo inhibition of human papillomavirus type 16 E6 and E7 genes. Cancer Res 55: 4599–4605
81. Tobery TW, Smith JF, Kuklin N, Skulsky D, Ackerson C, Huang L, Chen L, Cook JC, McClements WL, Jansen KU (2003) Effect of vaccine delivery system on the induction of HPV16L1-specific humoral and cell-mediated immune responses in immunized rhesus macaques. Vaccine 21: 1539–1547
82. Touze A, Coursaget P (1998) In vitro gene transfer using human papillomavirus-like particles. Nucleic Acids Res 26: 1317–1323
83. Trus BL, Roden RB, Greenstone HL, Vrhel M, Schiller JT, Booy FP (1997) Novel structural features of bovine papillomavirus capsid revealed by a three-dimensional reconstruction to 9 A resolution. Nat Struct Biol 4: 413–420
84. Unckell F, Streeck RE, Sapp M (1997) Generation and neutralization of pseudovirions of human papillomavirus type 33. J Virol 71: 2934–2939
85. von Knebel DM, Rittmuller C, zur Hausen H, Durst M (1992) Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6–E7 anti-sense RNA. Int J Cancer 51: 831–834
86. Varsani A, Williamson AL, de Villiers D, Becker I, Christensen ND, Rybicki EP (2003) Chimeric human papillomavirus type 16 (HPV-16) L1 particles presenting the common neutralizing epitope for the L2 minor capsid protein of HPV-6 and HPV-16. J Virol 77: 8386–8393
87. Villa LL, Costa RL, Petta CA, Andrade RP, Ault KA, Giuliano AR, Wheeler CM, Koutsky LA, Malm C, Lehtinen M, Skjeldestad FE, Olsson SE, Steinwall M, Brown DR, Kurman RJ, Ronnett BM, Stoler MH, Ferenczy A, Harper DM, Tamms GM, Yu J, Lupinacci L, Railkar R, Taddeo FJ, Jansen KU, Esser MT, Sings HL, Saah AJ, Barr E (2005) Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. Lancet Oncol 6: 271–278
88. Volpers C, Unckell F, Schirmacher P, Streeck RE, Sapp M (1995) Binding and internalization of human papillomavirus type 33 virus-like particles by eukaryotic cells. J Virol 69: 3258–3264
89. Wakabayashi MT, Da Silva DM, Potkul RK, Kast WM (2002) Comparison of human papillomavirus type 16 L1 chimeric virus-like particles versus L1/L2 chimeric virus-like particles in tumor prevention. Intervirology 45: 300–307
90. Yan M, Peng J, Jabbar IA, Liu X, Filgueira L, Frazer IH, Thomas R (2004) Despite differences between dendritic cells and Langerhans cells in the mechanism of papillomavirus-like particle antigen uptake, both cells cross-prime T cells. Virology 324: 297–310
91. Yan M, Peng J, Jabbar IA, Liu X, Filgueira L, Frazer IH, Thomas R (2005) Activation of dendritic cells by human papillomavirus-like particles through TLR4 and NF-kappaB-mediated signalling, moderated by TGF-beta. Immunol Cell Biol 83: 83–91
92. Yang R, Murillo FM, Cui H, Blosser R, Uematsu S, Takeda K, Akira S, Viscidi RP, Roden RB (2004) Papillomavirus-like particles stimulate murine bone marrow-derived dendritic cells to produce alpha interferon and Th1 immune responses via MyD88. J Virol 78: 11152–11160
93. Yang R, Murillo FM, Lin KY, Yutzy WH, Uematsu S, Takeda K, Akira S, Viscidi RP, Roden RB (2004) Human papillomavirus type-16 virus-like particles activate complementary defense responses in key dendritic cell subpopulations. J Immunol 173: 2624–2631
94. Yoon CS, Kim KD, Park SN, Cheong SW (2001) alpha(6) Integrin is the main receptor of human papillomavirus type 16 VLP. Biochem Biophys Res Commun 283: 668–673

95. zur Hausen H, de Villiers EM, Gissmann L (1981) Papillomavirus infections and human genital cancer. Gynecol Oncol 12: S124–S128
96. zur Hausen H (2002) Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2: 342–350
97. Zhang H, Fayad R, Wang X, Quinn D, Qiao L (2004) Human immunodeficiency virus type 1 gag-specific mucosal immunity after oral immunization with papillomavirus pseudoviruses encoding gag. J Virol 78: 10249–10257
98. Zhang H, Huang Y, Fayad R, Spear GT, Qiao L (2004) Induction of mucosal and systemic neutralizing antibodies against human immunodeficiency virus type 1 (HIV-1) by oral immunization with bovine Papillomavirus-HIV-1 gp41 chimeric virus-like particles. J Virol 78: 8342–8348
99. Zhang LF, Zhou J, Chen S, Cai LL, Bao QY, Zheng FY, Lu JQ, Padmanabha J, Hengst K, Malcolm K, Frazer IH (2000) HPV6b virus like particles are potent immunogens without adjuvant in man. Vaccine 18: 1051–1058
100. Zhao KN, Sun XY, Frazer IH, Zhou J (1998) DNA packaging by L1 and L2 capsid proteins of bovine papillomavirus type 1. Virology 243: 482–491
101. Zhao KN, Hengst K, Liu WJ, Liu YH, Liu XS, McMillan NA, Frazer IH (2000) BPV1 E2 protein enhances packaging of full-length plasmid DNA in BPV1 pseudovirions. Virology 272: 382–393

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