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Human Dendritic Cells Are Activated by Chimeric Human Papillomavirus Type-16 Virus-Like Particles and Induce Epitope-Specific Human T Cell Responses In Vitro

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Human papillomavirus (HPV)-derived chimeric virus-like particles (VLPs) are the leading candidate vaccine for the treatment or prevention of cervical cancer in humans. Dendritic cells (DCs) are the most potent inducers of immune responses and here we show for the first time evidence for binding of chimeric HPV-16 VLPs to human peripheral blood-derived DCs. Incubation of immature human DCs with VLPs for 48 h induced a significant up-regulation of the CD80 and CD83 molecules as well as secretion of IL-12. Confocal microscopy analysis revealed that cell surface-bound chimeric VLPs were taken up by DCs. Moreover, DCs loaded with chimeric HPV-16 L1L2-E7 VLPs induced an HLA-\*0201-restricted human T cell response in vitro specific for E7-derived peptides. These results clearly demonstrate that immature human DCs are fully activated by chimeric HPV-16 VLPs and subsequently are capable of inducing endogenously processed epitope-specific human T cell responses in vitro. Overall, these findings could explain the high immunogenicity and efficiency of VLPs as vaccines.

class I-restricted T cell responses capable of eliminating HPV-transformed cells. We reported earlier that HPV16 L1L2-VLPs induced MHC class I-restricted, HPV-capsid protein-specific human T cell responses in vitro (40). Still, these results did only prove the potential of the human immune system to mount a cellular immune response against capsid proteins, but could not explain the high immunogenic potential of VLPs. Therefore, we investigated the possibility of loading in vitro-generated human DCs with chimeric L1L2-E7 VLPs to use the loaded DCs as primary APCs in vitro. The results presented here show that human monocyte-derived DCs bind HPV-16 VLPs, thereby up-regulating activation-specific cell surface markers as well as secretion of IL-12 (p70). Additionally, human DCs did take up VLPs as seen by confocal microscopy with directly labeled VLPs. Furthermore, chimeric L1L2-E7 VLP-loaded DCs were able to induce HLA-A*0201-restricted, E7-specific T cell responses in vitro as determined by enzyme-linked immunospot (ELISPOP). Overall, the results show the binding to and activation of DCs by VLPs and explain the high immunogenicity of VLPs. Furthermore, the system allows the mapping of endogenously processed T cell epitopes derived from the fusion protein of chimeric VLPs.

Materials and Methods

Abs and reagents

Monoclonal anti-HPV16 L1 Ab as well as Abs to human CD14-FITC, CD19-FITC, CD40, CD54-FITC, CD80-FITC, CD86-FITC, CD83, Isocontrol types, streptavidin (SA)-APC, and HLA-DR, DQ, DP-FITC were purchased from BD Pharmingen (San Diego, CA), goat anti-mouse-FITC, goat anti-rabbit-HRP, and goat anti-mouse-IgG-biotinylated were purchased from BioSource (Camarillo, CA), HLA-A, B, C-PE was purchased from Dako (Glostrup, Denmark). Abs for FACS analysis were used at 1:50 dilutions in PBS containing 1% FCS and 0.01% NaN3. Polyclonal rabbit Abs and reagents were used at 1:50 dilution for Western blot for presence of L1, L2, and in the case of human DCs, AB serum containing 1000 U/ml rhu-GM-CSF. Cells were harvested, washed with PBS, and subsequently stained for FACS analysis.

Measurement of endotoxin levels and removal of endotoxins

Endotoxin levels of serial dilutions of VLP preparations were detected and semiquantitated against an endotoxin standard (Sigma) in a limulus assay (E-Toxate; Sigma) following manufacturers instructions.

Confocal microscopy

For confocal microscopy, DCs were loaded with VLP-GFP particles at a concentration of 10 μg/10^6 cells in 1 ml of PBS for 1 h at room temperature. Cells were subsequently incubated for 48 h in 15 ml of RPMI 1640 plus 5% human AB serum containing 1000 U/ml rhu-GM-CSF. Cells were harvested, washed with PBS, and subsequently stained for FACS analysis.

Activation and binding assay

DCs were collected and incubated with VLPs at a concentration of 10 μg/10^6 cells in 1 ml of PBS for 1 h at room temperature. Cells were subsequently incubated for 48 h in 15 ml of RPMI 1640 plus 5% human AB serum containing 1000 U/ml rhu-GM-CSF. Cells were harvested, washed with PBS, and subsequently stained for FACS analysis.

D C generation

Frozen PBL were thawed and washed once with RPMI 1640 containing 10 mM pyruvic acid (Life Technologies, Gaithersburg, MD), 10 mM nones- sential amino acids (Life Technologies), 100 μg/ml kanamycin (Sigma, St. Louis, MO), 5% human AB serum (Sigma), and plastic adherent cells were selected by plating 150 × 10^6 cells/ml in 175-cm² tissue culture flasks for 2 h at 37°C. The nonadherent cells were carefully washed away with PBS, and remaining adherent cells were cultured for 4 days (for activation studies) or 7 days (for binding/uptake studies) in 1000 U/ml rhu-GM-CSF and 1200 U/ml rhu-IL-4. Every other day, one-half volume medium was replaced by fresh medium supplemented with 800 U/ml rhu-GM-CSF and 1000 U/ml rhu-IL-4.

Virus-like particles

HPV-16 L1L2-VLPs were produced as described earlier (37). Each batch was tested by Western blot for presence of L1, L2, and in the case of chimeric particles, also for L2 fusion protein (E7 protein). Each batch was tested by transmission electron microscopy as described (37) for the presence of particles. For production of green fluorescence protein (GFP)-VLPs, the GFP gene was cloned from pEFG-plasmid (Clonetech, Palo Alto, CA) with the primers (GFP forward) 5’-ATGCTATGACAAAGGCAGAGG-3’ and (GFP reverse) 5’-CGGTCTAGATGTTAGGAAC-3’. The mutated L2 gene (lacking the stop codon) was cloned by PCR with the primers (L2 forward) 5’-CAGCCAAAGAGACATCTG-3’ and (L2 reverse no stop) 5’-CAGCCAAAGACTGCAAA-3’. PCR products of both reactions were subsequently subcloned into pFASTBAC from where chimeric VLP particles were produced as described previously (1). Presence of L2-GFP fusion protein in purified chimeric VLPs was confirmed by Western blot analysis with anti-GFP Ab (BD PharMingen). Abs for FACS analysis were used at 1:50 dilution for detection of GFP-positive cells at 100 x magnification.

VLP-induced activation of DCs

DCs were collected and incubated with VLPs at a concentration of 10 μg/10^6 cells for 1 h at 4°C. Cells were transferred to 37°C, and aliquots were sampled at indicated time points and immediately fixed with an equal volume of 2% paraformaldehyde solution. Fixed cells were dried overnight onto microscopic slides and subsequently treated with 0.5% Triton X-100 for 15 min and counterstained with anti-actin-Cy3 Ab (Sigma). Slides were analyzed with a Zeiss LSM-510 laser-scanning confocal microscope (Carl Zeiss, Jena, Germany) with the Ar 458/488 and HeNe 543 lasers as well as bright-field differential interference contrast. Data was analyzed by Zeiss-LSM Image Browser, version 2.3.

In vitro immunization assay

In vitro immunization assays were performed with 1.2 × 10^6 DCs loaded with 10 μg of chimeric HPV16-L1L2-E7 VLPs for 1 h at room temperature. Washed, and mixed with 2 × 10^5 nonadherent autologous cells. Cells were cultured in 48-well plates (Costar, Cambridge, MA) at 0.5 × 10^6 cells per well in RPMI 1640 plus 5% human AB serum for 7 days at 37°C. Restimulation after 7 and 14 days was done with 0.5 × 10^6 cells per well of nonadherent, autologous PBL, loaded with 10 μg/ml chimeric HPV16-L1L2-E7 VLPs for 1 h at room temperature. PBL were subsequently washed with PBS, irradiated (25 Gy), and added to the cultures. For control experiments, 1.2 × 10^6 DCs either were left untreated or loaded with 10 μg of L2E7 protein and used in control experiments described as above. Restimulation of control experiments was done with autologous PBL alone or PBL loaded with L2E7 protein and subsequently treated as described above. For restimulation, the medium was supplemented with IL-2 at 40 U/ml at 2 and 4 days after restimulation. After 28 days, the effector cells were pooled, purified over Lymphoprep (Nycomed), and tested for IFN-γ production by ELISPOP. Next, 96-well multiscreen HA plates (Millipore, Bedford, MA) were coated with 5 μg/ml anti-human IFN-γ Ab (BD Pharmingen) overnight, washed, and blocked for 4 h with RPMI 1640 plus 5% human AB serum at 37°C. Cells (250,000 per well) were incubated in the presence or absence of peptide for 40 h at 37°C. Wells were washed and

Donor material

PBL from normal, healthy HLA-A*0201-positive donors were obtained by leukapheresis. Leukocytes were purified by Ficoll gradient (Nycomed, Oslo, Norway) and stored in liquid nitrogen for further use.

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plates were incubated with biotinylated anti-human IFN-γ Ab (BD PharMingen) and SA-alkaline phosphatase (Sigma) and spots were counted after staining with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. The average of the background counts was subtracted from the average of the sample counts. Statistical analysis was performed with the Microsoft Excel program (Microsoft, Redmond, WA).

**Results**

**Binding of VLPs to human DC**

HPV-derived VLPs, either as empty particles consisting solely of capsid proteins or as chimeric particles consisting of a fusion between L2 capsid protein and either an Ag or peptides thereof, have been shown to be highly immunogenic in vivo even without the use of adjuvants. The reason for this phenomenon is not fully understood, and presumably these particles are taken up by professional APCs, which subsequently would prime immune responses. Therefore, we investigated whether human DCs generated in vitro could bind VLPs. For that purpose, we generated and purified VLPs as described (37) and found all the batches of VLPs positive for the presence of capsid proteins L1, L2 and, in the case of chimeric particles, E7 fusion protein by Western blots (data not shown). In addition, all batches of VLPs were routinely tested by transmission electron microscopy for the presence of actual particles (Fig. 1A). This quality control process is essential because we have shown earlier that human PBL do respond to intact particles but not to capsomers or individual capsid proteins (40), and furthermore, capsomers or capsid proteins could inhibit interaction of VLPs with cells. Therefore, we included the quality control tests for all our VLP batches used in subsequent experiments.

To test for binding of intact VLP particles to human DCs, we generated, by incubating adherent cells from normal donor PBL in IL-4 and GM-CSF for 7 days. The DC population was harvested, incubated for 1 h with biotinylated HPV-V.LL2-VLPs on ice, and subsequently stained with SA-APC. A distinct binding of VLPs to the DC population was observed by FACS analysis (Fig. 1B, top). The VLP binding cells expressed high levels of MHC class II molecules and CD86, indicating that the VLP binding cells are indeed DCs (Fig. 1, bottom). The VLP binding cells were negative for CD3, CD14, CD16, CD19, and CD56, excluding the presence of B cells, T cells, NK cells, or macrophage/monocytes in the VLP binding cell population (data not shown). The VLP-binding DCs also expressed intermediate levels of CD49f (α6 integrin) on their surface, which has been described as a potential receptor for papillomavirus (41). Overall, these results clearly demonstrate that HPV-16 VLPs bind to the cell surface of in vitro-generated human DCs, possibly via CD49f.

**IL-12 (p70) secretion of human monocyte-derived DCs induced by HPV-VLPs**

Because VLPs did bind to DCs, we investigated whether the binding of VLPs to human DCs could activate DCs. For induction of effective T cell responses, in particular Th1-type T cell responses, the production and secretion of IL-12 is of crucial importance. Thus, we incubated DCs for 48 h either with medium plus GM-CSF alone or medium plus GM-CSF and LPS, L2E7 protein, VLPs, or VLPs that had been heated for 10 min at 95°C. Subsequently, the supernatants were tested for the presence of IL-12 (p70). Indeed, DCs incubated with VLPs secreted IL-12 (p70) into the supernatant, as did the DC plus LPS-positive control. In contrast, low levels of IL-12 secretion were detected in supernatants from untreated DCs, DCs plus L2E7 protein, and DCs plus heated VLPs (Fig. 2). This result indicated that VLPs actually activated human DCs, and therefore, we further investigated the observed activation by analyzing the cell surface expression of various markers known to be activation-dependent on DCs.

**Activation of human DCs by HPV-VLPs in comparison to LPS or TNF-α**

We investigated the potential of VLPs to activate human monocyte-derived DCs in vitro and compared the activation by VLPs with the activation potential of known activating agents for human
DCs. Bacterial LPS as well as rhu-TNF-α have been described to induce activation in human DCs and are commonly used in vitro. We generated human DCs as described above, harvested the DCs after 3 days, and incubated equal number of cells with either 10 µg of VLPs or excess doses of LPS (10 µg/ml) or TNF-α (100 ng/ml) for 1 h in a small volume of PBS. The VLP-loaded cells were subsequently transferred into medium containing GM-CSF. Meanwhile, the LPS or TNF-α exposed cells were transferred into medium containing GM-CSF and either 10 µg/ml LPS or 100 ng/ml TNF-α, respectively. As a control, an equal number of DCs were handled exactly the same way, but no activating agents were added. After 48 h, the cells were harvested and stained for FACS analysis as described. The results of the staining are presented in Fig. 3. Untreated DCs expressed already high levels of MHC class I and class II molecules as well as intermediate levels of CD83 and already high levels of CD86, but low levels of the costimulatory molecule CD80. After exposure to VLPs for 48 h we detected a significant up-regulation of CD80 as well as CD83 on the cell surface of DCs, indicating an activation and maturation of the DCs. Because levels of CD86 were already high, probably because of the manipulation during harvesting and subsequent reincubation of DCs for 48 h, no significant increase in cell surface expression of CD86 could be observed after incubation with VLPs. When DCs were generated by incubation of adherent cells for time periods shorter than 3 days in IL-4 and GM-CSF before collection and exposure to VLPs, no activation effects by VLPs were seen. The up-regulation of CD80 and CD83 was most pronounced when using DCs after 3–4 days in IL-4 and GM-CSF before incubation with VLPs (data not shown). Thus, all subsequent experiments were done with DCs harvested after 4 days of culturing in IL-4 and GM-CSF.

In comparison to excess amounts of LPS or TNF-α, it is obvious that VLPs are activating human DCs in vitro to the same extent as LPS or TNF-α at the concentrations used (Fig. 3). The up-regulation of CD80 as well as CD83 are equally efficient for all activating agents and clearly above the background expression seen on untreated cells. In contrast, CD86 expression is not changed significantly by VLPs, but LPS or TNF-α treatment induced slight up-regulation of CD86 in a part of the DCs. Therefore, the result demonstrates that VLPs have similar effectiveness in activating human DCs in vitro as high amounts of LPS and TNF-α.

The observed activation of monocyte-derived DCs on binding of VLPs was attributable to interaction of VLPs with DCs because 1) the endotoxin level in the VLP prep as determined by Limulus assay was very low (0.085 EU/10 µg ± 0.006 EU/10 µg VLPs) and we found that such low levels of endotoxin could not activate DCs in our experimental setting; 2) a sample of VLPs heated to 95°C for 5 min did not activate DCs, ruling out nonproteinaceous components as activating agents; and 3) a crude lysate of Sf9 insect cells (which are used to produce VLPs) pretreated with an endotoxin binding resin (END-X B15) did not activate DCs, ruling out any additional protein contaminants from the manufacturing process still remaining after the purification steps as activators for DCs (data not shown).

Taken together these results clearly show that VLPs interacting with DCs activate human DCs.
Uptake of chimeric GFP-VLPs by human DCs

We further investigated what happened to VLPs after binding to the cell surface of DCs. For that purpose, we constructed chimeric HPV16-L1L2 VLPs containing GFP fused to the L2 protein, allowing us to directly detect the chimeric VLPs without further staining procedures. After pulsing DCs with chimeric GFP-VLPs for 1 h on ice, loaded DCs were washed and shifted to 37°C. At specific time points (t = 0, 30, 60, 90, and 120 min) samples were removed, fixed with paraformaldehyde, and dried onto slides. After counterstaining with an anti-actin Ab, the samples were analyzed by confocal microscopy for the localization of the GFP-VLP (see Fig. 4). Right after loading, the entire GFP-VLP signal was detected on the cell surface (Fig. 4A), whereas after 30 min at 37°C the GFP signal was found focused into distinct spots on the cell surface (Fig. 4B) and after 120 min at 37°C the GFP signal was detected inside of the cell (Fig. 4C). Treatment of DCs with cytochalasin D, a fungal toxin that disrupts actin filaments, inhibited the focusing of the chimeric GFP-VLPs as well as the uptake of GFP-VLPs during the incubation of a 2-h incubation period at 37°C (see Fig. 4D). The inability of DCs to take up chimeric VLPs because of disruption of the actin cytoskeleton indicated an active uptake mechanism by DCs for VLPs.

Induction of epitope-specific T cell responses by DCs loaded with chimeric VLPs

It has been reported that DCs are capable of channeling exogenous Ags into the MHC class I presentation pathway, and therefore we investigated whether the uptake of chimeric VLP by DCs would lead to induction of peptide-specific, MHC class I-restricted T cell responses in vitro. We chose to use chimeric HPV16-L1L2-E7 VLPs because two HPV16-E7-derived HLA-A*0201-restricted CTL epitopes are well characterized. The HLA-A*0201-restricted peptides 86–93 (TLGIVCPI) and 11–20 (YMLDLQPETT) are known to be exclusively recognized by human CD8+ T cells, to be immunogenic and endogenously processed (42). Therefore, the two CTL epitopes offer an excellent model system for testing the possibility of using chimeric VLPs for the mapping of endogenously processed epitopes. DC generated from PBL collected from healthy HLA-A*0201-positive donors were loaded with HPV16-L1L2-E7 chimeric VLPs, washed, and cocultured with nonadherent autologous PBL. Subsequently, the culture was restimulated twice with autologous, irradiated nonadherent PBL loaded with HPV16-L1L2-E7 chimeric VLPs. For control purposes, cultures were set up in parallel with either DCs loaded with L2E7 protein alone or unloaded DCs for priming and subsequent restimulation with PBL loaded with L2E7 protein or unloaded PBL, respectively. Seven days after the last round of restimulation, the cells from each culture were collected and tested for specific responses to HLA-A*0201-restricted E7-derived peptides by IFN-γ ELISPOT. The experiments were repeated twice with different donors, and the results are presented in Fig. 5. The figure shows the average number of spots above background (spontaneous IFN-γ release from cells not stimulated with peptide) derived from three independent experiments. The statistical analysis demonstrates that we were able to detect an E7 peptide 86–93 (TLGIVCPI)-specific response as determined by IFN-γ ELispot when DCs loaded with chimeric L1L2-E7 VLPs were used for priming. There was no specific response against E7 peptide 86–93 (TLGIVCPI) detected when DCs loaded with L2E7 protein alone were used for priming in vitro. The analysis demonstrates that DCs indeed do take up and process the VLP particles for presentation, indicated by the induction of a specific CTL response in vitro against the immunodominant, HLA-A*0201-restricted CTL epitope. Overall, these results obtained from the in vitro immunization experiments indicate the potential use of chimeric VLP-loaded DCs for in vitro immunization for the definition of T cell epitopes of endogenously processed Ags.

**Discussion**

VLPs are the lead vaccination approach for prevention of HPV infections as well as chimeric L1L2-E7 VLPs for the treatment of...
cervical cancer (43). VLPs have been shown to be highly immunogenic in vivo in animal models as well as in human clinical trials. Our results described here provide an explanation for the observed immunogenic potential of VLPs in vivo. Our initial observation that human DCs get activated by and rapidly take up HPV-16 VLPs demonstrates the interaction of human DCs with VLPs. Furthermore, we present evidence that the viral capsid proteins or fusion proteins thereof can be channeled into the MHC class I presentation pathway, resulting in mature DCs capable of inducing MHC class I-restricted, peptide-specific T cell responses. The experimental assays with immature human DCs is likely to reflect an in vivo situation in which VLPs bind to and activate immature DCs present at the site of injection. After migration of VLP-loaded DCs to secondary lymphoid organs, the fully activated DCs are involved in the induction of humoral and cellular immune responses against capsid proteins as well as fusion proteins.

Ag-loaded, activated DCs are the most potent APCs, and therefore only a limited number of VLP-loaded DCs needs to reach the secondary lymphoid organs to induce a strong immune response (44). For example, a clinical study reported that a single injection of HPV-6 VLPs in human patients with genital warts induced neutralizing Ab titers in most patients (36). The VLP vaccine was administered in the absence of an adjuvant and the Ab titers induced showed the high immunogenic potential of VLPs in humans. These results are in line with similar results obtained in animal models, where vaccination experiments showed that VLPs not only induce Ab responses but also cytotoxic T cells capable of rejecting tumor cells. For example, in a tumor challenge model with chimeric, tumor Ag containing VLPs, a single injection of chimeric VLPs was sufficient to induce protective T cell responses against a challenge with the specific tumor Ag-expressing tumor cell line (37–39, 45). Taken together, the results derived from vaccination experiments demonstrate the potential of VLPs not only to induce protective Ab responses but also the capability to induce specific T cell-mediated cellular immune responses. In particular, the fact that a single immunization with chimeric VLPs was enough to induce protective immune responses indicates the involvement of specialized APCs like DCs. The fact that human DCs bind VLPs in vitro allows the speculation that human DCs also are involved in the induction of immune responses in vivo.

The expression of costimulatory molecules and the stage of maturation of human DCs largely determine the potential of DCs as APCs in vitro as well as in vivo (46). Activation of DCs leading to maturation of DCs by a wide range of different substances (like bacterial endotoxins, nucleic acids, cytokines or heparan sulfates, CD40L interactions, etc.) has been described, and the activation of DCs by noninfectious viral particles also was demonstrated (47). In the latter study, the authors presented data demonstrating activation of DCs by attenuated whole influenza virus. Nevertheless, viral particles consisting of hemagglutinin and neuraminidase proteins alone did not induce up-regulation of MHC class I molecules and costimulatory molecules or cytokine production. Therefore, they concluded that the observed activation of DCs by attenuated viral particles was attributable to the presence of the viral genome; meanwhile, viral capsids alone were not able to activate immature DC. This is in contrast to our results, which show the activation of DCs by VLPs devoid of any viral genome. The observed activation of DCs by VLPs was clearly dependent on VLPs, because endotoxin levels detected in the VLP preparations are too low to activate DCs. We also ruled out that contaminating proteins derived from infect cell cultures used for production of VLPs are responsible for the observed activation of DCs, because crude insect cell lysates after removal of endotoxins were not able to induce the observed effects on DCs. Furthermore, heat denaturation of VLPs abolished the activating potential, and therefore we excluded any nonproteinaceous components in the VLP preparations as activating agents for human DCs. A comparison between different activating agents showed that VLPs were as potent activators as high doses of LPS or TNF-α, as shown in Fig. 4. Thus, this result allows the conclusion that the observed activation of DCs was not induced by an indirect mechanism via cytokines derived from cells other than DCs present in cultures. Overall, the evidence clearly shows that VLPs specifically activate human DCs after interacting with a potential cell surface-expressed receptor on DCs. Indeed, human DCs express CD49f, which was described previously as interacting with VLPs (41) and conferring binding of VLPs to CD49f-negative cells after transfection (48). Therefore, CD49f could serve as a binding receptor for VLPs to DCs. Taken together, our data allow the conclusion that the observed activation of DCs by VLPs resulted from specific interaction of DCs with VLPs and not from any contaminating substances in the VLP preparations or any indirect effect leading to activation of DCs.

Several viruses, such as influenza virus, herpes simplex, or measles virus (49–51) have been shown to infect DCs and lead to inhibition of activation of DCs. Therefore, such an infection could constitute an immune evasion mechanism (52–54). Because papillomaviruses are epitheliotropic viruses binding to cells in the epithelium, the majority of viral particles will infect epithelial cells after entry into the body. Shortly after infection, primarily the early genes of HPV are expressed, and only later on are the capsid proteins L1 and L2 expressed in mature keratinocytes. An immune

![Graph](http://www.jimmunol.org/)
response induced by DCs taking up viral particles at the site of infection would primarily be directed against capsid proteins rather than early gene products. In contrast, an active infection of DCs by HPV could be detrimental for the virus, because expression of early proteins in the cytoplasm would finally lead to the induction of an immune response by infected DCs specific for early gene products. Subsequently, the immune system recognizes and destroys HPV-infected epithelial cells producing new viral particles. Therefore, it seems unlikely that DCs would serve as target cells for infection by papillomaviruses. The fact that the observed uptake of VLPs by DCs in vitro was dependent on an intact actin-cytoskeleton supports the notion of an active process like endocytosis for uptake.

Our results presented here provide evidence that after uptake of chimeric L1L2-E7 VLPs by immature human DCs, the activated DCs are capable of inducing E7-derived peptide-specific T cell responses in vitro. Class I-restricted presentation is in general associated with degradation of cytoplasmic proteins, which is considered inaccessible to exogenous Ags. Nevertheless, there is evidence that DCs could channel exogenous proteins after uptake into MHC class I presentation pathway and induce specific T cell responses (18, 55). This is best seen in cross-priming effects in vivo (4), where exogenous Ags, for example, derived from tumor cells, are taken up by APCs and presented to cells of the immune system. It also has been postulated that Ag transfer between DCs might be essential for the efficient stimulation of Ag-specific immune responses (56). Furthermore, the enhancement of MHC class I presentation by exogenous particulate Ags also has been described (57). Because VLPs represent a structure similar to particulate Ags, a similar process leading to MHC class I presentation as described for particulate Ags could be envisioned for VLPs. Our results show that, indeed, loading of DCs with chimeric L1L2-E7 VLP led to E7-specific T cell responses. Chimeric VLPs are devoid of any viral genome, and no expression of E7 protein could take place in the cytoplasm of DCs exposed to VLPs. Therefore, all peptides presented in an MHC class I-restricted fashion are derived from exogenous chimeric VLPs added to the culture, demonstrating the ability of DCs to channel exogenous proteins into the MHC class I presentation pathway. The fact that chimeric VLPs lead to induction of peptide-specific T cell by an in vitro immunization system described here indicates that such a system could allow the identification of endogenously processed epitopes of any Ag delivered to immature DCs by the use of chimeric VLPs. Therefore, chimeric VLPs not only represent an efficient vaccination approach in vivo but also a tool for identification of endogenously processed T cell epitopes.

Chimeric VLPs are explored in clinical trials as vaccine for the induction of tumor Ag-specific immune responses. The induction of tumor Ag-specific cellular immune responses in vivo is critically dependent on several factors: the presentation of tumor Ag-specific peptides, the expression of costimulatory molecules by the APC, and the cytokine environment during the interaction of APCs with specific T cells. A tumor-specific CTL response is most efficiently induced by APC providing stimulatory signals via costimulatory molecules as well as a favorable cytokine environment. The importance of costimulatory molecules like CD80 is undisputed in the induction of effective anti-tumor immune responses (58, 59). Furthermore, a potent anti-tumor effect of IL-12 has been described, either by systemically administered IL-12 (60) or by vaccination with poorly immunogenic tumor cells transfected with IL-12 genes (29, 61). Furthermore, synergistic effects of expression of CD80 simultaneously with the secretion of IL-12 in inducing protective immunity against poorly immunogenic tumors in vivo have been demonstrated (62, 63). Therefore, the ability of chimeric VLPs to deliver tumor Ags to DCs and to induce CD80 expression as well as simultaneous secretion of IL-12 renders VLPs powerful tools for vaccination approaches against cancer. Several clinical trials are currently exploring the potential of chimeric L1L2-E7 VLPs to induce cellular immune responses against E7-expressing cells. Immunotherapy of cancer in general is critically dependent on the vaccine delivery system, and chimeric VLPs could, indeed, be a universal carrier for tumor Ags for the treatment of a variety of malignancies by specifically activating DCs.

The central role of DCs in controlling the induction of immune responses renders DCs a highly attractive target for immunotherapy. The evidence we present here indicates the versatility of chimeric VLPs to deliver specific Ags like E7 of HPV to immature DCs. Our data provide insight in the mechanism underlying the high immunogenic potential of VLPs in vivo and could help in the future to design better vaccination strategies for immunotherapy of cervical cancer.

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